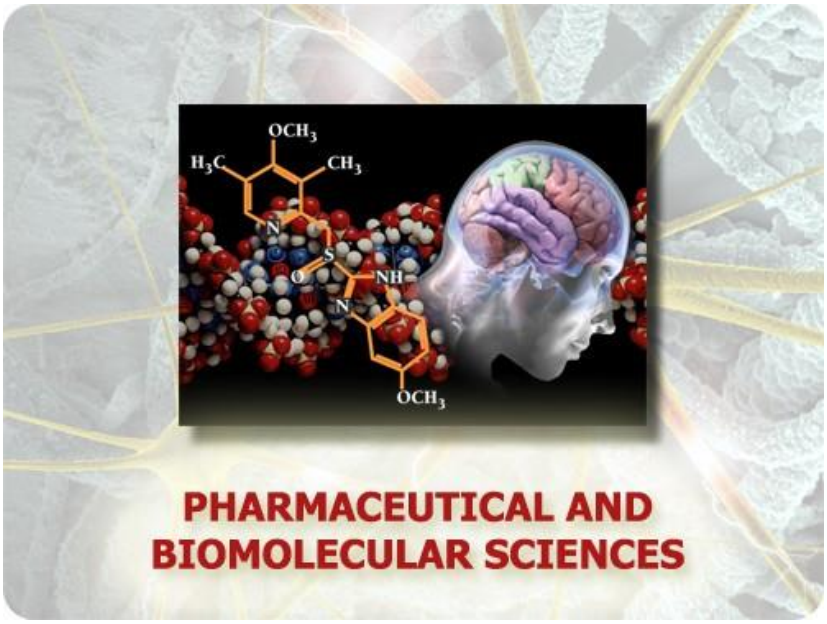
Università degli studi di Torino

PhD in
Pharmaceutical and Biomolecular Science
XXXV



"Omics approaches to study the chemistry behind the different aspects of coffee quality"

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1. Introduction

1.1 Food quality: I see, I like and therefore I drink it!

Food quality is a complex, dynamic and multifaceted concept that is affected by a wide variety of contextual and situational factors. Moreover, its definition has witnessed an evolution in time and embracing nowadays different aspects covering the whole food chain production (Total quality) (figure 1.1).

Figure 1.1 Schematic illustration of the different aspects covering the concept of food quality.

The concept has been defined in the 24th Food and Agricultural Organization of the United Nations (FAO) Regional Conference for Europe and introduced in the International Standardization Organization (ISO 9001:2015) (Food and Agriculture Organization of the United Nations (FAO), 2004; "ISO - ISO 9001:2015 - Quality management systems — Requirements") Historically, quality has been interpreted as the lacking of defect, fraud and adulteration. During time, quality have to guarantee the expected properties such as sensory and nutritional characteristics or resulting benefits. The most recent standard, the ISO 22000:2018 extends the concept joining the unavoidable food quality and safety management systems ("ISO - ISO 22000:2018 - Food safety management systems — Requirements for any organization in the food chain," 2018).

Dimension of quality depends from consumers' expectations and from producers' views.

While the quality sought by industries is an objective standard type that is well defined, measurable and verifiable, with respect to certain standard parameters in offering products and services in conformity with customers' needs, the quality required by the consumer is, instead, a perceived standard, determined by subjective factors dependent on the moment and / or the situation (Illy, 2005).

The quality is formerly measured by three criteria:

- Suitable value: that is restricted to a group of target people as producers, processors and traders that must respect the stability, the transport, the processing of the product. As part of market value, time, is increasingly paid attention to, since it incorporates the terms *preparation* and *consumption* of the food (ready-to-eat food and fast food).
- Sensory value: is the aspect that is considered most important by the consumers, since in comparison to other quality categories, the relevant properties of this quality class can be easily detected with the senses.
- Health value: another consumer utility based on the sum of essential nutrients content (Leitzmann, 1993)

1.1.1 The value of the sensory experience for the consumers

Sensory quality can be defined as a product's ability to satisfy consumers' hedonic needs. It is therefore subjective because it is interpreted by each consumer and cannot be measured in absolute terms. Sensory quality is influenced both by the individual genetic characteristics of the sense organs and individual's ability to use them, and by the traditions of the various regions in the world.

In the choice of consuming a food rather than another, the sensory experience, and so the association to quality, have a very specific effect on the consumer option, influencing their decision. Consumer preference is used in this context to refer to an hedonic evaluation of the food, to distinguish the attractiveness or the displeasure associated with the selection of the product itself.

Sensory evaluation is used by the human brain to analyze and interpret reactions related to the enjoyment of food. Like any complex human behavior, food choice is influenced by three different factors: emotional aspects; physiological effects; and economic, social and cultural environment (Clark, 1998).(Clark, 1998). The field of sensory evaluation grew in parallel with the processed food industries; since product flavor quality drives consumer in the choice of the food, industries require knowledge and control over the sensory impact that a food promotes. These sensory measurements have become a powerful tool for:

- Quality monitoring
- Product and innovation development
- Recipe optimization
- Lowering costs (Lawless, 2010; Heymann, 2016; Regueiro, 1985).

In this context, aroma has a special fascination to humans; it is one of the main attributes of food quality evaluated by consumers at the time of purchase. Beyond the fact that food is essential to survive, the hedonic side considers food as an emotional source. It has been underlined that food reward can be explained by the concepts of appetite/incentive motivation and pleasure/palatability (Kringelbach, 2004). The pleasure connected to eating foods, consists of multiple brain networks that involves the classical sensory input among which, sight, hearing, smell, taste and touch (Kringelbach, 2015). While smell and taste are classified as “chemical senses”, due to the conversion of the chemical signals into action potential in sensorial neuronal fibres, the other senses, are elicited by “physical forces” such as light, sound, temperature or pressure (Buettner, 2017). The balance of the two chemical senses, smell and taste, forms the most important factor of what is defined as *flavour* perception (Kringelbach, 2015; Prescott, 2015; Sunarharum, 2014). Particularly, flavour consists of the combination of *non-volatile* taste, perceived in the mucosa of the oral cavity and the *volatile* aroma, perceived nasally (Folmer, 2016; Shepherd, 2006) and they are reciprocally affecting each other. The sense of smell is the unique perception having a dual nature: *orthonasal* feeling, that occurs when volatiles organic compounds (VOCs) are inhaled through the nose and interact directly with the olfactory system in the absence of an oral stimulus; and *retronasal* sensation, that occurs when VOCs, released from food during ingestion are moved from the back of the throat to the nasal cavity through the pharynx,

to the olfactory epithelium. The signal transmission from the olfactory nerves pass to the olfactory bulb and then to the brain, where it was elaborated as an odorous information (Folmer, 2016; Shepherd, 2006; Sunarharum, 2014) (figure 1.1.1.1).

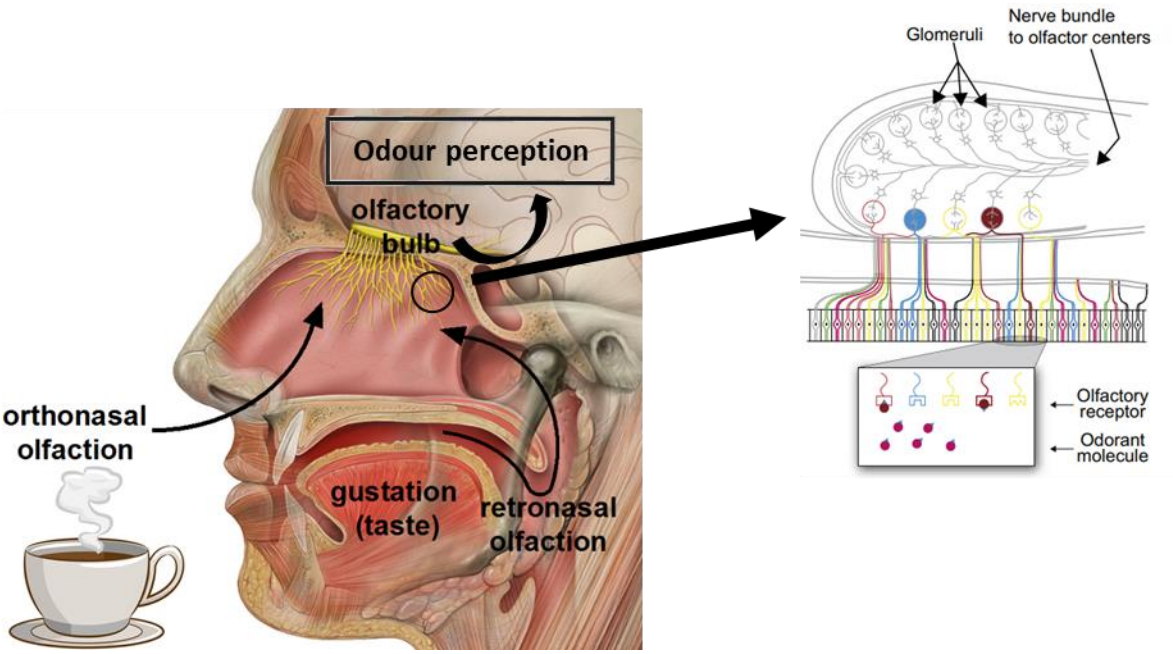

Figure 1.1.1.1 Schematic illustration of the olfactory system.

Since the volatile molecules arise in the mouth, the retronasal sense, is attributed both to the taste sensation and to the smell feeling (Shepherd, 2006). In fact, the retronasal olfactory pathway, is combined with taste, touch, sound and active sensing nerve tract, forming a flavour system. The sense of taste, detected from buds located on surface of the tongue, represent the most obvious component of food flavour, eliciting the basic emotions of pleasure (sweet), disgust (bitter), sour, salty and savoury (Kringelbach, 2004; Shepherd, 2006; Sunarharum, 2014). While sipping a cup of coffee, the perception detected stimulates a complex multisensory experience that involves sensory sensorial characteristics. Particularly, the smell sensation, perceived through the VOCs, is achieved both from the nasal cavity, through inhalation, and from the oral cavity, during the exhalation. The gustation, perceived from coffee beverage, is linked to saltiness, sweetness, sourness, bitterness and savouriness. Besides aroma and taste, texture, mouthfeel and chemesthesis are other sensorial additional modalities perceived from coffee, influenced by the interaction between the beverage with the lining of the mouth during consumption. These somatosensory sensations include body (viscosity), described as heavy or light, astringency, roundness, oiliness, intensity, smoothness, sharpness and creaminess. To complete the scheme of the

senses, there are the physical senses of sight (the aspect of foam and the colour) and the hearing (the call of the whistle of the Moka machine) (figure 1.1.1.2). (Buettner, 2017; Folmer, 2016; Sunarharum, 2014).

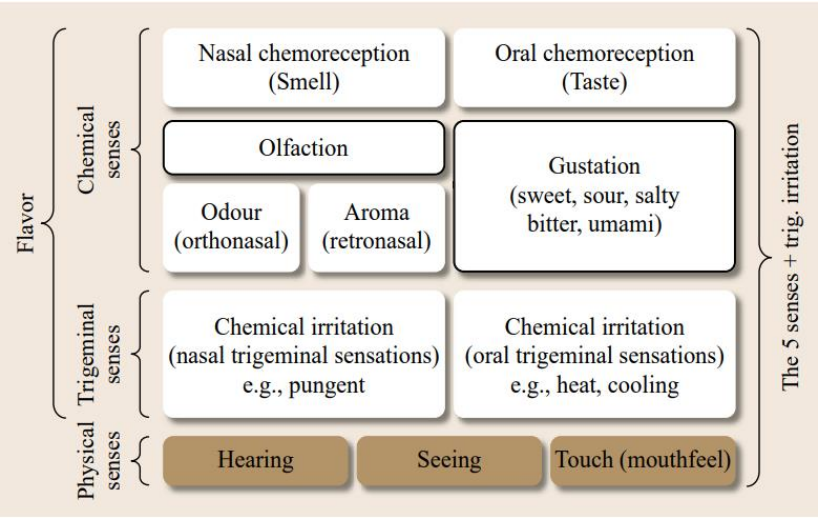

Figure 1.1.1.2 Schematic illustration of the flavour composition.

Coffee become one of the major beverage of consumed for its energizing effect and for its socialization capacity. The pleasure to drink a cup of coffee is mainly linked to its aroma and flavour.

Consumers, in industrialized countries, are asking high quality coffee products, at a competitive prices. Coffee quality could be perceived from an objective and subjective dimension: the first one refers to the physical characteristic, as appearance, texture, flavor and nutritional content and the second one, concerned how the consumers perceived the beverage. The two statement are strictly related and are the base of its economic value.

A part of the economic coffee market is based on the knowledge of the benefits that costumers derived from the product. These benefits are directly related to distinctive sensorial attributes, nutritional properties and the sense of social status. In fact, coffee evolved, during years, from a commodity product, to a high-quality artisanal food, capable of stimulating sensory and nutritional satisfaction to the body. This switch has been possible due to three approaches that currently characterize the consumer coffee: *pleasure*, *health* and *sustainability*. The *pleasure* is linked to the sensory attributes, that an individual can experience directly by consuming the coffee. Those are directly dependent from variety of origin, species, geographical location, harvesting and processing methods and they are the main reason why the price of food is strictly dependent on the attributes of reputation, create by sensory quality experts. Hedonic evaluation is an aspect that can be evaluated in marketing as it remains a link to communicate the quality index to consumers. Through the sensory characteristics, for example, it is possible to define arabica coffee species, which possess superior sensory characteristics, as a high-cost product, compared to robusta, which is more resistant, but has a lower production cost, resulting in a lower quality of the cup (Illy, 2005). Understanding the sensory determinants, allow us to estimate the fundamental market prices for the development of sustainable supply chains. The preferential evaluations of consumers/buyers, as well as the information on prices acknowledgement, allow the orientation of manufacturers on best production practices to achieve the desired sensory attributes in the coffee market. So, sensory analysis provides a method for examining the relationship between the price of an asset and the qualitative attributes it enjoys that good (Donnet, 2008; Samoggia, 2018). In recent years, there is a constant growing in the focusing of the constituents of the human diet and of pollutants in food and beverages, in order to avoid health risks. Health, in fact, is considered connected not only to the nutritional

properties related to product, but also to food safety connected to the internal and external contaminants, that could be generated both from the environment and during post-harvest processing. Action against contaminants is important to protect the economies of producer countries, the farming population and the product quality. In fact, industries are trying to ensure the maximum stability, quality and safety to the food cycle, to satisfy the consumer expectation and to improve the food welfare, increasing the investments in the control of health risk. Nowadays, purchases are more based on the concept of *sustainability*. With the increase in coffee consumption around the world, the environmental impact has played an important role (Samoggia, 2018). Industries are trying to apply solutions to remain competitive helping to improve the sustainability of their business, adopting the concept of circular economy: "…*use and then recycle, rather than throw away..*". This occurs in a context in which the growing consumer demand for the individually packaged products (Gandia, 2018; Marinello, 2021). The concepts of safety and sustainability, will be addressed in the following chapters.

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1.2 Aroma and flavour descriptors: take time to stop and smell a cup of coffee

Coffee beans are obtained from the two commercial type of tropical species of the *Rubiaceae* family: *Coffee Arabica* and *Coffee canephora*, which main variety is *robusta* (Folmer, 2016; Bröhan, 2009; Toledo, 2016). The two species, which cover 70-80% and 20% of the commercial market respectively, differ in geographical origin, climate conditions and altitude (Folmer, 2016; Kulapichitr, 2019; Sunarharum, Williams, 2014). *Arabica* grows well at medium/high altitudes (1000 to 2100 m) with daily average temperatures of around 18 to 22 °C, typical of equatorial regions, developing a most distinctive, tasteful and intense aroma which results in a clear preference by consumers (Moeenfard, 2020). In contrast, *Robusta coffee*, that is less vulnerable to pests and diseases, is less requested than *Arabica* cultivars, with a better resistance to hot and humid climate (Moeenfard, 2020; Toledo, 2016).

The ripe red cherry fruits, produced by coffee shrub, usually contain two seeds, known as "green coffee". Coffee cherries have several layers covering the seeds: the outer skin (pericarp), the pulp (mesocarp), the mucilage, the parchment (endocarp) and the silver skin that is directly in contact with the bean (Folmer, 2016; Buffo, 2004) (figure 1.2.1).

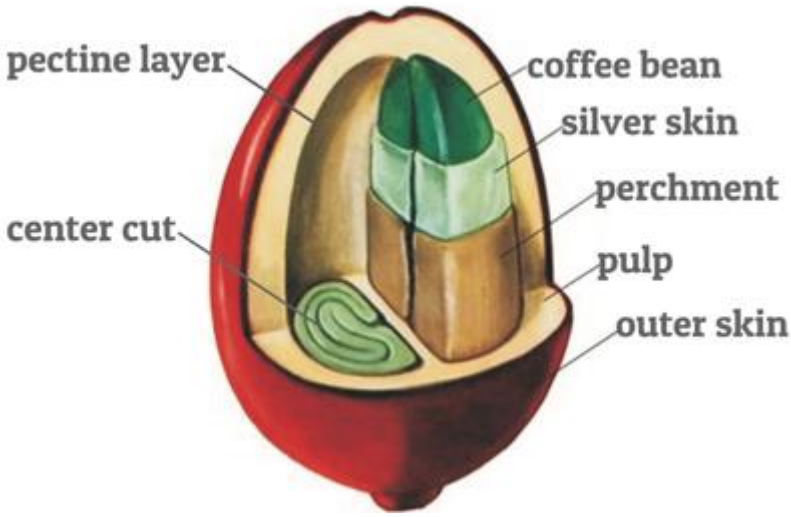

Figure 1.2.1: Coffee cherry anatomy. Source: https://essense.coffee/en/coffee-the-journey-from-the-seed-to-your-cup/.

Coffee harvesting occurs between December and February and between May and August with two collection systems: by *hand picking*, in which only the ripe cherries are collected

(more time-consuming) and *mechanical harvesting*, in which all cherries, damaged or not, are collected (cheaper and less accurate) (Flament, 2002). The fruits, once harvested, are washed with water in order to separate the impurities and then are depulped using a *dry* or a *wet method*: the first one consists on sun drying the fruits including with the outer skin, pulp and mucilage, which are removed only once dried, the wet procedure consists in removing the pulp prior, allowing the mucilaginous layer to be removed by natural fermentation. After washing, the so-called parchment coffee is dried by sun or hot air drying and the silver skin and the parchment removed (Folmer, 2016; Flament 2002; Sunarharum, 2014; Toledo, 2016). The choice of the type of postharvest processes choice tends to be cultural and associated with the country of the coffee's origin, due to the different flavour obtained: dry processing produces a 'hard' coffee with a medicinal flavour, heavy body, sweet, smooth, and complex attributes, while wet processing yields a better quality coffee with less body, higher acidity and more aroma (found application for *Arabica* coffee) (Sunarharum, 2014). Different green bean origins are blended and roasted together to produce the characteristic aroma, colour, brittle, porous texture, developed in chemical reactions during the roasting process (Buffo, 2004; Marin, 2008; Toledo, 2016). The *roasting procedure* is characterized by three phases, during which temperatures vary between 180°C and 240°C in a period between 8 and 15 minutes:

1. An initial phase, *endothermic*, during which moisture is eliminated. Heat from hot gases or hot metallic surfaces is transported into the green bean with a temperature up to 100°C. The water starts evaporating and the gaseous reaction products like carbon dioxide and steam quit the bean.
2. The *exothermic phase* (170°C-220°C) during which a number of complex pyrolytic reactions take place and the release of many compounds from them leads to appearance of characteristic aroma and coffee taste. The beans change colour to a dark brown.
3. A final rapid *cooling phase* to stop the roasting process and reactions (figure 1.2.2) (Folmer, 2016; Buffo, 2004; Clarke, 2014; Sunarharum, 2014).

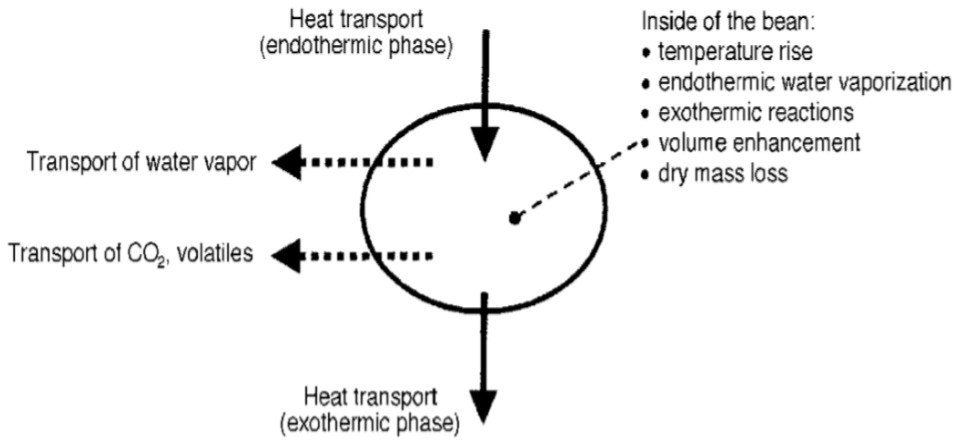

Figure 1.2.2 Roasting of coffee beans, from (Clarke, 2014).

During roasting coffee beans swell, increase in volume and change colour and acidity. Once the roasting degree is achieved a fast quenching, using a cold air method or the water method, is necessary to avoid over-roasting and to stop exothermic reactions (Baggenstoss, 2007).

The quantity of heat transferred to the beans during roasting is an influential parameter to produce different aroma profiles, from the light roasting that produces sweet, cocoa and nutty aromas to the dark roasting responsible for burnt/acrid, ashy/sooty, sour and pungent coffee (Sunarharum, 2014).

Roasting is a very important step of the whole coffee production chain, primary in obtaining a high quality coffee aroma.

Non-volatile compounds

The basic taste sensation of coffee beans is given by non-volatile compounds, which determine principally bitterness, sourness and astringency. Consequently to roasting, there is a considerable degradation of polysaccharides, sugars, amino acids, chlorogenic acids and a moderate relevant increase of organic acids and lipid content (Buffo, 2004; Flament 2002).

The main reactions are schematized in figure 1.2.3

Carbohydrates: this class is essential in the aroma formation compounds, mainly by *pyrolysis* of the low molecular weight sugars and by *Maillard reaction* with the amino acids.

Monosaccharides and the *disaccharide sucrose*, that are present in green coffee beans, the 55% of total carbohydrates, are very fragile to heat treatment, thus playing an important role as a precursor in the flavour generation. *Polysaccharides* represent the main fraction of green coffee beans. Among monosaccharides dominate *arabinose*, *galactose*, *mannose*, *mannans* and *cellulose*; in roasted coffee, they take part in withholding volatiles and in contributing to the viscosity of the coffee brew (Folmer, 2016).

Acids: they include, to a greater extent, *chlorogenic* and *quinic acids* and, to a lesser extent, *citric, malic, oxalic, tartaric, pyruvic acids*. Some acids come from carbohydrate precursors during roasting process. In particular acids such as *formic, acetic* (increase up to a maximum of 240°C, then degrade), *glycolic* and *lactic* (continue increasing up to 280°C) in the free form, but also, in the form of lactones and esters. The higher content of carbohydrates present in *C. Arabica* determine a high acid concentration compared to *Robusta* (Clarke, 2014). Citric and malic acids, with the roasting, degrade to citraconic, glutaric, itaconic, mesaconic and succinic acids from citric acid, fumaric and maleic acid from malic acid. Quinic acid remain relatively stable during roasting, while the content of chlorogenic acids (CGAs) decrease, since it hydrolyzes to quinic acid, precursor of guaiacol, 4-ethyl- and 4-vinylguaiacol key volatile odorants and *caffeic acid*, responsible of the formation of phenolic volatiles and to the corresponding lactones. These acids, in general, contribute to acidity, bitterness and astringency in coffee brew (Folmer, 2016; Clarke, 2014).

Lipids: this class is majorly present in *Arabica coffee* than in *Robusta* and most of lipids are located in the endosperm of bean and a small part, also, in the wax, outside the coffee bean. Lipids can be classified into saponifiable fraction for 75% constituted by *triglycerides (TAGs)* and unsaponifiable one for the other 25% composed by 19% of *free fatty acids (FFA)* and *esterified diterpene*; by 5% of *free and esterified sterols* and the remainder, by substances such as *tocopherols (α-tocopherol)* and *squalene* (Clarke, 2014). Lipid fraction has the function of conveying the active compounds and, among them, the aroma components, contributing to the perceived texture and mouthfeel in the brew (Moeenfard, 2020). Furthermore, this fraction is responsible of the crema emulsion in espresso coffee. Lipids are not stable to high temperatures and so they could be used as markers for the shelf-life definition of roasted

coffee since they observed a continuous decrease in TAGs content during storage associated with a strongly increasing of FFA (Cincotta, 2020).

Aminoacids: free forms are less than 1% of green coffee, although they are fundamental in Maillard reactions and Strecker degradation. Glutamic acid, aspartic acid, leucine and asparagine are the main free amino acids. During roasting serine, cysteine, methionine and lysine are reduced and arginine disappears, reacting with amino-carbonyl of reducing sugars to form a pool of compounds as furfurylthiol, thiophenes, thiazoles and heterocyclic compounds such as alkylpyrazines, pyrroles, pyrrolizidines, pyridines. Glutamic acid, alanine, glycine, leucine and valine, instead, are the most stable and increase (Flament, 2002). Due to the Strecker degradation, chemical components containing carbonyl compounds can also react with free amino acids, resulting in the formation of aldehydes, ketones, ammonia, and carbon dioxide (Buffo, 2004; Holscher, 1992; Marin, 2008; Toledo, 2016).

Nitrogen-containing compounds: as *melanoidins*, that impart the color to roasted coffee, *caffeine*, who contributes to the strength, body of coffee brew, not being influenced by the roasting process and *trigonelline*, who on the contrary, is rapidly degraded during roasting being involved into the formation of nicotinic acid and volatile compounds such as pyridines and pyrroles (Folmer, 2016; Buffo, 2004; Sunarharum, 2014).

Free sugars	Fragmentation			
	→	Aliphatic acids		
	Dehydration			
	→	volatile heterocyclic compounds		
	Maillard reaction			
	→	melanoidins		
		Furans		
	Thermal degradation	Ketones		
	→	Furanones		
		Aldehydes		
		Pyridines	Oxidation	
		Pyrroles	Aldehydes →	Acids
		Oxazoles	Strecker degradation → Ketones	
	Maillard reaction	Pyrazines	Ammonia	
	→	Thiazoles	CO_2	
	with free aminoacids	Furans		
		Furanones		
		Dicarbonyl		
		Thiophenes		
Trigonelline	Thermal degradation	Nicotinic acid		
	→	Pyridines		
		Pyrroles		
CQAs	Thermal degradation			
	→	Phenolic compunds		
	Hydrolisis	Quinic acid		
	→	Caffeic acid →	Phenolic compounds	
		Lactones		
Lipids (unsaturated FA)	Oxidation	Furans		
	→	Aldehydes		
TAGs	Hydrolisis			
	→	FFAs		
Aminoacids: cysteine and methionine	direct degradation			
	→	Thioles		
	or with reducing sugars	Sulphides		
Aminoacids: valine, isoleucine and leucine	→	Aldehydes		
Aminoacids: serine and threonine	→	Alkylpyrazine		
Citric acid		Succinic acid		
	Thermal degradation	Citraconic acid		
	→	Glutaric acid		
		Itaconic acid		
		Mesaconic acid		
Malic acid	Thermal degradation	Fumaric acid		
	→	Maleic acid		

Figure 1.2.3: Schematic principal reactions that can occur during roasting process.

Volatile compounds

Coffee is especially drunk for its flavour and mostly for its aroma. The pleasant coffee aroma is due to the presence of more than 850 volatile compounds that belong to different chemical families, including furans, pyrazines, ketones, pyrroles, phenols, hydrocarbons, acids and anhydrides, aldehydes, esters, alcohols, sulfur compounds, anisoles, pyridines, thiazoles and thiophenes.

Volatile organic compounds (*VOCs*), are the most quality-determinant of coffee, that range in concentration from part per million (ppm) to part per trillion (ppt). However, among the all number of compounds that are considered as VOCs, only few, called *key aroma compounds*, are capable to impact on coffee flavour, presenting perceivable odours. The characteristic of key odorants is such as to be capable to stimulate the olfactory receptors and to be both volatile and hydrophobic, so that to be recognized as an odour, being present in concentrations exceeding their smell threshold (Blank, 1991; Bröhan, 2009; Caporaso, 2018; Fisk, 2012; Glöss, 2014; Grosch, 1998; Maeztu, 2001; Marin, 2008; Semmelroch, 1996; Sunarharum, 2014; Toledo, 2016).

These key odorants are of a great importance, since their loss or a small change in their composition will result in coffee aroma defect or off-flavour (Regueiro, 2017).

Furans: jointly with pyrazines, this class is quantitatively more abundant in coffee. Furans are formed in all food submitted to roasting, promoting the characteristic malty and sweet roasted aromas with a high sensory threshold. This class is generated for thermal degradation of carbohydrates, oxidation of polyunsaturated fatty acids and decomposition of ascorbic acid or its derivatives (Bicchi, 2011; Sunarharum, 2014).

Pyrazines: jointly with furans are the major abundant class in coffee and together with thiazoles have the lowest sensory threshold concentrations, even if they contribute as potent key odorants to coffee aroma. Pyrazines exhibit nutty, earthy, roasted, green aroma, formed during the Maillard reaction between amino acids and reducing sugars (Lolli, 2020; Pickard, 2013; Sunarharum, 2014).

Aldehydes and **ketones**: aldehydes are Strecker degradation and autoxidation of unsaturated fatty acids (FA) products, responsible of the malty coffee note. Ketones are produced from the pyrolysis of carbohydrates and impart mushroom, but even caramelised sweet notes (Lolli, 2020; Sanz, 2001).

Sulfur-containing compounds: they result from the Maillard reaction of cysteine and methionine with reducing sugars. The main class of compounds are thiols, which are the major contributor to coffee aroma despite their presence at relatively low concentrations. These compounds are characterized by roasted, sulphur and meaty notes (Bressanello, 2017; Lichtenberg, 2007).

Furanones: are a significant group of volatiles in coffee in terms of abundance and potency. This class impart sweet and caramel note to coffee aroma.

Phenolic compounds: these compounds originated from the thermal degradation of CQAs and are characterized by a spicy phenolic aroma and vanillin note.

Trace amounts of **esters, pyridines, pyrroles, terpenes, oxazoles, lactones, alkanes, alkenes, benzenic compounds** and **acids** were also detected in roasted ground coffee (Sanz, 2001).

All together, these components and their relative ratios that can be affected from post-harvest treatments, by the storage conditions and shelf-life and by the brew preparation impacting on coffee aroma perceived. A summary of the most important classes of volatile compounds is reported in table 1.2.1

Table 1.2.1: a summary of individual compounds important to the flavour of roasted coffee powder, together with aromatic notes, sensory threshold concentration and key odorants (Bicchi, 2011; Blank, 1991; Bressanello, 2017; Clarke, 2014; Gemert, 2011; Grosch, 1998; Lolli, 2020; Maeztu, 2001; Pickard, 2013; Ribeiro, 2009; Semmelroch, 1995; Sunarharum, 2014).

VOCs	Aromatic note	Sensory threshold (ppb)	Key odorants
Furans			
Furfural	Sweet woody almond fragrant baked bread	280	
2-((methylthio-methyl)furan	Smoke roast		
2-furanmethanol acetate	Sweet fruity banana horseradish		
2-methylfuran	Ethereal acetone chocolate		
5-methyl-2-furancarboxyaldehyde	Caramel	6000	
Furfurylformiate			
Furfurylmethyl ether	Sweet spicy		
Furfuryldisulfide	Roasted coffee, sulphurous cooked meat and liver, onion and garlic nuances		
2,5-dimethylfuran			
Vinylfuran			
2-(methoxymethyl)furan			
2-furfurylfuran	Roast		
2-(2-furanylmethyl)-5-methyl-furan	Hearthy, mushroom		
2,2'-methylenbis-furan	Roast		
Pyrazines			
2,3-dimethylpyrazine	Musty, nut skins, cocoa powdery and roasted with potato and coffee nuances	800	
2,6-dimethylpyrazine			

2,5-dimethylpyrazine	Nutty, peanut, musty, earthy, powdery and slightly roasted with cocoa powder nuance	80	
2,3-diethyl-5-methylpyrazine	Nutty, roast, earthy	0.09	Key odorant
2-ethenyl-3,5-dimethylpyrazine	Earthy	0.000012	
2-ethenyl-3-ethyl-5-methylpyrazine	Earthy	0.000014	
2-ethyl-3,5-dimethylpyrazine	Nutty, roast, woody, papery, earthy	0.04/0.16	Potent key odorant
2-ethyl-3,6-dimethylpyrazine	Earthy	8.6	Key odorant
2-methoxy-3,5-dimethylpyrazine	Earthy	0.006	
2-methoxy-3,2-methylpropylpyrazine	Green earth		
2-methoxy-3-isobutylpyrazine	Pea earthy beany chocolate nutty		Key odorant
2-methoxy-3-isopropylpyrazine	Earthy roasty	0.002	Key odorant
3-ethenyl-2-ethyl-5-methylpyrazine	Earthy		
3-isobutyl-2-methoxypyrazine	Peasy, earthy	0.002/0.005	Key odorant
2-isobutyl-3-methoxypyrazine	Green, earthy		
6,7-dihydro-5-methyl-5H-cyclopentapyrazine	Nutty, roast		

Ethylpyrazine	Nutty, musty, fermented, roasted, cocoa and meaty nuance	4000	
Methylpyrazine			
2-ethyl-6-methylpyrazine	Earthy, musty		Potent key odorant
2-ethyl-5-methylpyrazine			
2-ethyl-3-methylpyrazine			
2,6-diethylpyrazine			
3-ethyl-2,5-dimethylpyrazine			
2-ethenyl-5-methylpyrazine			
Trimethylpyrazine	Earthy roast		
5-methyl-5-cyclopentapyrazine	Roasty, sweet		
2-ethylpyrazine	Earthy, musty		Potent key odorant
2,3,5-trymethylpyrazine	Roasty, earthy		Potent key odorant
3,5-dimethyl-2-ethylpyrazine	Earthy		Key odorant
Aldheydes			
2-methylbutanal	Malty	1.3/1.9	Key odorant
2-methylpropanal	Buttery oil		Key odorant
3-methylbutanal	Malty, fruity, almond, aldehydic	0.35/0.4	Key odorant
(E)-2-nonenal	Fresh, woody, fatty	0.08	
3-methylpropanal	Pungent, fruity		Key odorant
Acetaldehyde	Malty	0.7/10	Key odorant
Methylpropanal	Minty	0.7	Key odorant
p-anisaldehyde	Sweet fruity, honey like	27	
Phenylacetaldehyde	Fruity		
Propanal		10	Key odorant

Hexanal			
2-(methylthiol)propanal			
Phenylacetaldehyde			
5-methylfurfural	Caramel		
Furfural			
Butanal			
Benzaldehyde	Strong sharp, sweet, bitter, almond, cherry		
2-methylbenzaldehyde			
2-methyl-3-(2-furyl)acrolein			
1H-pyrrole-2-carboxaldehyde	Musty		
Ketones			
1-octen-3-one	Mushroom like	0.0036	
2,3-hexadione	Sweet, creamy, caramellic, buttery with a fruity jammy nuance		
2,3-butanedione	Buttery oil	0.3/15	Key odorant
2,3-pentanedione	Buttery oil	20/30	Key odorant
3,4-dimethylcyclopentenol-1-one	Caramel like, sweet	20	
4-(4'-hydroxyphenyl)-2-butanone	Sweet fruity, (rasberry)	From 1 to 10	
2-hydroxy-3-methyl-2-cyclopenten-1-one	Slight rum like		
1-hydroxy-2-butanone	Caramellic-spicy, maple like		
3-hydroxy-2-butanone	Sweet coffee musty grain malt butterscotch		

2-hydroxy-3,4-dimethyl-2-ciclopenten-1-one	Buttery		
2-butanone	Caramel like	20	
2-pentanone			
3-penten-2-one			
1-(furan-2-yl)ethanone			
1-(furan-2-yl)butan-2-one			
1-(1-methylpyrrol-2-yl)ethanone			
Furfural acetone derivative			
1-hydroxy-2-propanone	Caramel		
Acetoxyacetone	Fruity		
Sulphur compounds			
3-mercapto-3-methylbutylformate	Green blackcurrant, catty, roasty	0.0035	Key odorant
2-methyl-3-furanthiol	Meaty, boiled	0.007	Key odorant
3-mercapto-3-methylbutylacetate	Roasty		
3-methyl-2-butene-1-thiol	Amine-like	0.0003	Key odorant
Methanethiol (methyl mercaptan)	Putrid,sulphurous, cabbage-like	0.02/0.2	Key odorant
2-furfurylthiol	Roasty	0.01	Key odorant
3-methyl-2-butene-1-thiol	Meaty	0.0003	Key odorant
3-methylthiophene	Roasty		
4-methyl-4-furanthiol-(furfurylmercaptan)			
2-furanmethanethiol			
3-mercapto-3-methyl-1-butanol	Meaty (broth)		
3-methylthiophene			Key odorant

2-thiophenecarboxaldehyde			
5-dimethyl trisulfide	Cabbage like	0.001/0.01	Key odorant
bis (2-methyl-3-furyl)disulphide	Meaty	0.00076	
Methional	Boiled potato like	0.2	Key odorant
Dimethyldisulphyde			
Furfuryl-methyl-sulphide	Vegetable		
Thiophene			
Thiazole			
2,4-dimethyl-5-ethylthiazole	Earthy, roasty, meaty		
4-methylthiazole			
Trimetilthiazole	Earthy, roasty		
Furanones			
Dihydro-2-methyl-3(2H)-furanone	Sweet and solvent like with a brown rummy and nut like nuance	0.005	
2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone (EHMF)	Sweet caramel	20/1.15	Key odorant
3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolone)	Sweet caramel, seasoning like	20	Key odorant
4-hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol)	Sweet caramel	10	Key odorant
5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone (abhexon)	Caramel like, seasoning like	7.5	Key odorant
5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone	Sweet caramel	1.15	

5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone	Sweet, fruity, sweet caramel with a maple like nuance		
2,5-dimethyl-3(2H)-furanone	Fruity, caramelic		
Phenolic compounds			
4-ethylguaiacol	Spicy	25/50	Key odorant
4-vinylguaiacol	Spicy, woody	0.75/20	Key odorant
2-methoxy-4-methylphenol	Phenolic, burnt		
2-methylphenol (o-cresol)			
3-methylphenol (m-cresol)			
Vanillin	Vanilla like	25	
Guaiacol	Phenolic, burnt, spicy	2.5	Key odorant
Acids			
2-methylbutyric acid	Acidic, fruity, dirty, cheesey with a fermented nuance	10	
3-methylbutyric acid	Cheese, dairy, acidic, sour, pungent, fruity, sticky, ripe fatty and fruity notes	700	
2-methylbutanoic acid	Sweaty		
3-methylbutanoic acid	Sweaty		
Acetic acid	Sharp pungent sour vinegar		
Glycolic acid			
Lactic acid			
Formic acid			
Esters			
Ethyl-2-methylbutyrate	Fruity	0.5	
Ethyl-3-methylbutyrate	Fruity	0.6	
Methyl acetate			
Ethyl acetate			

3-methylester-propanoic acid			
Methyl isovalerate			
Butyl butanoate			
Prenyl acetate			
Furfuryl formate			
2-oxobutyl acetate			
2-furfuryl-propanoate			
Pyridines			
Pyridine	Sickening sour putrid fishy amine	77	
4(H)-pyridine			
3-ethyl-pyridine	tobacco		
Pyrroles			
Pyrroles	Defective beans, negative notes		
1-methyl-1H-pyrrole	Powerful smoky woody herbal		
1H-pyrrole			
1-furfurylpyrrole			
2-acetyl-1H-pyrrole			
1-methyl-1H-pyrrole-2-carbaldehyde	Musty		
Terpenes			
Linalool	Flowery	0.17	
Limonene	Terpene pine herbal peppery	4	
Geraniol	Floral, sweet, rosey, fruity and citronella like with a citrus nuance	1.1	

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1.3 Does coffee go bad?

The role of Shelf life in coffee aging

The flavour importance in the sensory acceptance of coffee drove the research toward in-depth investigations on the aspect of aroma and taste. In fact, after roasting process, coffee undergoes to an evolution of the flavour and rapid loss of quality. due to physical and chemical changes. These reactions change the overall food appearance, texture and flavour. Some of the most common deteriorative changes include:

- Volatilization, during which, odorant molecules are lost together with carbon dioxide (CO_2).
- CO_2 release.
- Surface oil migration.
- Intrinsic reactivity of aroma components with compounds naturally present in coffee.
- Oxidative reactions with the formation of off-notes and rancidity development (Folmer, 2016; Kong, 2016; Manzocco, 2016; Ross, 2006).

With the migration of the oil towards the surface of the coffee bean and with the greater exposure of lipids to oxygen of the air, this fraction can easily undergo auto-oxidative phenomena. The auto-oxidation take place when the oxygen is activated by chemical catalysts, such as transition metals (Fe, Cu, Mn, which in turn, coffee is rich) present in food, or from light or other radiation. The formation of an activated form of oxygen determines the transformation of unsaturated lipid molecules, easily attacked, due to the presence of double bonds, into radicals. The radicals as highly reactive and unstable compounds, are transformed into hydroperoxides. The decomposition of the latter, leads to the formation of numerous and important secondary oxidation products, such as aldehydes, ketones, epoxides, alcohols, hydrocarbons, and acids with a characteristic off-flavour note and, in extremes cases, with carcinogenic and toxic effects. Hydroperoxides can also polymerize to give dark colour to food, oxidize proteins, vitamins and pigments (figure 1.3.1) (Belitz, 2009; Flament, 2002).

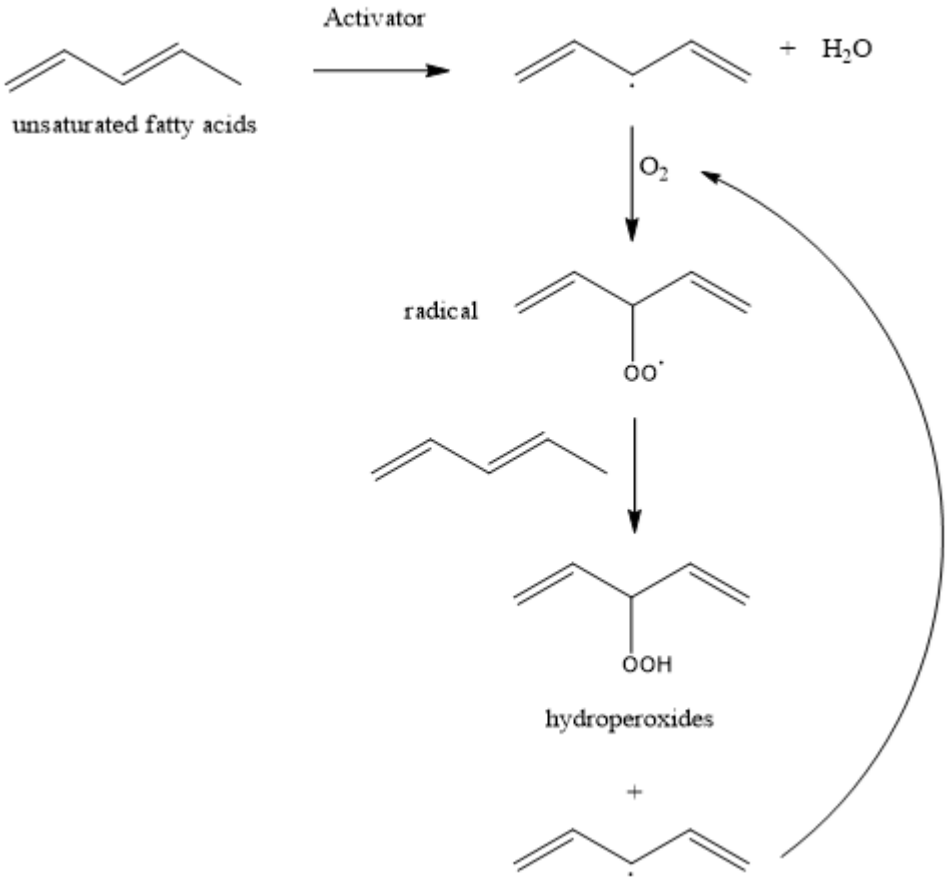

Figure 1.3.1: Lipid oxidation mechanism.

Oxidation is affected by the presence of transition metals and favoured by heat. Oxidation can also develop at low temperatures as the activation energy (E_a) is relatively low. So even when a food is stored at low temperatures, but in contact with oxygen, its lipid fraction can be affected by oxidative phenomena. These phenomena are emphasized by the pH of the medium, the presence of free water to induce reactions and on how the lipid fraction is distributed. When the presence of water is very low, the oxidation proceeds slowly because the water molecules hydrate the metal salts, reducing their catalytic activity or forming hydrogen bonds with hydroperoxides, thus hindering their decomposition and the consequent formation of new free radicals. A progressive increase in a_w, on the other hand, leads to an increase in the oxidation rate because it increases the mobility of the reactants in the medium, with migration of the hydroperoxides and with a consequent better contact with the transition metals present. For the pH, the acidic environment generally favours oxidation. The chain reactions that characterize lipid oxidation can be limited or reduced in time by the action of antioxidants (Belitz, 2009). Antioxidative activity of a coffee brew depends on phenolic compounds, melanoidin and phenylindans. All the antioxidative factors seem to be involved in chelation of transition metals, radical scavenging in chain reactions, trapping of active oxygen, and so on (Clarke, 2014).

These reactions are strongly affected by environmental conditions such as oxygen availability, temperature, relative humidity (UR%), water content of beans and pH.

Grinding the roasted coffee beans, exposes a greater surface area of them to oxygen, allowing a loss of odorants with lower molecular weight such as sulphur-containing compounds, and the increasing of oxidative reactions with the implemention of off-flavour (Ross, 2006). The coffee sensitivity towards oxidative reactions are due to the presence of a large number of strongly active volatile and non-volatile compounds, clearly prone to the reaction with oxygen, like aldehydes, ketones, thiols and lipid fraction (Folmer, 2016; Buffo, 2004; Kong, 2016; Makri, 2011; Manzocco, 2016; Marin, 2008; Ross, 2006). The staling effect, due to the loss of freshness, has been extensively studied as it determines the consumer's acceptability and determines the *shelf life* of the product (Flament, 2002). What it is defined is the time after production during which the product still retains a good level of quality without being affected by a series of variables that imply a loss of acceptability. This quality level is defined as *acceptability limit* and the time to reach this limit, during which the

perfectly packed product remain safe, known as *primary shelf life*. It is very important to focus on understanding how packaging properties could influence the coffee permeability in different environmental conditions, in order to increase the shelf life of the product (Anese, 2006; Cardelli, 2001; Manzocco, 2016). In fact, although many physical and chemical changes occur in coffee during storage, the rate at which they take place depends on environmental variables that could, as far as possible, be limited by the use of proper materials, correct packaging methodologies and of storage conditions. However, the quality decay during storage is rapidly accentuated during the *secondary shelf life*, when the pack was opened by consumer and a series of environmental conditions become the driving force leading to an acceleration of the rate of quality decay (figure 1.3.2) (Anese, 2006; Cardelli, 2001; Nicoli, 2012).

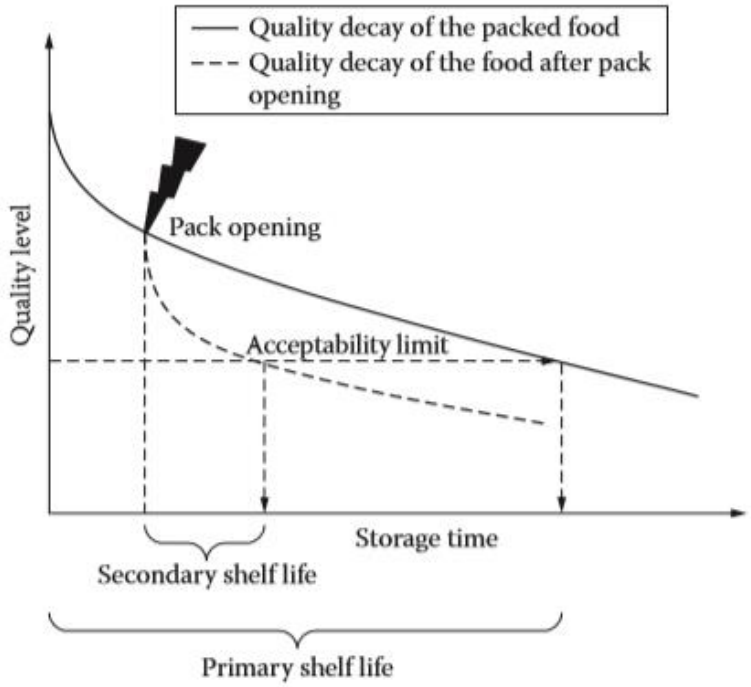

Figure 1.3.2: Diagram of quality decay of a general packed food versus after pack opening. From (Nicoli, 2012).

The preliminary step is to identify which are the principal factors (microbial, chemical or physical factors) that could influence shelf life, defining the acceptability limit. These events can be linked to the food formulation or related to environmental factors. The decay of food quality is monitored by testing it under two well-defined storage conditions: actual (real time test) and accelerating conditions test (ASLT). This implies that the experiments have to be carried out under environmental conditions chosen to simulate the realistic behaviour during the trade market. Real-time shelf-life testing is a procedure theoretically applicable for shelf-life estimation of food in particular for those which quality decay occurs in a rather short time. On the contrary, the prediction of long-term shelf life, in stable foods, is traditionally obtained by accelerating shelf-life experiments, performing tests under environmental conditions able to speed up the quality deterioration (Calligaris, 2016). Finally, data are modelled on the acceptation of a sensory panel to obtain a shelf life estimation.

The kinetic of deteriorative reactions is caused not only by intrinsic factors, typical of the food product itself, but also by extrinsic reasons linked to storage environmental conditions

and packaging properties (Kong, 2016; Manzocco, 2016). Among the extrinsic factors that could increment the rate of deterioration are:

- Temperature: during storage, coffee is subject to fluctuating ambient temperature, that can accelerate the chemical reactions rate. This happens especially when temperature increases, resulting in a volatiles loss, associated to a change in roasted coffee freshness. As observed by Ross *et al.* (Ross, 2006), the changes are slower when the coffee is stored at a low temperature.

  The relationship between the effect of the temperature oscillation and the deteriorative reactions is described by Arrhenius equation (1889):

  $$\mathbf{K = K_0\, e^{-Ea/RT}}$$

  K: reaction rate constant

  R: molar gas constant (8.31 J K^{-1} mol^{-1})

  T: absolute temperature

  $\mathbf{E_a}$: apparent activation energy (J mol^{-1}); constant independent of temperature, dependent of water activity (a_w), moisture content, solids concentration and pH

  $\mathbf{K_0}$: pre-exponential factor; constant independent of temperature

  The equation highlights how K grow up in exponential manner with the increase of T and to the decrease of E_a. The Arrhenius equation is the most employed model describing the temperature dependence of chemical reactions that occur in foods during storage (Kong, 2016).

  Normally, temperature is chosen, among all environmental factors, as factor to be modulated in accelerating test to simulate an oxidative reaction, as food decay. This is not only due to the fact that temperature is one of the most critical factors affecting reaction kinetics in food, but also to the availability of a theoretical basis for the development of a mathematical description of the temperature sensitivity of quality loss rates (Calligaris, 2016; Nicoli, 2012).

- Moisture: The relative humidity (RH or UR) of the environment, migrating into the product, directly affect its physical and chemical properties, impacting on the texture and on the chemicals' stability. The RH is defined as "the ratio of the vapour pressure of air to its saturation vapour pressure". It is a real number that indicates how humidity amount present in food at given temperature and pressure conditions. When coffee is exposed to a constant humidity, the product will adsorb or lose moisture until the equilibrium is reached (ERH). The presence of water gained or present inside the food can lead to the natural deterioration of the product: this is not so much in terms of quantity, but mainly in terms of availability of water free from direct interactions with the soluble food constituents. Free water, results available for biological and enzymatic processes and it is expressed by the parameter of water activity (a_w).

a_w is defined as:

$$a_w = P/P_0$$

P is the vapour pressure of the water in the product
P_0 is the vapour pressure of the pure water

a_w can increase or decrease according to the RH environmental conditions and to the water vapour resistance of the packaging material used. The migrating phenomenon is modulated by a driving force corresponding to the difference of water vapour pressure at both sides of the packaging material. Inside the package, the water vapour pressure is related to the balance between the product and the gas phase at the specific temperature, outside it depends on the environmental RH and the temperature.

- Light: during the supply chain, food may be exposed to natural or artificial light, accelerating the oxidative reactions with implementation of off-flavour, colour change and rancidity.

- Oxygen: just in the same way light operates, also the migration of oxygen into packaging can accelerate oxidative reactions, with consequent reduction of coffee quality (figure 1.3.3) (Kong, 2016).

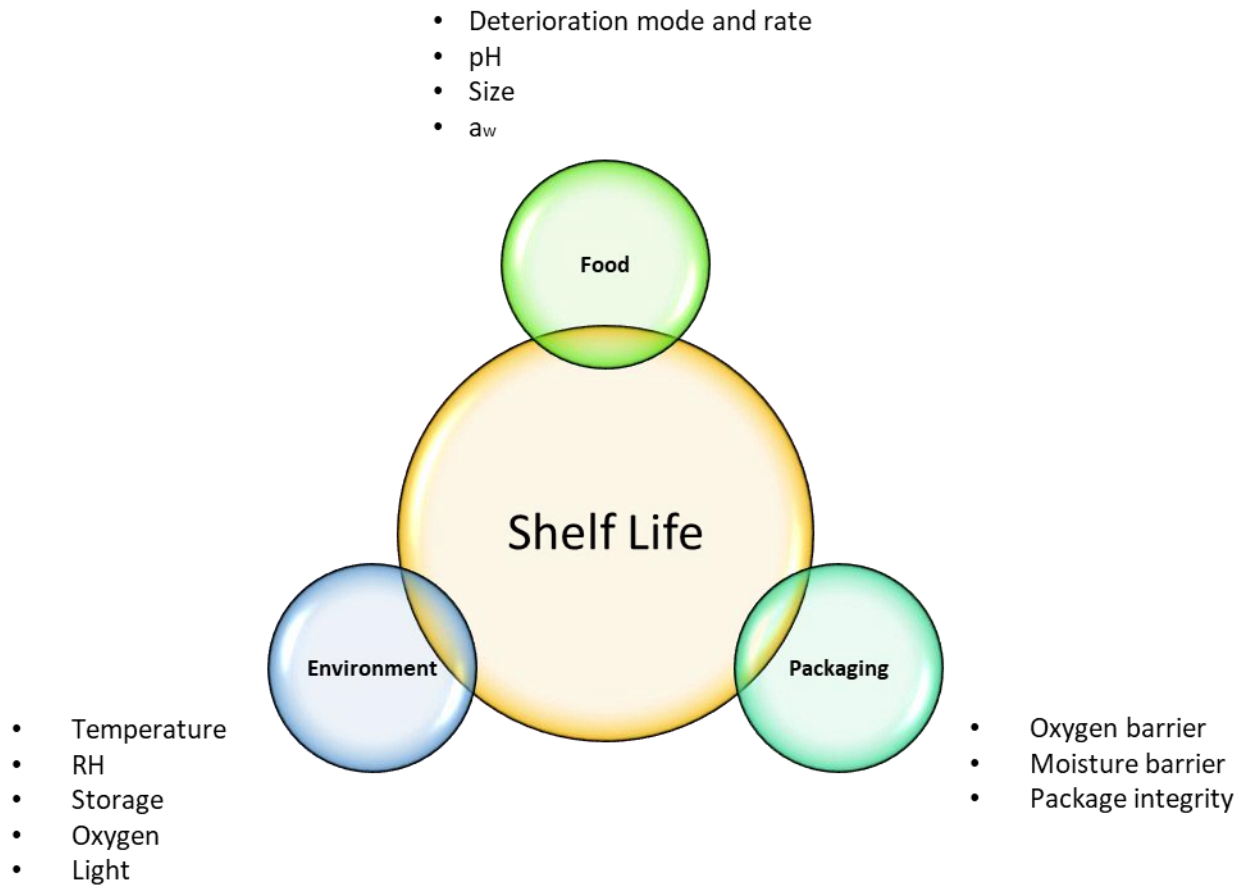

Figure 1.3.3: The three main factors that could influence the shelf life.

Approaches to slow down deterioration of foods and extend shelf life include modification of formulation, of the processing, packaging, and of the storage conditions. Nowadays, optimization of packaging technologies enables to greatly slow the staling phenomena. The principal task of packaging is to protect coffee from light, oxygen, temperature, moisture, and microorganisms. Furthermore, packaging preserves from mechanical damage and protects coffee from shock and vibration that might occur during manufacturing process (Anese, 2006). The material and the technological methods of packaging, are the most common ways to decrease the impact of product deterioration.

Materials that have traditionally been used in coffee packaging include metals, aluminium or glass cans, tinplate and plastic, providing a total barrier from water, gases and volatile compounds, with a high cost and greater fragility for glass containers. Modern coffee wrapping often combines several materials: polymer–aluminium multiply laminates, which

ensure an efficient barrier, waterproof film and a rigid strength. Coffee pods and capsules are quite a recent innovation, which contain the optimal quantity of coffee for a single-cup preparation. In order to obtain the required shelf life of roasted coffee, the choice of packaging technique is crucial. Controlled and modified atmosphere packaging (MAP), including vacuum packaging, nitrogen or CO_2 flushing, is used to slow down undesirable reactions by limiting the availability of oxygen, humidity and flavour loss. The prevention of increased internal pressure may be overcame by fitting a one-way valve on the package, preventing the swelling, releasing the CO_2 without letting air to go in. The pressurization is the best technological method to protect aroma longer, even when packaging is opened. Under this condition, the degassing decreases reducing oil migration from the cell to the surface, while avoiding a contact with oxygen and thereby with the oxidative reactions. Furthermore, the pressure inside the package is higher than that of volatiles, dissolving them in the lipid phase (figure 1.3.4) (Kong, 2016; Robertson, 2011).

The kinetic of coffee degradation reactions, however, may increase after the packaging has been opened by consumers. Once the pack is opened, coffee is not immediately consumed and the degradation reactions may therefore proceed with higher rates due to the changed and not appropriate storage conditions (Anese, 2006).

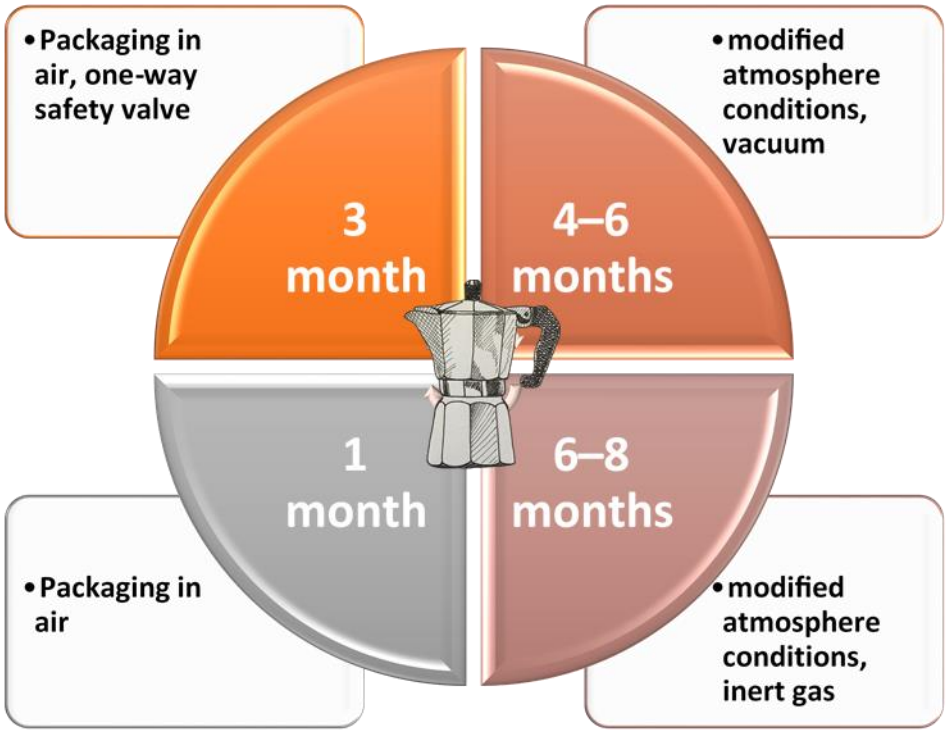

Figure 1.3.4: The different packages can influence the rate of coffee shelf life when closed.

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1.4 Safety in a cup:

Contaminants and safety

The need of food and nutritional availability have been the driving forces behind human evolution and civilization. Nowadays, however, consumer concerns about food safety has flanked the need for good quality food, and transformed and evolved food choices and, thereby, industrial production as well. Our food system is facing different types of risk, often narrowly comprised as foodborne illness and risks directly related to food ingestion. Food safety refers to handling, preparing and storing food in a way to best reduce the individuals' risks becoming sick from foodborne illnesses. A safe food supply is essential for a healthy society. (FAO https://www.fao.org/3/cb8667en/cb8667en. Thinking about the future of food safety A foresight report). As living standards have improved, concerns about both food safety in general and the presence of potential contaminants, more specifically, has gained high interest among consumers. The latter rely on the government for assurances that the products sold are safe. This is achieved through regulations and monitoring that reduce the risk of hazards in the supply chain, from the agricultural production to the market distribution (Fung, 2018). In the recent years, the complex food supply chains are increasingly vulnerable due to extreme climate changes, conflicts and complications that are putting at risk the food integrity. The different number of food contaminants and residues (pathogens, microbes, parasites, mycotoxins, antibiotic drug residues or pesticides), their different physicochemical behaviour, the complexity of the matrices in which they are present and their low concentration have increased the need for their monitoring. Industries are thus trying to ensure the maximum stability, quality and safety to the food cycle. This requirement come from three statements:

- Consumer expectation of a food that reflects the quality and the safety values.
- Adoption of various quality and safety assurance standards and systems with the aim to improve food welfare, restructure the food inspection system and try to enhance consumer information.
- Globalisation with an increase in the complexity of the supply chain industries, with a major inputs, processes, outputs and actors that can affect the food integrity (Ravelo-Pérez, 2009; Röhr, 2005; Wahab, 2019).

The global environment in which the food field operates through policies, regulations and guidelines, seeks to ensure that our foods are safe and are continuously developed or updated. The governments of many states around the world have established methods for regulating the food safety by increasing investments in the control of health risks. However, a harmonized legislation around the world is not yet available (Ravelo-Pérez, 2009; Unnevehr, 2020).

As with food in general, coffee requires a monitoring system from a safety perspective, industry-wide. Moreover, with the increase in its consumption, the need for greater control has also increased, both in terms of consumption of the beverage itself and of the entire coffee production chain. Despite these potential effects, green coffee beans and roasted coffee could present a number of risks related to environmental contaminants, pathogen attack and, in the case of roasted coffee, the formation of new contaminants during thermal processing. The contaminants, which are potentially harmful and most prevalent in coffee, can be divided as follows:

1.4.1 Natural contaminants

1.4.1.1 .Mycotoxins

Green coffee beans are prone to fungal attack, which can produce poisonous substances, called mycotoxins, that can cause acute or chronic intoxication and damage (Soliman, 2002). Mycotoxins are secondary metabolites produced, principally, in the field and during storage conditions. The ability of fungi to produce mycotoxins is positive correlated to the increase of temperatures, rainfalls, relative humidity and stress conditions. Nowadays, mycotoxins, are one of the most severe hazards in food due to the amplified global climate change, which may facilitate appearance and dissemination of new toxins (Levi, 1980; Mujahid, 2020). In fact, in coffee plantations, the presence of these contaminants can usually be found, due to production in tropical and semi-tropical areas (Leitao, 2019). But even the post-harvest methods, like the wet and dry processing or the storage and transport conditions, could influence the fungal growth.

For coffee the main mycotoxins that can be found is **Ochratoxin A (OTA).** It is a secondary metabolite, consisting of an amino acid phenylalanine and a dihydro-isocoumarin linked by a

peptide bond (figure 1.4.1.1.1). It is the most toxic member of the ochratoxin group, to which are added ochratoxin B (OTB) and ochratoxin C (OTC). OTA was classified by the International Agency for Research on Cancer (IARC) as a possible carcinogen (Group 2B), damaging DNA, compromising the immune system, leading to liver cancer and neurodegenerative diseases. Furthermore, OTA displays a vast toxicity even as teratogenic compound. Although humans can be exposed to OTA by inhalation or dermal contact, dietary intake is the principal source (Leitao, 2019). The European Union (EU) Legislation set up a maximum level for OTA in roasted coffee of 5.0 µg/kg (Humaid, 2019).

Even if the heat treatment could reduce the presence of mycotoxins, until the 84%, (with a combination of dark roasting and longer times of treatment), the roasting process can induce the formation of other toxic contaminants (Al-Ghouti, 2020; Van der Stegen, 2001). Many approaches can be used to control the OTA content, including organic and inorganic adsorbents, irradiation, high pressure processing (HPP), ultrasound and ozone. The lasts two approaches, in particular, exhibit a potential in reducing OTA inactivating the filamentous fungi. Even gamma radiation and HPP, at different power, inhibit fungal growth (Leitao, 2019).

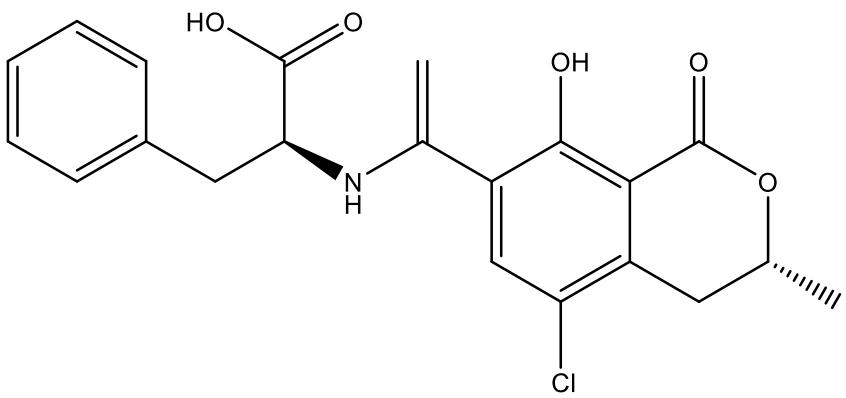

Figure 1.4.1.1.1: Chemical structure of the ochratoxin A.

1.4.2. Antropic contamination

1.4.2.1. Pesticides

Pesticides have been widely used since ancient times, but only recently have their actual economic benefits been seen for industrial production and the quality of agricultural products

have increased, sometimes becoming indispensable. This has caused much concern because of their level of toxicity, which is still not fully understood (de Queiroz, 2018). In fact, inappropriate methods of use and their widespread use have often put the health of many consumers at risk, especially with regard to exposure to a different combination of substances (Ravelo-Pérez, 2009). Whether pesticides are used in crops, leading to the formation of surface residues, or whether they are absorbed by plants and thus form internal residues, the risk to the consumer is the same, and exposure to that contaminant will depend on the persistence time of the pesticide and its concentration in food. Sometimes, however, pesticide residues are also found in organic crops, meaning that environmental contamination occurs, leading to the presence of residues even when the pesticide has not directly been applied. For these reasons, when pesticides are approved for use, they must I) predict their residence time on crops and II) define the time period when it is safe to consume the crop. Many countries implement control programs to ensure that pesticides are used properly and that residues in food are minimal so that consumers are not exposed to acute and chronical poisoning risks, due to inhalation, contact and ingestion (Farah, 2019; Shaw, 2016). Many organizations, such as the World Health Organization (WHO), the Environmental Protection Agency (EPA) in the United States of America (USA) the Environmental Protection Agency (EPA) in the European Union (EU) recommend the classification of pesticides according to their degree of hazard, keeping in mind that insecticides, fungicides and herbicides are the most commonly used pesticides in coffee crops. Pesticides include, insecticides, fungicides and herbicides

The approved **Insecticides** generally act on the nervous system of target organisms. Among those most commonly used in coffee are:

- Chlorinated hydrocarbons, that are prohibited.
- Organophosphates and carbamates compounds, which act by inhibiting the activity of cholinesterase enzymes in the nervous system of insects and mammals. Compared with previous one, they are less persistent, but can induce acute toxicity. Recently, they have been classified as carcinogenic to human (Group 2A and 2B) by IARC.
- Pyrethroids that interfere with the balance of sodium ions in the nerve junctions of insects, without having toxic activity on mammals.

The applied **Fungicides** include sulfur, aryl- and alkyl-mercurial compounds, bis-dithiocarbamates, and chlorinated phenols. They generally act on the cellular growth functions of the parasitic compounds. Most fungicides, however, are only minimally toxic to mammals.

Herbicides are generally the least toxic pesticides according to WHO, causing contact dermatitis as the main toxic activity. Glyphosate is among those most commonly used worldwide and exerts a broad-spectrum herbicidal effect Coffee plantations might be treated with glyphosate for keeping weeds under control. However, this compound is found to be probably carcinogenic to humans (Group 2A), according to IARC (Farah, 2019). Glyphosate is a controversial chemical substance and has been under attack, in recent years, from politicians and environmentalists alike. Studies are on-going for coffee in order to understand potential effects on coffee brew consumers. A very recent publication explain how the levels of glyphosate in green beans do not represent a minor exposure for coffee consumption (Delatour, 2023).

1.4.2.2 Mineral Oil Hydrocarbons (MOHs)

Behind the packaging of a food product, there is a choice of a packaging system I) to be chemically and physically compliant with the food, to maintain its sensory quality; II) to maintain the barrier property, to avoid the permeability of adverse factors; III) to maintain the stability of the product; and IV) to avoid the migration of toxic packaging material with the food contact phase. This last point, is of utmost concern in the selection and use of packaging materials for food packaging. Today, there is an increased attention to the migration of mineral oils from packaging materials. These are the so-called mineral oil hydrocarbons (MOHs), a group of substances consisting of chains of carbon and hydrogen atoms, containing from 10 to about 50 carbon atoms. They are generally derived from petroleum and include saturated or unsaturated compounds with linear, branched, cyclic and aromatic structures. The main ones are:

- MOSH (Mineral Oil Saturated Hydrocarbons), saturated aliphatic hydrocarbons
- MOAH (Mineral Oil Aromatic Hydrocarbons), aromatic hydrocarbons.

The main MOH contamination (related to the volatile component) occurs through cardboard food packaging, which allows these hydrocarbons to reach food through an evaporation and condensation mechanism, even in the presence of plastic barriers. But cardboard is not the only source of contamination; in the early 1990's, MOSH were discovered to be able to migrate into foods from jute bags carrying principally coffee, cocoa, soybeans, peanuts and grains. Jute fibres, in fact, were mixed with mineral oil to improve their spinning properties, and the volatile part of this oil was transferred to food. Special criteria for the production of jute bags were adopted in 1998, and to date, bags destined for the EU market are treated with vegetable oils or completely replaced (Hochegger, 2021).

1.4.3 Processing contaminants

During roasting of green beans, the colour and the flavour change for a series of chemicals processes, including Maillard reactions, Strecker degradation, caramelization, lipid oxidation and degradation of chlorogenic acids (Schouten, 2021). Besides the development of the desired physicochemical and sensory properties, the roasting, induced the formation of undesired compounds including polycyclic aromatic hydrocarbons (PAHs), acrylamide (AA), 5-hydroxymethylfurfural (5-HMF) and furans.

1.4.3.1 Acrylamide

Acrylamide (AA) is a neurotoxin, generated principally via the Maillard reaction, during roasting process (figure 1.4.3.1.1). It is naturally present in many everyday food products and most industrial and commercial cooking methods can cause its formation (frying, roasting, baking). Acrylamide is generated at high temperature and low humidity, by a chemical reaction, which transforms sugars and amino acids naturally present in many food products. The amount of acrylamide of a given product is linked to how it was cooked and to its basic ingredients.

According to European Food Safety Authority (EFSA), coffee beverage is one of the major sources of acrylamide considering its high daily consumption rates, leading to 20–30% of AA total daily intake (Hamzalıoğlu, 2020; Lachenmeier, 2019).

In June 2015, EFSA published a document regarding the risk for the health concerning acrylamide in food. EFSA experts confirm that acrylamide in food can increase cancer risks for consumers of all age and promote the reduction of the exposure to the molecule through the adoption of strong legally binding maximum levels in foods. It is classified as "a possible carcinogenic" by the International Agency for Research on Cancer (IARC) (Group 2A), mainly damaging nervous system (Knight, 2021; Strocchi, 2022; Zhu, 2022). Many approaches for the AA mitigation were adopted, avoiding the formation of unpleasant colour, taste and flavour. Among them, the use of asparaginase and acrylamidase, two enzymes that with different strategies, limited the AA formation in green coffee. Another strategy is to optimize the roasting time and temperature conditions, lowering the amount of AA in roasted coffee beans (Strocchi, 2022). In next chapters an insight about this contaminant should be presented.

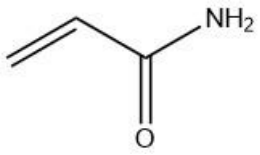

Figure 1.4.3.1.1: Chemical structure of the acrylamide.

1.4.3.2 Furans

Furans (C4H4O), are oxygenated heterocycles that, together with a series of homologues, occurs in the volatile fraction of a wide variety of foods and drinks. They are formed during thermal treatment of most food crops and drinks, as one of the Maillard reaction products. Its generation is mainly due to thermal degradation of carbohydrates, oxidation of polyunsaturated fatty acids and decomposition of ascorbic acid or its derivatives (Bicchi, 2011). Their toxicity is linked to the possibility of being potentially carcinogenic, according to the IARC (Group 2B) (Park, 2021; Zhu, 2022). However, the volatility for furan and 2-methyl furan is so high that they escape from the brew and in cup it remain in very low amount.

Among furans, **5-Hydroxymethylfurfural (5-HMF)** is a furanic aldehyde formed as an intermediate compound in coffee roasting processes, from the decomposition of sugars or through the Maillard reaction (figure 1.4.3.2.1). Some factors that influence the rate of 5-HMF formation include the processing temperature, type of sugar, pH and water activity. The amounts of furan and 5-HMF and furans in coffee are affected by the roasting temperature and time, so their content decrease, by increasing the time and temperature of the heat procedure (Park, 2021). HMF, have an effects on health that is still controversial. 5-HMF may be irritant to eyes, upper respiratory tract and skin and may be considered potentially carcinogenic for humans, hepatotoxic and nephrotoxic (Hamzalıoğlu, 2020; Lee, 2019; Park, 2021; Zhu, 2022). UV irradiation, addition of phenolic compounds, vacuum treatment and microwave heating could be used, as a strategy for the reducing of HMF (Lee, 2019).

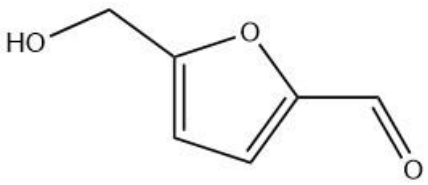

Figure 1.4.3.2.1: Chemical structure of the 5-Hydroxymethylfurfural.

1.4.3.4 Polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are constituted by a group of more than 200 hydrophobic compounds, which structure include two or more fused aromatic rings (figure 1.4.3.4.1). PAHs derived from the incomplete combustion and pyrolysis of organic matter, presented in the pollution environment, but even derived from industrial food processing, domestic cooking procedures, packaging materials and roasting processes. Low levels in green coffee indicate that compounds are mainly formed during roasting process, whose variations in time and temperature lead to different PAHs contents, growing with increasing of temperatures and roasting time. Unfortunately, no studies are available so far on possible methods of reducing PAHs during coffee roasting. These compounds are potentially genotoxic and carcinogenic to humans, inducing mainly breast, lung and colon cancers. Inhalation, ingestion and dermal contact are the main pathways of exposure for humans In accordance

with these premises, the sum of PAHs in foods, should not exceed 10 µg/kg for oils and fats, 35 µg/kg for cocoa beans and derived products, 30 µg/kg for meat and fishery products. The total content in roasted coffee range between 3.5 and 16.4 µg/Kg but no regulations indicated the maximum quantity of PAHs recommended in coffee and coffee substitutes. (Binello, 2021; Rattanarat, 2021).

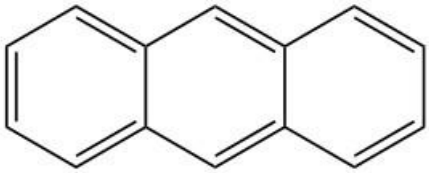

Figure 1.4.3.4.1: Chemical structure of one of polycyclic aromatic hydrocarbons (Anthracene).

Ensuring safe food is a complex task. This is because food is increasingly traded globally, new ingredients and new products are developed and different pathogens arise every day. This is why the food industries must be constantly monitored and coordinated on a global level, to guarantee the consumer the protection of the food they eat. Public authorities must also ensure that risks are constantly assessed and that arrangements and control laboratories are constantly adapted to new findings.

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1.5 Good for you, good for the environment

The coffee's evolution from a simple commodity to an elite product is linked to a mutation of the coffee concept over time, that have led the product from being a simple drink of consumption in the 60s to the specialty coffee in 90s, and the expansion of coffeehouse chains have managed to better respond to the sensory needs of consumers (Samoggia, 2018). This transformation of consumer's behavior has been possible due to three main reasons: pleasure, health and above all, sustainability (Samoggia, 2018). The term "sustainability" is popular today because of issues such as environmental deterioration, climate change, overconsumption and unlimited economic expansion. The interconnection of the environmental, social and economic fields defines sustainability, participating together in the definition of well-being and progress. These three goals are essential to addressing today's environmental and climate challenges because they enhance the potential the Earth has to offer us to meet our needs without compromising global natural resources (Folmer, 2016). The three sustainability fields promote this goal by harnessing ecological resources, financial support, technological progress and institutional change, with the goal of preserving ecosystem stability and biodiversity. As the world's population has grown, companies have incentivized an increase in food production. However, this progress has had impacts on the ecosystem and biodiversity of flora and fauna on which humanity depends. Like other human activities, coffee cultivation is affected by deforestation, loss of biodiversity, climate change, and problems with water quality and availability. As result, coffee plantations are increasingly attacked by pests and diseases that damage the plants, limiting their ability to absorb essential nutrients and are subject to temperature fluctuations and water that can hinder the development and maturation of the plants themselves, reducing the yield and quality of the beverage (Folmer, 2016). It is therefore feared that future climate change may greatly reduce the areas suitable for coffee cultivation, especially with seasonal temperature increase (Nab, 2020).

In response to environmental and market challenges, more and more coffee companies and global organizations are implementing sustainability programs aimed at identifying the best adaptations to climate change to manage the production chain, the domestic supply chain and to help farmers through their means of subsistence. Furthermore, recognizing the importance of coffee for the economies of many countries heavily dependent on its cultivation and export earnings, it is of fundamental importance to promote sustainable development and poverty reduction in producing countries through projects whose main

beneficiaries are Africa, Asia and Latin America (Folmer, 2016). On the other hand, the Food and Agriculture Organization of the United Nations (FAO) has established standards to be followed for more sustainable and intelligent consumption, which avoids waste. All these actions allow consumers to play an active role and help to reduce environmental impact (de Figueiredo Tavares, 2020). As customers are increasingly aware of the environmental impact due to their habits, to remain competitive in the market, manufacturers have increasingly opted for sustainable production, without creating food waste and using materials with little or no pollution. About 100 million tons of food waste is produced each year in the EU, which is expected to rise to 200 million by 2050. The production of these wastes and by-products has significant negative environmental effects in terms of land use, carbon emissions and water benefits. Therefore, not only effective waste management is needed, but also a better use of renewable resources. In recent years, efforts have being made to establish a plan to make food packaging more environmentally sustainable, moving from the simple concept of "take, use and throw" to "take, use and recycle." Ethical production of innovative and sustainable materials and the implementation of circular economy (CE) have thereby received increasing attention from small consumers and big food companies (Sisti, 2021). The CE concept, globally, is based on the idea of maximizing the value of the material throughout the industrial chain, recognizing the limited availability of natural resources, paying attention to the value of corporate resources, material flows and waste minimization (Abuabara, 2019). When a product reaches the end of its life, not only the product itself, but also the packaging, if possible, must be reused in a productive way. Thus, we talk about not only a reduction in consumption and waste of environmentally polluting materials, but also a reduction in emissions of CO_2, nitrogen oxides and CH_4 gas emissions, promoting better waste management and less use of productive resources (such as energy, water, land and materials), with positive impacts on climate and habitat (Furfori, 2014; Levine, 2016; The British Standards Institution, 2011). Since coffee is a complex food system with a multi-level supply chain involving not only agriculture, trade, and production, but also the production of the beverage with its consumption and the disposal of packaging waste, greenhouse gas (GHG) production also occurs in the preparation and consumption stages of the beverage. In this context, the coffee brewing method and the energy efficiency of the appliance used have a significant impact on GHG. In particular, studies have shown that, compared to espresso preparation, mocha preparation leaves a smaller carbon footprint. This is true even though

the quality of a cup of moka depends largely on the skill of the person preparing it and the maintenance of the coffee machine (figure 1.5.1) (Cibelli, 2021). So, the basic concept of these multi-size aspects is that of reuse, repair and recycling, both at the industrial and household level (figure 1.5.2).

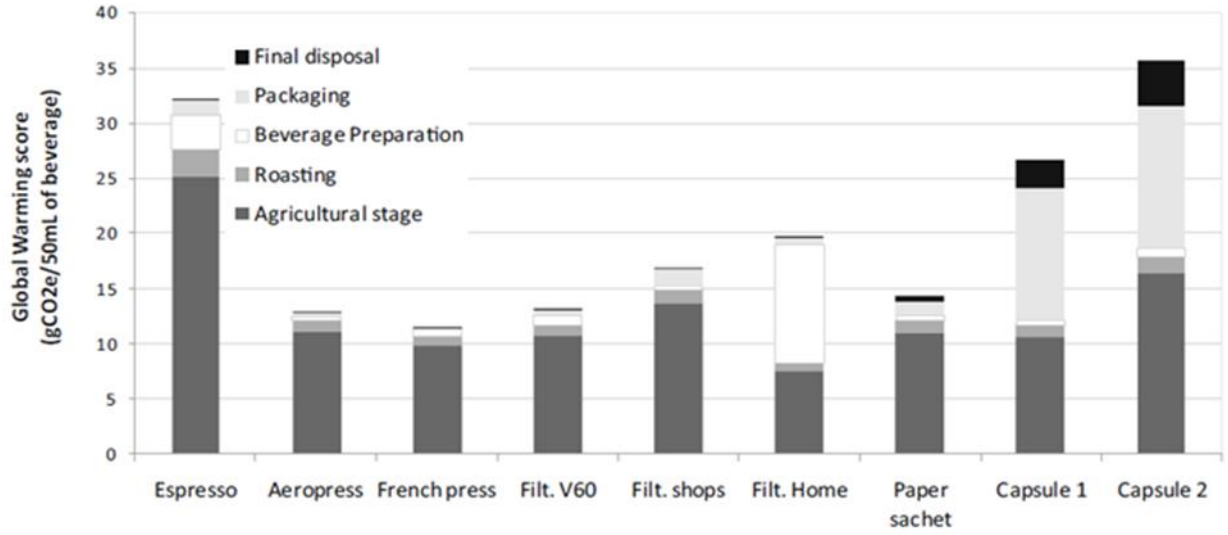

Figure 1.5.1: Contribution analysis for Global Warming score in the different stages for each method of beverage preparation (de Figueiredo Tavares, 2020).

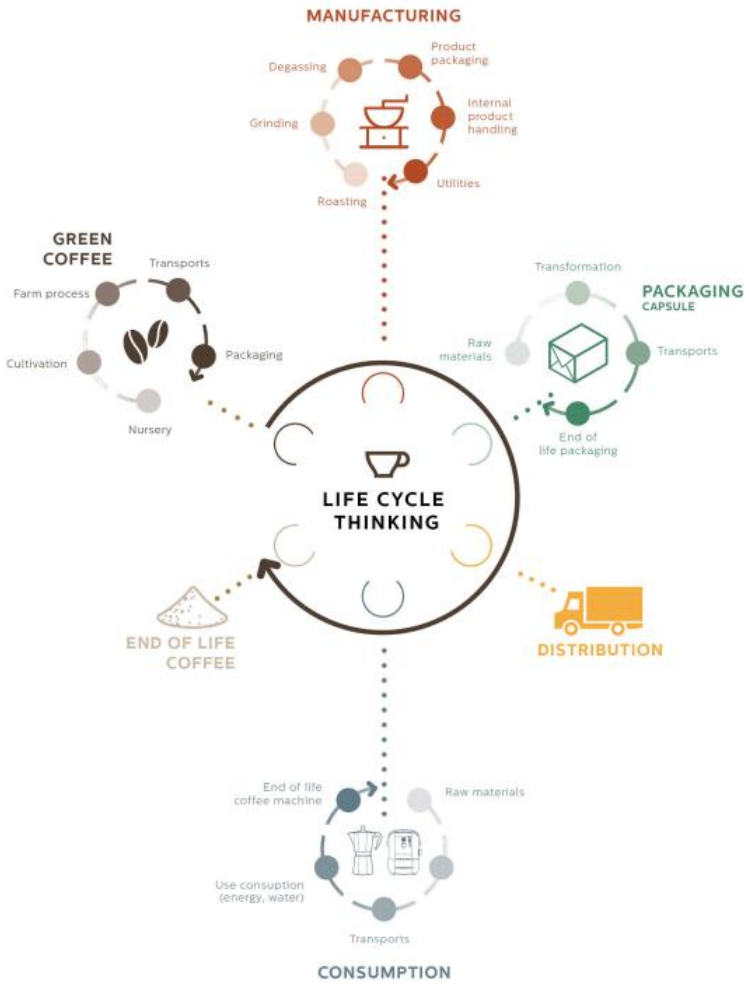

Figure 1.5.2: Greenhouse effect on all the coffee supply chain (Furfori, 2014).

This has made it possible to: (I) mitigate the environmental impact of packaging; (II) avoid waste by ensuring good storage, preserving freshness in airtight packaging and safeguarding food quality; and (III) use recyclable and renewable materials, prioritizing reuse, recycling and composting (Bilancio di Sostenibilità 2019 Lavazza; Bilancio di sostenibilità 2020 Lavazza; Eiermann, 2020). To reduce the environmental footprint of a product, both its intrinsic and extrinsic attributes (e.g., packaging) must be changed. By-products rich in molecules with functional properties, such as coffee silver husk or used ground coffee (SCG), could be recycled as a source of sustainable energy and incorporated into new industrial supply chains (fuel, soil fertilizer or food fiber) (figure 1.5.3) (Magnier, 2016; Oliveira, 2020; Sisti, 2021).

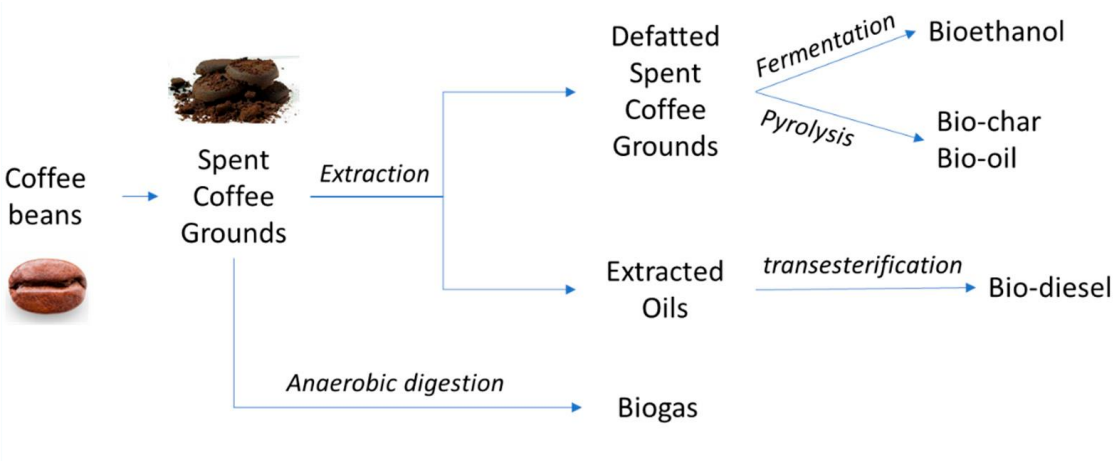

Figure 1.5.3. Intelligent reuse of spent ground coffee in energy production (Sisti, 2021).

But even packaging materials themselves could be reused. More environmentally friendly technologies and materials are being adopted, especially for ready-to-drink and single-serve products, which have been very successful in recent times. In today's contexts, the growing demand for single-serve coffee products has overtaken mocha brewing reflecting how new consumer behaviors are veering toward a constant search for convenience, practicality and "ready-to-use." A products of increasing popularity in coffee drinks, are capsules and pods, which contain the exact quantity of coffee, but, simultaneously, exalt the aroma and the ease of the beverage preparation (Gandia, 2018; Marinello, 2021). By 2025, the market for capsules is estimated to grow to $29 billion, due to their ability to preserve the freshness of the ground product from oxygen and moisture permeation and to be quickly usable (Abuabara, 2019; Cincotta, 2020; Eiermann, 2020). Although the socio-economic profile of single-serve coffee consumption is emerging, espresso capsules have been found to be the most environmentally impactful in terms of both production of gas emissions, water consumption, and energy waste, as well as the huge production of plastic-aluminum packaging waste, which is at odds with the industries' shift toward a more sustainable and CE. Indeed, capsules commonly consist of plastic or aluminum material, contain about 7 g of single-serving ground coffee, and are sealed with a laminated protective film used as a barrier against light, gases, and water vapor, preserving the quality of the coffee and, increasing the shelf life of the product at the same time reducing flavor loss. The basic problem of the single served coffee is mostly the waste disposal, i.e. the need to discard aluminum and plastic in the appropriate collections after separation from the coffee cake residue. However, the differential separation of the diverse materials related to the spent coffee and packaging, is

not always done correctly ending up in the undifferentiated waste (Marinello, 2021). To overcome these critical issues, industries are trying to apply solutions to improve sustainability by producing 100% compostable and degradable material. Unlike compostable capsules, those made of multilayer material with aluminum would have to be properly separated or delivered to certain collection points for recycling, still creating both recycling and disposal problems. For these reasons, in recent years fossil-based plastics have been replaced with bio-based solutions (about 2.43 million tons in 2024) to preserve the quality of the terrestrial and marine environment. Compostable capsules are made from biodegradable polymers that can preserve aroma and serve a good barrier function, being disposed of entirely in organic waste. However, striking a balance between increasing environmental demands, purchasing biodegradable or recyclable products, and promoting the maintenance of food quality and safety is a real challenge. The quality of packaging materials affects the shelf life of packaged foods, so attention must be paid to the choice of materials during production. Packaged products must meet a number of standards and be convenient, efficient and effective, for instance, i) they must ensure the absence of impurities and the safety of food during marketing, ii) they must ensure the preservation of fragrances and the protection of food from moisture, radiation and light. Therefore, efficient packaging must maintain food quality and increase product shelf life, as well as act as a barrier, be impermeable, non-toxic and inert (figures 1.5.4). Furthermore, material used for capsules need to be tested for coffee brewing conditions (temperature and pressure) of the machine used to avoid the release of packaging residues into the brew. Although much research has focused on improving the characteristics of biopolymer-based packaging films, their mechanical, thermal and physical properties are still unsatisfactory and often difficult to be industrially applied. Indeed, capsules with compostable material should be able, similarly to aluminum material, to preserve aroma and act as a barrier, but because they are "compostable," they have a lower capacity to preserve the coffee quality and thereby, potentially, to reduce the shelf life (Al-Tayyar, 2020; Cappiello, 2022; Ibrahim, 2022).

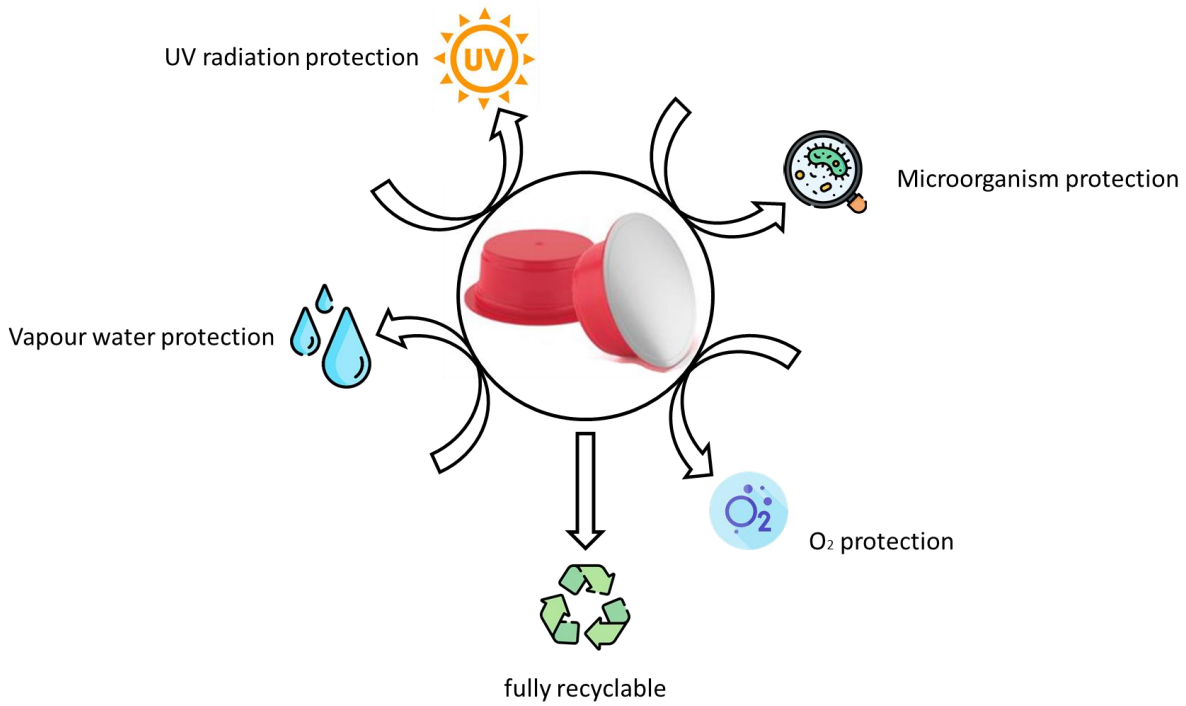

Figure 1.5.4 Importance elements of biodegradable food packaging materials.

It is a common understanding that the production of packaging, and particularly packaging waste, has a significant impact on the degree of eco-efficiency of food products. Consequently, the creation of sustainable packaging has attracted the attention of food authorities and research organizations, which have also focused the role of shelf-life and how it too can reduce potential food waste. Indeed, a eco-friendly, but not durable, packaging could similarly impact an aluminium/plastic packaging, the latter minimizing food loss, but strongly impacting on the environment

Therefore, a sustainable approach to food packaging should balance and minimize both food loss and packaging waste. Various materials, solutions, and systems are currently available on the market for food packaging with variable effects on the preservation of food and potential dietary risks. Today, life cycle assessment (LCA) methodology is widely applied in the packaging industry with the aim of highlighting critical environmental issues and identifying the most environmentally friendly solutions through benchmarking (Casson, 2022). Not only, LCA studies are also exploited to identify GHG emissions associated with the use and post-consumer phases of a product. LCA aims to minimize not only the environmental impact, but also the social and economic effect that a company's entire operations may produce both outside the production chain and within the company itself, with the intent of

aligning marketing strategy with product sustainability strategy. The company has to consider both the direct environmental aspects associated with activities, product development, and services the organization can control, and the indirect aspects related to potential activities over which the organization does not have direct control, but that could affect positively or negatively on the sustainability.

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1.6 The role of chemical analysis in flavour studies

Food analysis nowadays is important for a series of *i) quality assessment* ii) *legal factors*, such as detecting the authenticity of a product or revealing a food fraud; iii) *sanitary factors*, linked to the harmful effects that spoiled food or contaminants can have on human health; iv) *environmental factors*, related to the impact of the packaging on the welfare nature. Furthermore, food design and analysis need to be accompanied by the evaluation of the sensory impact as a primary parameter in the qualitative assessment, and some productions actually provide for legal standards based on the sensory quality of the food (i.e. extra virgin olive oil). In fact, the term quality encompasses a whole range of characteristics that food must have, including being safe. For this reason, food quality is to be intended as nutritional and sensorial value. If on the one hand, consumers pay attention not only to the number of calories introduced through food but also to the quality of the nutritional components supplied to the body, on the other hand, consumers are attentive to the hedonic aspect that a food product can satisfy them (Illy, 2005).

With the exponential growth of consumer interest in food safety and nutrition, the response with the development of increasingly modern analytical techniques has increased in parallel, to ensure safety, quality and food traceability. Furthermore, such requests have increased the need to develop more sophisticated instrumental methods, able to offer results of better quality and in higher quantity, by increasing the sensitivity, precision, specificity and speed of analysis.

Currently, food is not just a way to feed, but a science, that connect the product, as such, to individuals, diet, safety, health, environment and so on. We can therefore talk about Foodomics, i.e. a discipline defined in 2009 "*… that studies the food and nutrition domains through the application and integration of advanced-omics technologies to improve consumer's well-being, health and knowledge..*" (Cifuentes, 2009).

In this context, Foodomics is a discipline in which food, advanced analytical techniques and data analysis are combined. It is an approach that is not easy to use, because it requires complementary knowledges from different fields, like analytical chemistry, statistics, bioinformatics, medicine and biology. In fact, the nutrigenomic approach made it possible i) to tackle important problems in the field of diseases; ii) to understand the biochemical mechanisms, which arise as a result of intolerances to some food components; iii) to determine genetic differences between individuals when stimulated by the same nutrient,

and iv) to understand how a personalized diet, studied on a specific individual, can prevent some food intolerances. The knowledge of food chemical composition is essential also to understand all factors impacting the product shelf life, during the industrial chain, from raw materials to packaging, to improve food hygiene, processing and conservation (García-Cañas, 2012).

Determining flavour-active chemicals is a common basis for methodologies used to examine the quality and authenticity of meals, drinks, and their constituents. "Omics" sciences such as flavoromics and “Molecular Sensory Science” or sensomics, could be a choice for a comprehensive method of evaluating the quality of the meal. These approaches use advanced data processing techniques, multivariate statistics and advanced data mining techniques to address the large amount of data involved in fingerprinting/profiling methodologies due to the evolution of modern high-resolution analytical techniques and new capabilities to handle large data sets. Molecular descriptions of the sensory properties of food are the aim of sensomics and flavoromics. Their ability to disseminate knowledge about the sensory properties of ingredients and how they work in food products, the effects of different raw materials, and the effects of technological procedures on the sensory properties of meals and beverages is their main strength. These methods are commonly used in food flavour research because they provide a biological dimension to the information conveyed by sensory perception (Vrzal, 2019).The concept of flavour involves a holistic description of food perception and is the complex combination of the olfactory, gustatory and trigeminal sensations perceived during tasting (Chartier, 2012; Spence, 2015). The volatile fraction of a food is at the origin of its aroma, which can be defined as that combination of volatiles that can be perceived both orthonasally and retronasally by the odour receptor sites of the smell organ.

The chemical compounds that define both aroma and taste, are of crucial interest in this scenario. They required methods able to detect and distinguish between different aroma and taste compounds and to quantify them in order to facilitate description of the perceived product attributes. Two main approaches are applied for the characterization of food flavour (Murray, 2001; Regueiro, 2017):

Sensory evaluation

Sensory method provides useful information about the human perception of food flavour. These evaluations focus on the degree of consumer acceptability regarding product attributes. Sensory food analysis plays a principal role in industry to ensure safety and reduce the risk regarding ingredient changes, to investigate the effects of raw materials, to formulate and launch new products, to create sensory maps, to track product changes over time, to better understand the stability and effects of packaging, preventing consumer complaints (Murray, 2001; Regueiro, 2017).

Tasting, by trained (or not) experts, is a daily routine industrial method, grown in second half of the twentieth century and implemented at all the stages of the food processing to evaluate impact and environment effects, technological processes, packaging and shelf life on the final product. Sensory evaluation is a scientific discipline that "evoke, measure, analyse and interpret" the human reaction to the food tasting perceived by the principal senses (Stone, 2004).

Three types of method are commonly used and could be differentiated according to the different goal:

- *Discrimination test*: This is the simplest sensory test, based on the comparison of two or more samples, with the aim to perceive a difference existing between them. Common methods, include the triangle, the duo-trio test and the paired comparison test.
- *Descriptive test*: Consists in the quantification of the perceived flavour note of the product, providing a sensorial description of differences among the products. The most famous method is the quantitative descriptive analysis (QDA). The determination of flavour attributes, take advantage of a widely sensorial vocabulary attribute to describe a product. Furthermore, this method promote quantitative attributes by evaluating an aromatic note in terms of intensity
- *Hedonic test*: it is a method that allow to quantify the degree of liking of a product.

Nowadays it is possible to use methods that have the advantages to be flexible, fast, performed by both trained assessors and untrained consumers. The discrimination test and the hedonic test are the two methods in which the consumers could be called to express an opinion, while the descriptive test requires trained panellists (Folmer, 2016; Lawless, 2013).

Since the consumption of food is connected to the pleasure perceived during tasting, sensory evaluation methods have become an integral part of quality control, at the beginning of the industrial chain, during the entry of raw materials, up to the use of programs of control over the shelf life of the product.

Although sensory evaluation is carried out by trained panellists and it is the method preferred for evaluation of odour (Chambers IV, 2013) there are some limitations such as low repeatability and reproducibility linked to the subjectivity of the assessment, conditions of analysis, time and costs needed for its implementation and above all, the limits of human ability to distinguish components in a mixture (Chambers IV, 2013; Regueiro, 2017).

It is therefore important to establish a relationship between sensory results and instrumental measurements, to establish an objective evaluation of the sensory features of a food.

As coffee consumption has increased worldwide, there has been growing interest in the flavour component, which plays an important role in marketing the product. The evaluation of coffee aroma is a fundamental step of the entire coffee production chain from the selection of the raw material to the creation of the blends. In industries, sensory properties are measured through the use of the cup method. Although taste is a highly subjective matter and different tasters seem to have different opinions on the quality and value of a particular cup, in 1984 the Specialty Coffee Association of America (SCAA) proposed a detailed and standard protocol, adaptable to the adaptable to producers' standardization needs, for the definition of the sensory quality of coffee (SCAA, 2015; Sunarharum, 2014; International Trade Centre, 2011). The Coffee Cuppers' Handbook (1984), has helped to transform the work of cupping, based on the experience of the taster, into a science, in order to maximize the level of standardization of the method (Folmer, 2016).

The characteristics evaluated are:

- the *fragrance*, from the freshly roasted and ground coffee beans
- the *aroma*, from the extracted beverage coffee
- the *flavour* perception of the coffee brew given by the interaction between aroma and the taste
- the *aftertaste* of the coffee brew, derived by the residual taste compound on the back of the tongue
- the *acidity* and *body* of the brew
- the *uniformity*, *clean cup* and *sweetness* represented the quality of the green coffee's preparation during the harvesting and processing of the coffee beans

The protocol includes not only roasting and grinding parameters, but even temperatures, times and number of stirring between the steps. The procedure is reported below:

- Evaluation of the Fragrance/aroma is based on two estimation: by sniffing the dry ground coffee, after 15 minute of grinding and by inhaling the infusion with water.
- Evaluation of the Flavour, Aftertaste, Acidity, Body and Balance is based on the aspiration of the brew after 8-10 minutes of infusion at 70°C.
- Evaluation of Sweetness, Uniformity and Cleanliness is based on the rating of the brew at 37°C (protocol cupping).
- Evaluation of the overall is based on the scoring of all the attributes combined, of the liquor at reaching 21°C (Folmer, 2016; SCAA, 2015).

Instrumental evaluation

The objectification of aroma and taste on a molecular basis was proposed by Reineccius, Schieberle and Hofmann in 2012 (Charve, 2011; Dunkel, 2014; Jelen, 2012).

Flavoromics, is an "-omic" and "-holistic" approach focused on low molecular mass compounds (volatile and non-volatile) in untargeted mode and linking them to a defined sensorial perception, thanks to advanced chemometric techniques.

“Sensomics”, is an omic discipline whose aim is to identify key food aroma- and taste-compounds at the molecular level and to map their combinatorial code sensed by human chemosensory receptors, and integrated by the brain (a target approach).

In analytical terms, flavoromics and sensomics imply the comprehensive and quantitative analysis of the largest possible array of low-molecular-weight components in the investigated samples.

The approach to the analysis has radically changed with the rapid evolution of detection techniques, managing high numbers of samples. The deciphering of complex food chemical compounds, resulted in an increase in instrumental analytical performance, over the years (Folmer, 2016). The advent of modern chromatography, in the 50’s, changed food analysis, affording the separation of the components of a matrix. The development of detection techniques, has prompted the growth of new approaches for sample preparation and data elaboration. The chemical information deriving from the sampling, separation and identification of single compounds in a complex food matrix, are increasingly related to that obtained from the descriptive sensory analysis performed by a panel test. In this way, the two systems often tend to be integrated.

In this view, omics approaches have also seen the introduction of dedicated methods affecting the chemical analysis strategy, the best known are fingerprinting and profiling. Fingerprinting refers to general and rapid high-throughput screenings aiming to discriminate and classify samples; this can also be achieved with a non-separative approach, i.e. headspace-mass spectrometry (HS-MS), direct infusion-MS, nuclear magnetic resonance (NMR), Fourier transform infrared (FT-IR), near infrared (NIR), etc. combined with suitable statistical data processing. Profiling gives a detailed analytical profile of the sample by combining separative and spectroscopic techniques [e.g. gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), capillary electrophoresis-mass spectrometry (CE-MS) etc. in view of identifying and quantifying diagnostic components. Their application has led to the development of:

a) untargeted methods, more closely related to the fingerprinting approach, where the samples are compared via chemometric tools on the basis of their profiles produced by a diagnostic analytical method

b) to detect quali- and quantitative differences between them,

c) to classify them through the degree of similarity of their fingerprints,
d) targeted methods often associated to the profiling approach, in which the samples are compared on the basis of the chemical composition and/or distribution of known target analytes (markers).

In parallel with the introduction of analytical techniques, sample preparation, grew up. This step mainly involves the extraction of chemical components from a complex matrix, as first step of the analytical process. The interest on the use of sample preparation protocol is mainly related to the approach simplicity, flexibility, speed and sensitivity, indispensable for the subsequent analytical correct determination. Sampling is a complex process that depends on the nature of the matrix, and so, on the capability to isolate and purify the fraction of interest, on the concentration level of the target compounds in the sample and on the goal of the analysis. Advancement in sample preparation management protocols has resulted in minimizing artefacts formation, lower solvent consumption, sample deterioration, waste of time, automation and a low pre-analytical error (V. Soares Maciel, 2018).

Sampling techniques

In flavour analysis, the critical aspect is extrapolating volatile compounds from the food, because procedures adopted need to minimise/avoid the formation of artefacts, optimise the chemical-physical conditions, promote the reproducibility and improve adaptation to the sensitivity of the instrument.

Volatiles extraction has been performed by the use of techniques like distillation/extraction (SDE), solvent assisted flavour evaporation (SAFE) or with the use of microwave or ultrasound extraction. With the advance in the sampling techniques, a big breakthrough was given by the use of solid phase extraction (SPE), which is based on the use of a pre-packed column with several stationary phases, capable of selectively retaining the target analytes.

The headspace (HS) is the fraction providing the best picture of the volatiles released from a food, since it is the gaseous or vapour phase in equilibrium (or not) with a solid or liquid matrix. Every sample preparation method introduces a quali- and/or a quantitative discrimination: sampling should therefore be chosen to recover the fraction that better represents the property to be described through the chemical analysis and composition of

the investigated matrix, e.g. the volatile fraction of a food, in order to study its aroma. Retronasal perception is of at least equal importance and is more closely related to taste, i.e. to the multiple interactions with gustatory and trigeminal sensations (see above). The main challenge is therefore to develop sampling methods and techniques that can be used to obtain the headspace and characterise its composition in a meaningful and reliable way. Another advantage of HS sampling is that it is by definition a solvent-free and easy to automate method.

Generally, HS sampling is divided into two main modes: static (S-HS) and dynamic (D-HS). In the first case, analytes are partitioned in the headspace, from the matrix, driven only by their volatility and from the analyte partition coefficients between matrix and vapour phase. It refers primarily to a one-step gas extraction technique and is representative of the volatile fraction released by the investigated matrix. Stirring and heating improve the mass transport rate from the sample matrix to the headspace, which is particularly important for the extraction of low-volatility compounds. Dynamic HS sampling is a non-equilibrium continuous gas extraction process where the analytes in a continuous flow are extracted in a trap (Kolb, 1997). The D-HS composition provides qualitatively representative samples of the volatile fraction of the matrix under investigation and is characterised by very flexible sampling conditions and analyte concentration factors that influence quantitative recovery. The latter is the result of a compromise between the volatilities and polarities of the analytes and the sampling conditions (trapping material, temperature and purge gas volumes) (figure 1.6.1).

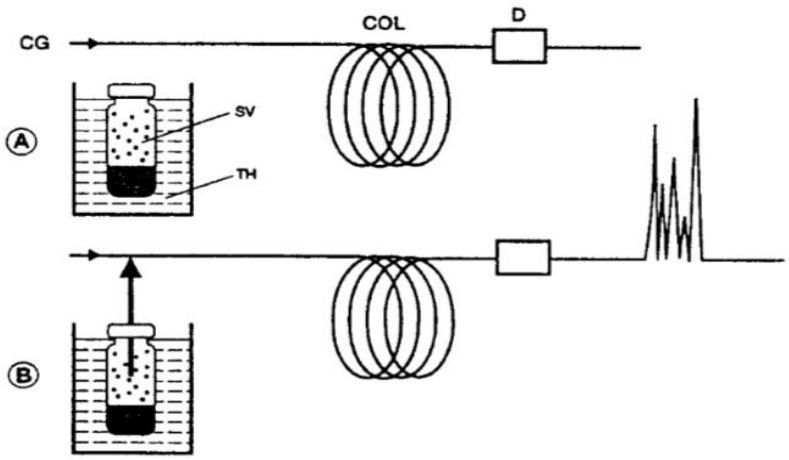


Figure 1.6.1: Schematic explanation of headspace-GC. (A) equilibrium between the solid phase and the gas phase in a general sample vial (SV). (B) sample transfer. CG= gas carrier, TH= thermostat, COL= gas chromatographic column, D= detector, from (Kolb, 1997).

Widely used in the food field, is the solid phase micro-extraction method (SPME), belonging to the techniques at high concentration capacity (HCC), which exploits the SPE procedure by increasing the sensitivity of the system. HCC techniques to extract volatile and semi-volatile analytes from a matrix, on an absorbent and/or adsorbent surface and then recover them by thermal or solvent desorption. Depending on the polymer matrix used, different groups of analytes will be extracted. Like a selective GC column, a polymer coating is chosen based on its discrimination for certain target analytes and their volatility ranges. The affinity of the coating for the target analytes is crucial in HCC sampling because both the matrix and the coating compete for the analytes. Often in HCC techniques, combinations of different polymeric coatings are used to obtain a greater range of selectivity on both polar and non-polar compounds, exploiting both absorption mechanisms, in which the analytes are partitioned in the polymeric material and adsorption, where the analytes are retained in the active sites of the polymeric surface (Ann Scheppers Wercinski, 1999; Pawliszyn, 2012). Generally, it is possible to speak of absorption in the case of liquid extracting phases and adsorption in the case of solid extracting phases (Conte, 2014). The headspace HS-SPME is the sampling technique used in the experimental part of the thesis dedicated to volatiles.

Solid-phase microextraction (SPME)

Solid-phase microextraction (SPME) is an analytical method, of HCC techniques, developed by Pawliszyn and co-workers in 1989, while the HS-SPME is an extension of SPME (Zhang, 1993).

SPME utilizes a thin rod of fused silica coated with an absorbent and/or sorbent polymer stable at high temperatures. Fused silica (SPME fiber) is attached to a metal rod protected by a metal cover. This assembly is placed in a fiber holder and collect in a modified syringe (figure 1.6.2).

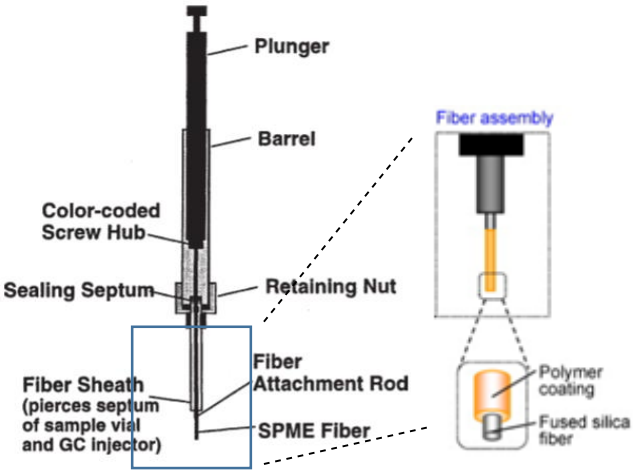


Figure 1.6.2: SPME fiber with syringe.

The SPME extraction technique consists of two processes:

- analytes partition/adsorption between the sample and the fiber coating
- on-line thermal desorption of the accumulated analytes from the coated fiber to the analytical instrument

During the extraction, the syringe, with the preconditioned fiber, pierces the septum of the closed vial in which it is contained the solid or liquid matrix to expose the fiber to the sample headspace, containing volatile organic analytes. The target analytes are extracted and concentrated into the fiber coating and transferred into the analytical system, principally by thermal desorption (Ann Scheppers Wercinski, 1999) (figure 1.6.3). In HS-SPME, analytes are recovered from the headspace of a matrix onto the fiber because of two closely-related but

distinct equilibria: i) the matrix/vapour phase equilibrium, which conditions the HS composition (measurable through its distribution coefficient, Khs), and ii) the HS/coating material equilibrium, which controls analyte accumulation onto the SPME fiber (expressed by its distribution coefficient, Kfh).

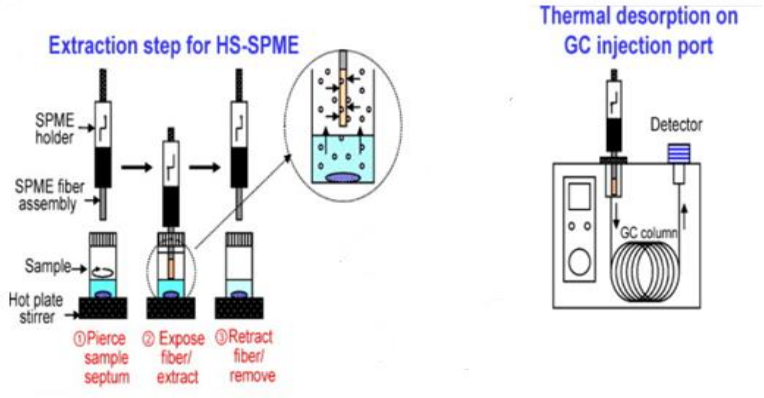


Figure 1.6.3: SPME headspace sampling.

Coatings for SPME can be liquid polymeric phase with a high molecular weight or a solid adsorbent of high porosity, to increase the surface available for adsorption. The largest producer of commercial fibers is Supelco (Sigma-Aldrich), which offers several coatings, consisting of one or more polymers with different polarity, and different coating thicknesses and lengths (Tranchida, 2019). Among the commercial fibers, the most widely used coating is polydimethylsiloxane (PDMS), which has high affinity for the extraction of non-polar compounds. The more polar polyacrylate (PA) or carbowax (CW) are more suitable for extraction of polar compounds. In contrast, mixed coatings blended with porous solid particles polydivinylbenzene (DVB), carboxen (CAR) or PDMS-DVB, CW-DVB, PDMS-CAR and DVB-CAR-PDMS, have more specificity with the polar compounds and have a widely surface area to extract volatile of low molecular-mass. (Kataoka, 2011). The thickness of the fiber determines its capacity of extraction and the time of extraction. A thin coating ensures fast diffusion and rapid release of the semi-volatile compounds during desorption.

Analytical platforms

In recent years, progress in technological analysis has been enhanced in all fields of science. In particular, the analytical tools were able to produce large amounts of information-rich data, especially for highly complex matrices, such as in food analysis. The analytical platforms combined with machine learning approaches are needed to cope with a huge amount of data of the great complexity. The increasing complexity of the data extractable from the matrices is a challenge from an analytical point of view, but the development of increasingly sophisticated methodologies made it possible to obtain performing models and much more accurate results to explain phenomena encrypted in them. Instrumental analysis becomes of fundamental importance especially in quality control. This area acquires particular interest in the field of food and drug analysis. Especially in food-omics, the control, authentication and characterization of the products of interest are of particular importance. In this area, although a lot has already been done over the years, there are still many open horizons, especially in an environmentally friendly direction. The most recent analytical approaches provides an highly informative power with a limited number of steps to meet the needs of reducing the carbon footprint going towards what has recently been called “white analytical chemistry” (WAC) (Nowak, 2021). WAC is a sustainable development of an analytical strategy in holistic view, in which a compromise between an unconditional increase in greenness not at the expense of functionality is searched (Bressanello, 2021; Liberto, 2019; Quintanilla-Casas, 2021; Scavarda, 2021). Hyphenated analytical techniques with high separation power and/or informative spectroscopic methods are of high interest, in particular the development of inclusive instrumentation in which the “sample preparation-analysis-data elaboration processing” sequence is merged on-line into a single step, that is the so-called “Total Analysis System” (TAS), (Dittrich, 2006; Manz, 1990).

Separation techniques

The advent of chromatography, in the 50’s, changed the approach to food analysis, allowing the separation of chemical compounds in the complex matrices. Mainly, the most used techniques of separation in flavour analysis are gas chromatography (GC) and liquid chromatography (LC). The introduction of GC allowed the separation, identification and determination of volatile and semi-volatile chemical compounds in complex mixtures. LC

performance has enormously increased with the introduction of HPLC (early 70') and more recently (late 90'), by ultra-high-performance liquid chromatography (UHPLC). Chromatographic methods are also readily applicable to the world of food-omics and in particular to food quality and safety control. They can be exploited for determination of the edible value of food, for product freshness, for identification of product authenticity, for control of both natural and technological contaminants, for analysis of veterinary drugs in food products and for control of food pollution due to packaging. Chromatography can be associated with a large array of detectors, able to identify a wide range of compounds with different chemical structure. In food analysis mass spectrometry is a legal standard mandatory to correctly identify chemical compounds in particular those related to food safety (de Hoffmann, 2019).

Gas Chromatography (GC)

GC is a potent analytical technique that offers high-resolution compound separation and enables the identification and measurement of trace components in complex samples. GC is one of the most widely used methods for the analysis of food samples and, in particular, for the characterization of the volatile fraction of food, for the identification and quantification of different pollutants and for the authentication of the origin of a specific matrix. This analytical technique, which was first discovered in the early 1950s, is able to separate components in a sample based on their different distributions between a liquid or a solid stationary phase and a gaseous mobile phase. The analytes must be vaporizable (or derivatized to make them volatile) and thermo-stable. In a GC system, the sample is introduced in the column through an injection system; the column is placed into a temperature-controlled oven where the separation take place: the end of the column is connected to a detector. The chemical components of a sample are volatilized in the inlet system. The splitless injection mode is more suitable for trace compounds, in this type of injection the carrier gas transfers the entire vapour cloud into the analytical column. In the split injection mode, only a part of samples are sent to the column, this modality affords to avoid column overloading improving the separation. Once in the column the compounds are move forward by the mobile phase and separated by the different interactions with the stationary phase, coated or bonded on the inner wall of the capillary column. There is a

constant exchange of solute molecules between the two phases, due to a continuous sequence of dynamic equilibrium, and the partitioning of the same between the stationary and mobile phases. The identification of analyte molecules as they elute from the column is the last step in the chromatographic process. The distribution of individual molecules within a chromatogram (qualitative information) and their abundance (quantitative information) can be determined using a variety of very accurate and sensitive detectors as FID (flame ionization detector), the O (olfactometry) or the MS (mass spectrometry) (Picó, 2012).
Flexibility and the relatively cheapness are important GC advantages; it can be coupled to several detectors with different characteristics.

Liquid Chromatography (LC)

In the disciplines of analytical chemistry, biochemistry, and chemical engineering, LC is a versatile separation technique used to separate a mixture of compounds in solution. Like GC, LC works in the same way, using the stationary phase and the mobile phase to separate analytes in a matrix, which interact differently between the two phases. The separation of compounds in chromatography is supported by different absorption mechanisms and intermolecular interactions between analytes with the stationary phase and the mobile phase. LC is used in many important fields such as pharmaceuticals, medicine, and food and environmental management. In addition, LC is recognized as one of the best separation and analysis methods because it allows separation of combinations of many chemical classes such as isomers, including structural, geometric, cis and trans, optical, etc.

There are several chromatographic analysis modes used in LC food analysis, the ion-exchange, the size exclusion, the hydrophilic and the chiral method, which exploit retention mechanisms differently (table 1). The most exploited separative methods are the *normal phase*, in which the stationary phase is hydrophilic and the mobile phase is non-polar. As the polarity of the mobile phase increases, the analytes are separated in order of increasing polarity. The *reverse phase*, in which the stationary phase is hydrophobic and the mobile phase is polar. The analytes are separated in order of decreasing polarity.

Table 1.6.1: Main methods of LC (adapted to (Picó, 2012)).

Methods of chromatography	Mechanism of retention
Normal phase	Adsorption
Reversed phase	Absorption, distribution
Reversed-phase ion pair	Ion exchange, absorption
Ion	Ion exchange
Ion exchange	Ion exchange
Size exclusion	Molecular-size effect, diffusion
Hydrophilic	Adsorption, absorption
Chiralic	Sorption on chiralic centers of stationary phase
Other methods: affinity, perfusion, micellar, hydrophobic, high temperature, countercurrent, ultrahigh pressure, etc.	

The column of an LC platform represents the heart of the separation system. Both retention and separation in the column occur because of the different rates of movement of the components on the sorbent layer. The efficiency of separation correlates with the column inner diameter, the length and particle size of the stationary phase.

Nowadays, the most modern columns have a high efficiency and analysis speed, in order to reduce solvent consumption as in the Ultra-high-pressure chromatography (UHPLC). They can often have a pre-column with the advantage of removing contaminants from solvents and minimizing stationary phase loss of efficiency of the analytical column due to stationary phase inactivation. Another parameter that influences retention is the composition of the mobile phase and the elution method applied: in isocratic mode the elution of the mobile phase remain constant during the separation process and it is exploited to separate combinations of compounds whose retention times do not differ significantly. In gradient elution, on the other hand, for which two or more solvents with different polarities are used, the composition

of the mobile phase changes. It is used for multicomponent solutions whose constituents have significantly different levels of sorption capacity. At the end of the column, the compounds separated are detected by different type of detection devices, and among them, in Table 2 are reported those most often applied to food analysis (Picó, 2012).

Table 1.6.2: Detectors combined with LC in food analysis (adapted to (Picó, 2012)).

Types of detector
Coulometric
Amperometric
Mass spectrometric
Fluorescence
Light scattering
Refractive index

The most widely used detectors for HPLC, are based on the absorption of light beam at a wavelength in the visible or ultraviolet (*UV-Vis*). The UV detector is the most used, but it suffers from some limitations linked to the lack, for some analytes, of chromophore groups. Despite this, it is versatile (λ from 190 to 550nm), sensitive (the optimal λ can be chosen for the analytes of interest) and selective (the λ can be varied to minimize the absorption of interferents). Photodiode array detector or diode array detector (DAD), in combination with HPLC, is the common UV detector that monitors the entire UV-vis spectrum using a photodiode imaging sensor. The detector can be used for quantification, identification and purity assessments of a peak, by superposition / comparison with in-memory UV spectra.
HPLC can also be combined with MS detectors, a key tool to identify the components of a sample, by the generation of a nominal mass molecular ions. The LC-MS has only been developed in last 40-50 years, due to the difficulty of introducing a liquid into a high vacuum system. Its development, as an elective diagnostic method, has made it possible to provide structural information on the analytes that are separated. Mass spectrometry guarantees a very high sensitivity enabling to detect analytes in very low or trace amounts (Weston, 1997). A better survey on mass spectrometry is reported below (Tranchida, 2020).

<u>Detection techniques</u>

There are different types of detectors classified according to their use. A universal detector responds to every component in the column effluent except the mobile phase (i.e. FID, MS). In contrast, selective detectors respond to a defined structural characteristic of the analytes eluting from the column (i.e. Nitrogen–phosphorous detector, Thermal conductivity, UV/vis (DAD), fluorimeter).

Mass spectrometry is among the most important detection system for chromatography. The MS as a result of its sensitivity and structure determination/identification potential in combination with separation techniques and data elaboration methods, is the technique of choice in food-omics and beyond, in -omics in general.

Flame Ionization Detector (FID), olfactometry (O) and mass spectrometry (MS)

In the FID detector the signal is produced by ions formed because of the combustion of organic compounds in a hydrogen-rich flame, which are then exposed to a polarizing voltage to produce a current proportional to the quantity of eluting analyte. This detector is one of the most used detectors for GC. It is employed in a variety of food analyses, especially when inexpensive, simple, and quick instrumentation, high sensitivity, repeatability, and stability were required.

It can be applied to a wide variety of organic compounds; provides analytes quantitation over a wide range of concentrations and accurate quantitative data. However, the detector only offers a limited amount of information regarding the chemical structure of the analytes actually separated, hence it is frequently used in conjunction with a GC-MS analysis to pinpoint target molecules.

Gas chromatography-olfactometry (GC-O), which combines GC separation with human nose detection, is a data dimension that is occasionally combined to FID or MS in the platform. In GC-O, skilled analysts (sniffers) identify and gauge the odor intensity of each peak that elutes from the GC column to provide a sensory assessment.

In GC-O, the stream of gas exiting from the chromatography column is often divided into two ports, the first one connected to the sniffing port and the second one connected to a FID or MS detector to get an instrument reference trace. Olfactometry is used for screening and it has a crucial role in defining the descriptive odor or potency of specific odorants or off-flavours that are discovered through a -omic investigation. Aroma extract dilution analysis

(AEDA) and combined hedonic aroma response measurement (CharmAnalysis) are the two primary screening methods that have been established. Using GC/O, both methods compare a number of samples of an original aromatic extract that have been sensibly diluted to determine which odorants best describe it. The effectiveness of the odorants is determined using this method based on the detection frequencies of the odorants noticed at the sniffing port. However, because it requires extensive analyst training and does not allow the rapid screening of a large number of samples, the GC-O is not considered as -omic friendly approach. Mass spectrometry (MS), on the other hand, is currently a crucial analytical technique on which the -omic approaches are based. It is used in conjunction with separation methods, as well as with statistical and bio-informatic tools (Brattoli, 2013; Durante, 2006; Gallo, 2016; Jacobson, 2013; Jiao, 2011; Jumtee, 2009; Ochi, 2012; Ribeiro, 2012). MS makes it possible to structurally identify molecules by ionizing and fragmenting them (through Electron Impact EI or Chemical Ionization CI) into ions with different mass-to-charge (m/z) ratios, in particular when combined with the separation capabilities of chromatographic techniques. The ions are then moved into the analyzers (as single quadrupole, ion trap (IT) or time of flight mass analyser (ToF)) and detected. Finally, the associated computer software converts this electronic signal into a mass spectrum.

GC-MS in general adopts electron ionization (EI), a “hard ionization technique”, to form ions and fragment them from neutral molecules (in the gaseous form). The gaseous or vaporized analytes ”collide” with an electron beam at 70 eV generated from a tungsten or rhenium filament to form an ionized molecule. The excess of energy accumulated in the resulting ion is also released (but not only) by its fragmentation with specific mechanisms depending on its structure and strength of chemical bond.

$$M + e^- \rightarrow {}^{\bullet}M^+ + 2e^-$$

Eq: 5

M: is the analyte molecule

${}^{\bullet}M^+$: is radical molecular ion with the same molecular weight as the molecule.

The ions created are accelerated toward the analyser where the ions are separated in agreement with their mass to charge ratio. Each compound's molecules undergo an ionization

process, hence the results of the mass spectral analysis for each compound are accurate indicators of that compound's identity.

The “soft” sources generate a limited fragmentation, providing information on the molecular mass. The different ionization methods generate the same types of ions:

a) "cationized" molecules, as a result of the attack of an H^+ or NH_4^+ ion, in the positive-ion mode
b) “anionized” molecules in the negative-ion mode

The soft ionization in general produce $[M + H]^+$ and $[M - H]^-$ respectively.

Electrospray ionization (ESI) and the atmospheric pressure chemical ionization (APCI) are among the most used ion sources for HPLC-MS. These techniques allow the introduction, in the ion source, of non-volatile molecules with a medium or low polarity, like peptides, proteins, phospholipids, polysaccharides and lipids. These interfaces made it possible to couple HPLC to MS that is made complex because HPLC operates with a mobile liquid phase under pressure, while the MS works in the gas phase under vacuum. For both ESI and APCI, the ionization occurs at atmospheric pressure and is obtained by a combination of high voltage and heat.

The principle of ESI is based on the high voltage (3-5 kV) nebulization of the liquid effluent from the HPLC and the subsequent generation of charged droplets (positive and negative) (figure 1.6.4a). The drops pass through a laminar flow of nitrogen heated, which removes the solvent molecules and, at the same time, increases the charge density, until the electric field at the surface of the drops is high enough to allow the expulsion of the desolvated molecular ions from the surface of the drops.

APCI is based on principles similar to chemical ionization (CI) for gas chromatography (figure 1.6.4b). The analytes are ionized via an ion-molecule reaction with a reagent gas that is sprayed, together with the liquid flow, in a heated vaporizer, where the ionization is initiated by corona effect at atmospheric pressure. In the APCI the desolvation process takes place for heating (500 °C), however the passage in the "heated cartridge" is fast, therefore, even

thermally labile molecules, can be analyzed with this technique (Korfmacher, 2005).

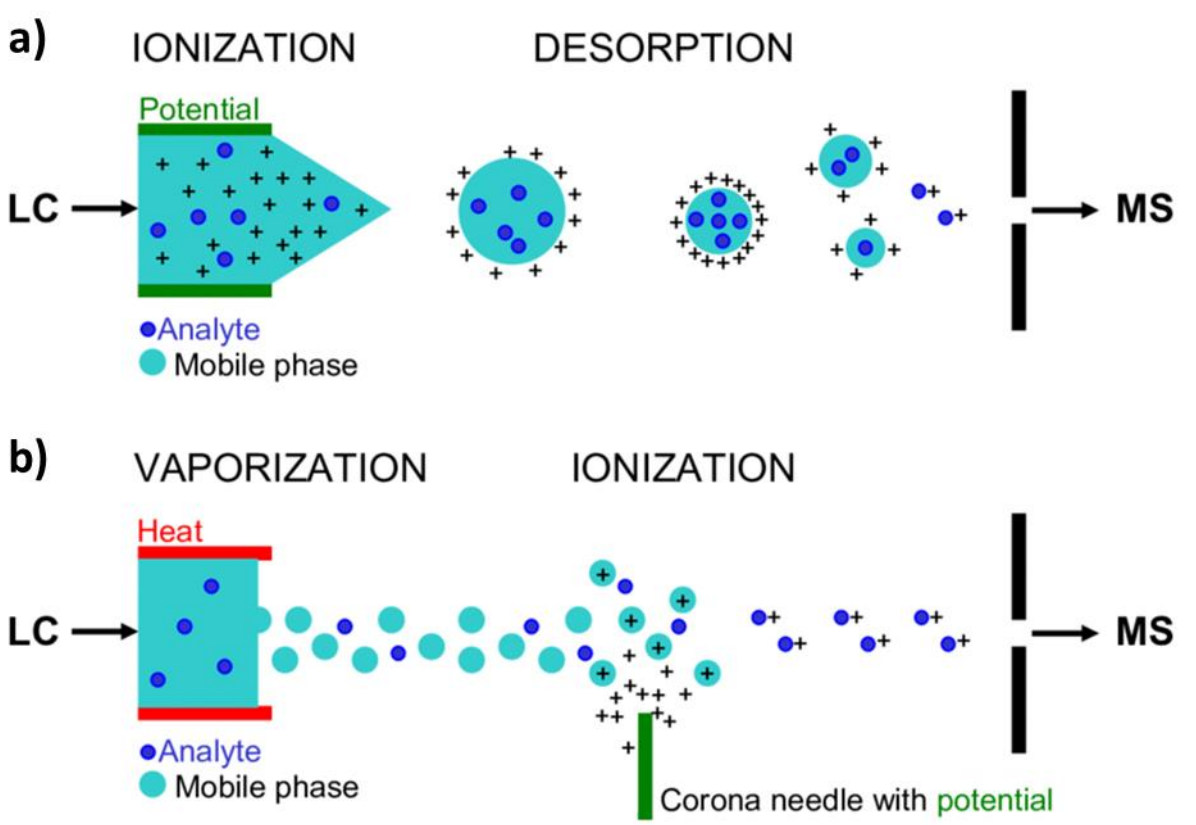


Figure 1.6.4a) and b) Schematic representation of two ionization interfaces: a) ESI ionization and b) APCI. Modified by Verplaetse et al., 2011 (Verplaetse, 2011).

The GC-MS market is currently dominated by the use of the quadrupole analyzer. Through this system, the separation of ions occurs through the combination of oscillating electric fields, applied alternately to two opposing pairs of circular section bars; when the two vertical bars have positive potential, the horizontal ones have negative potential and vice versa. The ion, inside the volume delimited by the 4 bars, assumes an oscillatory motion along the x axis of the quadrupole, with frequency and amplitude of oscillation that depend on the frequency and amplitude of the electric field, on the mass and on the charge of the ion. Then by modifying this field it is possible to scan the ions in function of their m/z. Only ions with a specific m/z ratio, compatible with the value of the oscillation frequency, will be able to assume a kinetic energy such that the motion becomes sinusoidal, without hitting the bars, and reaching the detector (figure 1.6.5).

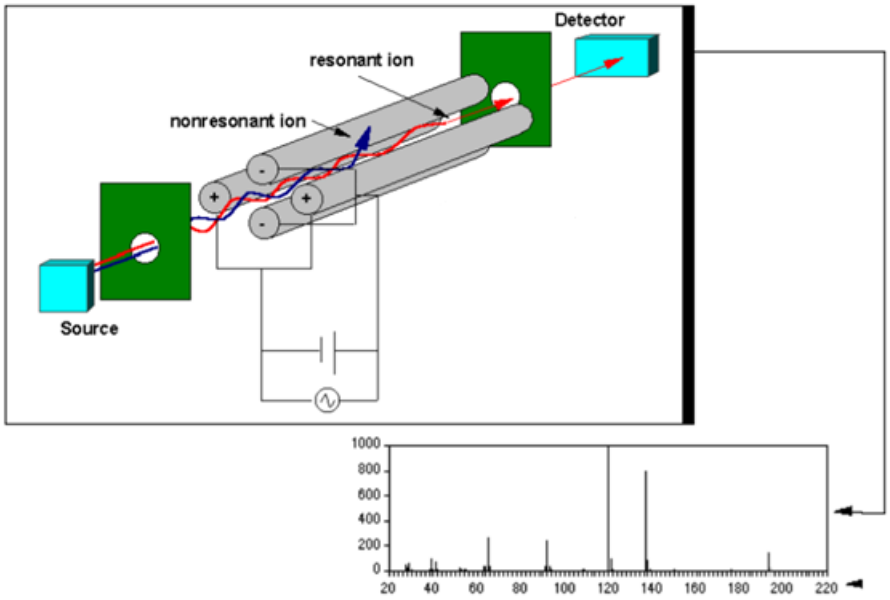
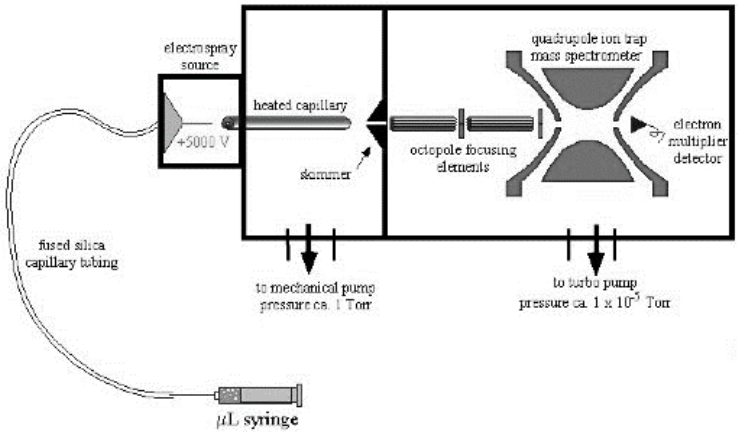


Figure 1.6.5: a schematic function of a quadrupole with ions that don't reach the detector and others that get out the quadrupole.

The quadrupole analyzer has a low resolution, but covers a mass range between 2 and 4000 amu, acquiring data in full-scan and/or in SIM scan mode. Last, but not least it is the relative cheap. The ion trap, instead, can be considered an alternative to the quadrupole analyzer (1.6.6). It uses three electrodes, two cap-shaped, inlet and outlet, and a central ring, to trap and accumulate ions, with the purpose of obtaining a high sensitivity. In particular, the ions oscillate between the two caps and inside the ring, remaining for a certain period of time within this space. The two side electrodes have a small slit in the center through which the ions pass. The mass spectrum is generated by varying the electric potential in order to eject, in sequence, the ions according to an increasing m/z value (Tranchida, 2020).



1.6.6: ESI Ion Trap Mass Spectrometer, modified by Challener 2010 (Challener, 2014)

Data science and artificial intelligence (AI)

Modern platforms today supply more and more measurable data during a single analytic run. So datasets analysed, normally, became not only larger, but also structurally complex and the information from hundreds or even thousands of data make exploration of data not-easy.
A data set is defined as complex when:

- it is common to observe high-dimensional data (i.e. large number of measured variables), mass of data (i.e. the number of samples is high), high variety of data (multiple data sources are available) and high velocity of data (fast and frequent data streams)
- there is an intrinsic complexity of the experimental problem, variables not known or interference on the system: experimental noise, synergistic or antagonistic effects

So the challenge is:

- the reduction of dimension set, in order to reduce the causes of complexity as much as possible
- the extraction of relevant information
- summary elements easier to interpret, looking for the similarity between data

A key step in omics studies is the interpretation of the huge amount of data of the high complexity in answering to a food question or problem.

Data science is an interdisciplinary field of science, based on combination of mathematical and statistical methods, information and computer processes to get new insights from available data. Depending on the field (i.e. information technology (IT), statistics and business) there are different terms and approaches used in data science and multiple definition of the same terms exist.

The use of a specific definition depends on the application environment i.e. academia *vs.* industry, the background and data science users. Scope of some terms is very similar and they are often used interchangeably i.e. data mining, pattern recognition, machine learning, artificial intelligence. All terms are commonly used in analytical chemical data analysis (Ayres, 2021; Szymańska, 2018).

However, there is not a complete compliance in the scientific world about these definitions that support open interpretation. In fact, a mathematical procedure is always applied to solve the problem if needed, and in analytical chemistry it could be summarized in Chemometrics (Amigo, 2021). The differences are the data and the information that can be retrieved from them. If data are not informative to answer your question, any kind of algorithm or tool can solve your problem. Data Mining can be defined as a set of methods used to extract usable information from large raw data sets. This definition implies that the usable information is already in the data and it does not include any procedure of Learning. Therefore, data mining is an unsupervised data processing.

Machine learning (ML) is dedicated to developing systems with the capacity to learn from a dataset and then provide reasonable outputs that can make informed decision based on what is learned. ML needs supervision therefore are supervised techniques. In addition, if data are comprehensively informative linear and easy algorithm can be enough without necessarily using more complex tools. These different visions can be represented from the figure 1.6.7, in the following discussion I will refer to the second vision (b).

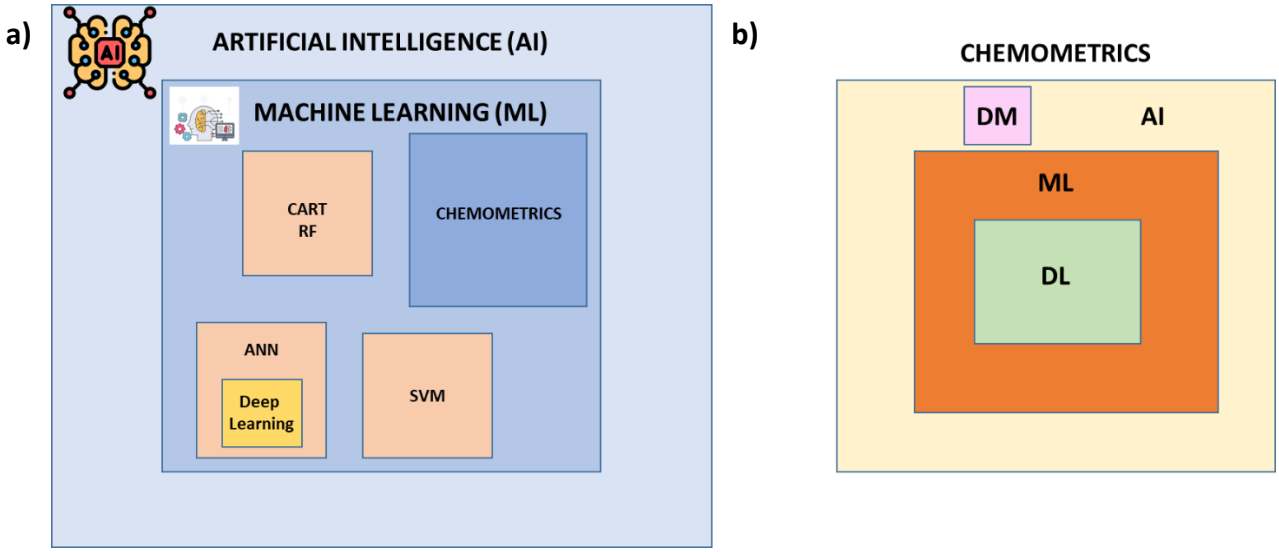


Figure 1.6.7: a) and b) the two different visions for AI, ML and Chemometric techniques. a) CART: Classification and Regression Tree; RF: Radio Frequency; ANN: Artificial neural network; SVM: Support vector machines; b) DM: Data Mining; AI: Artificial Intelligence; ML: Machine Learning; DL: Deep Learning.

Chemometric is mathematical-statistical disciplines that allows to face experimental problems complexes, trying to extract useful information from all data present on a set, in which experimental noise, redundant information, data not directly interesting for the studied problem, are presented.

Once analysed, the data are collected together in a *data matrix*, constituted of *objects* (samples, experiments, etc.) and *variables* that describe each observation (*multivariate system*).

A multivariate dataset can then be represented as in the figure 1.6.8.

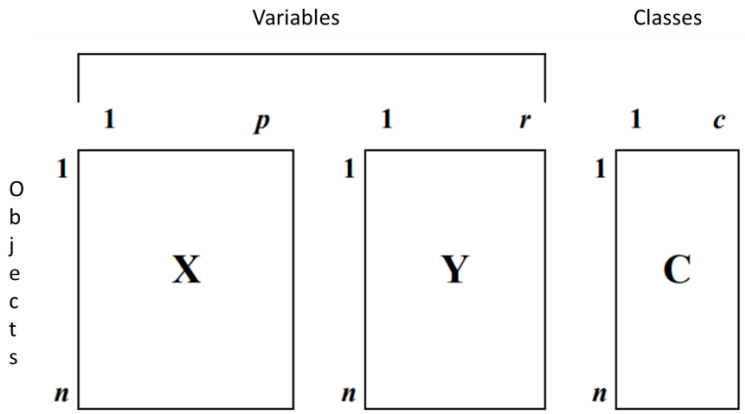


Figure 1.6.8: Scheme of model data matrix.

A common data table can therefore be represented with a matrix, whose n rows represent the objects and whose p columns, the variables.

The variables can be divided into two logical groups: The X block of *predictors* (the independent variables) and the Y block of *responses* (the dependent variables). Depending on the problem deal with, a variable can belong to one block rather than another.

Objects can be associated with a vector that contains the information of the belonging of each object to a predefined G *classes* (categories, groups). Each object corresponds to a number that identifies the membership class. There can be several classification criteria C and therefore C also becomes a matrix, in which, each column represents a different classification criterion.

The variables are the quantities that are used to study a given experimental phenomenon and to describe the observations as a whole. The variables are represented in the form of measurement scales, so before using data for the elaboration, it is necessary a preliminary analysis of data, to treat all data with the same dimension. So, a pre-processing tool, called *scaling*, is used before the treatment, to maximize the comparability between the variables.

The objects represent the samples needed to understand the phenomenon studied. Measurements made on a sample can be explained by one or more variables. The set of values that defines the object constitutes the *data*.

In many cases the available samples belong to different classes or categories. Classes are the expression of presence, in description of the object, of variables.

Chemometric tools can be classified into two main groups:

- *unsupervised* exploratory methodologies just showing the data as they are used to visualize the natural distribution of a sample in the multi-dimensional space. Its aim is to summarize, explore and discover data information without any deductive knowledge. Normally, it is the first step in data analysis helping to explore data.
- *supervised methods*, they look for determined features within data, explicitly oriented to address particular issues. The aim is to classify and predict a data set and determine a relationship between the response variables and the predictors, so that the dependent variables are used to guide the training of the models. They normally use a training set to build the model, a validation set to evaluate the model ability in future prediction and an external test set to prove the robustness of the model in prediction.

Validating a model is about building a structure that maximizes its predictive ability. The model created, must have stability characteristics that make it sufficiently independent from the data used to build it, furthermore the set of variables that bring information to the model, can lead to an increase in the complexity of the model, deteriorating its performance in prediction (overfitting). In general, in a model validation, a portion of the data is used to build the training set (set of learning), while the remaining is used to build the evaluation set (Berrueta, 2007; Ren, 2015; Todeschini, 1998).

Despite the technological development and the use of sophisticated chemometric tools to keep up with innovation, PCA and HCA are the most unsupervised chemometric approaches adopted in food field, while within the supervised pattern recognition methods Linear Discriminant Analysis (LDA) and Partial Least Square Regression (PLS) are the most used, the latter both in classification and in regression. These chemometric tools are employed to pursue the following pillars food analysis:

- Compositional properties and quality
- Processing and quality control
- Authentication & traceability
- Adulteration
- Prediction of food properties (figure 1.6.9)

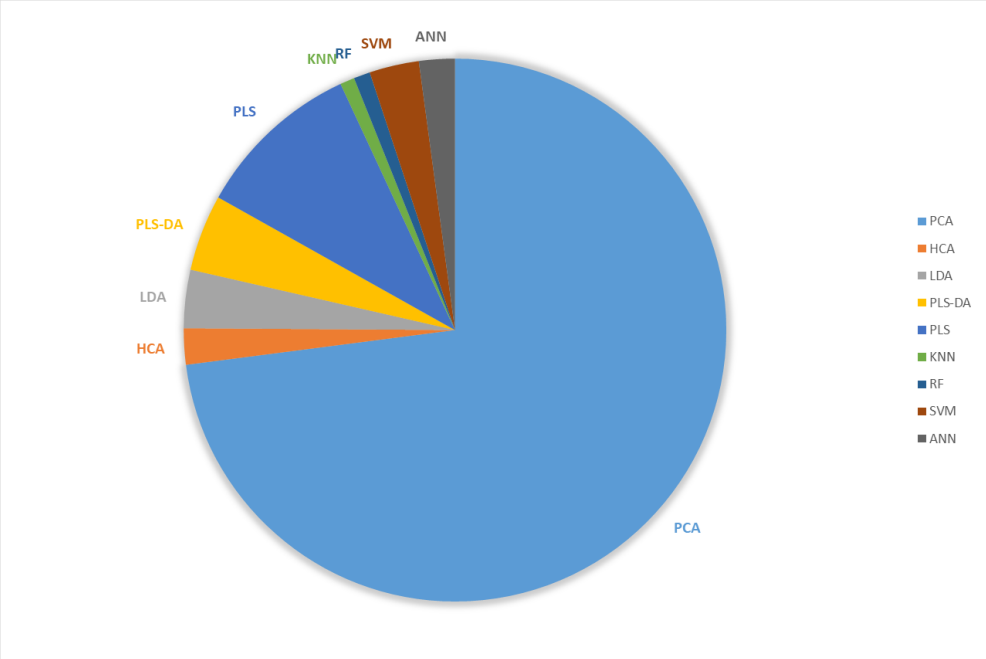


Figure 1.6.9: Overview of the data analysis tools most used in food analysis from 2010-2022.

Unsupervised data elaboration: display and clustering

Principal Component Analysis (PCA)

It is an exploratory technique that enables multivariate data to be shown on a two-dimensional plane so that decisions can be made depending on the outcomes that are seen. The PCA method can be used to compress data, reduce data dimension, and produce a new collection of uncorrelated variables in addition to data visualization.

PCA is a non-guided mathematical analysis of the data matrix whose purpose is to represent the dispersion present in the many variables, finding a few combinations of the original variables that better explain the total variation in the original dataset. This happens through a process of rotation of the original data, defined by a matrix x, in a new Cartesian system in which the data are sorted in variance descending order: so, the variable with the greatest variance is projected onto the first axis, the second on the second axis and so on. The reduction of the complexity of the matrix, occurs by limiting to analyse the main variables (by variance) among the new ones. In PCA, variability means information: so the first new axis is oriented in the direction of maximum variance of the data, the second is perpendicular to the first and both in the direction of the next maximum variance data and so on for all new p axes.

So the new dataset is described by less variables called principal components (PC1, PC2, PC3…). The first variable (PC1) must have the largest possible variance to cover the largest amount of variability inside of the data set. The second principal component (PC2) must be orthogonal to the first one in order to explain the larger amount of the remaining variance. The choice of number of PCs used, don't have a rule, so the decision is usually made by checking the variance explained measure mentioned above (figure 1.6.10).

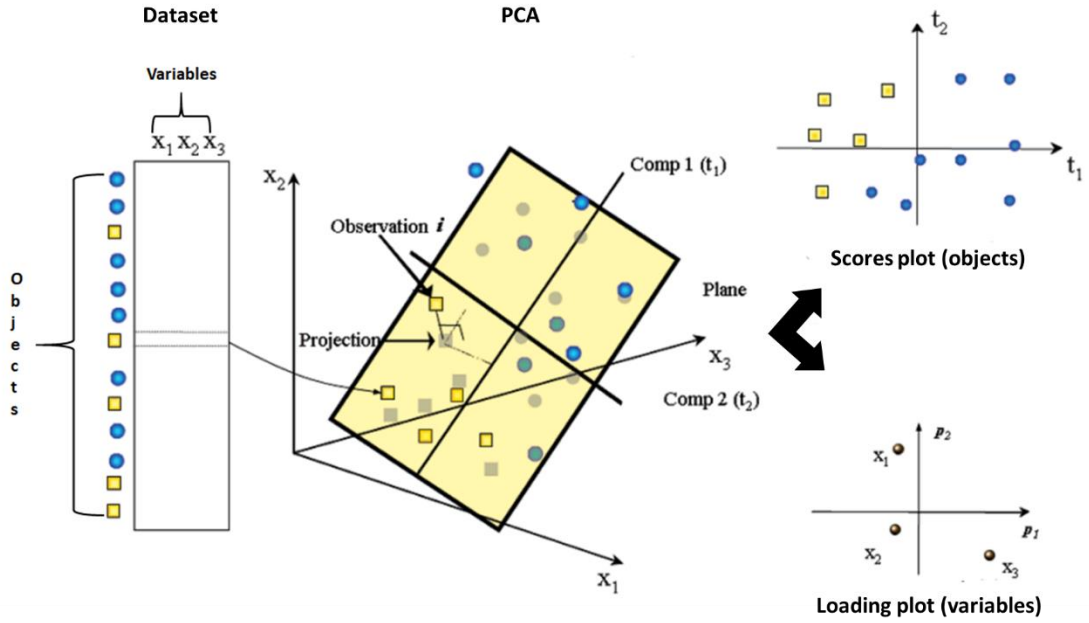


Figure 1.6.10: PCA elaboration scheme. From (Trygg, 2007).

PCA can be considered as the starting point of multivariate data analysis. With the Pcs chosen, the data can be visualized, in function of the variables or of the objects. So it is possible to talk about:

- Loadings plot: it is a diagram that plots the original variables on PCs choice. The projection of each variable into PCs, indicates the weight of the original variables in the definition of score samples.The loadings indicate which variables are most important for the differences observed between the samples.

- Scores plot: it is a plot that allow to analyse the objects behaviour in the different PCs. It is useful to see samples distribution or clusters of them and also the similarity between them.

It will be possible, even, to visualize at the same time objects and variables in order to value the relationships between the two. This type of plot is called Biplot.

It's possible to have other important information from PCA output:

Matrix correlation: it contains the information regarding the correlations between variables. Is a technique that measure the variance between two variables. The main diagonal is made up of values all equal to 1 because each variable is correlated with itself and provide the same information. All the other elements, that are symmetric up and down the diagonal, inform us about the correlation between variable couples. Each covariance value between two variance will be between -1 and +1. In the first case the correlation is inverse, in the second is direct. When the correlation is 0, there isn't a relationship between two variables (Todeschini, 1998).

Clustering methods

The goal of clustering techniques is to locate collections of related items or variables in a data matrix. In fact, the expanded groups can offer "categories" for additional supervised studies, including classification analysis. Principal component diagrams or specific techniques that operate on the entire data set can be used to conduct clustering. Clustering techniques can be divided into:

- Hierarchical Cluster Analysis (HCA): Agglomerative and Divisive
- Non-hierarchical method

Hierarchical agglomerative techniques are the most widely used. They are based on the ability to classify related units in a high-dimensional data analysis. Recently, HCA has been combined with heat map visualization, a clustergram. With stronger intensities of one colour to represent lower levels of the variable and increasing intensities of a different colour to represent higher levels, the clustergrams display each participant's row of data across each of the variables' columns as a colour block.

Supervised data elaboration

The most widely used classification techniques employ vote-based decision rules and discriminant analysis. While the later splits the samples into subsets depending on the values of specific variables, this procedure is repeated on each derived subset of samples, the former analysis entails drawing boundaries between the various classes specified by the training set. In both situations, probabilistic approaches to classification are used since voting or calculating distances determine whether a sample belongs to one class or another. The metrics used are based on either Mahalanobis, Manhattan or Euclidean distances.

- A sample is classified using K nearest neighbors (kNN) by the k samples in the training set that are the most similar to it. There are numerous possible techniques for determining nearness (i.e., similarity or distance) and for weighted voting, and the value of the number k is one of these alternatives. kNN classifiers are particularly useful for complex distributions that are challenging to describe because they don't presuppose any distribution or model for the data.
- Decision tree learning creates a tree that arranges a set of “tests” that direct the process in steps leading up to its conclusion. Although decision trees are easy to comprehend and relate to the features, they are sometimes not strong, which poses a risk for small training sets with many features. Ensemble learning construct numerous decision trees with boosting (emphasizing incorrectly modelled samples) or bagging to address these issues (resampling training data). Multiple decision trees are used by random forests (RF) to make a choice.
- For categorical predicted variables, there is a variation known as partial least-squares discriminant analysis (PLS-DA).
- For classification, regression, and other pattern recognition issues, support vector machines (SVM) build a hyperplane in a high-dimensional space. The decision function is flexible in that a variety of kernel functions can be supplied. Even for very huge feature spaces, SVM can function reasonably effectively.
- Artificial neural networks (ANNs) is one of the most popular ML topics and in particular of the deep learning, but it is best suited for very large data sets (Stilo, 2021).

Partial least square regression (PLSr) and Partial least squares-discriminant analysis (PLS-DA)

PLSr is a recent technique that generalizes and combines features from PCA and multiple regression. In particular, PLS modelling is a multivariate projection method for modelling a relationship between dependent variables (Y) and independent variables (X). The principle of PLS is to find the components in the input matrix that describe as much as possible the relevant variations of variables, giving less weight to the irrelevant or noisy variations. PLS simultaneously models both X and Y to find the latent variables in X that will predict the latent variables in Y. An important feature of PLS is that it is suitable for data sets with fewer objects than variables and a high degree of inter-correlation between the independent variables (Berrueta, 2007). When the Y matrix is formed by qualitative variables, the PLS is used for discrimination purposes and takes the name of PLS-DA (Trygg, 2007). The PLS-DA aims to find the variables and directions in the multivariate space which discriminate the stablished classes in the calibration set. Variable importance in projection (VIP), for example, is a well-known method for finding significant variables in complex data. This method selects variables from the PLS model by calculating the VIP score for each chemical variable, VIP score below a predefined threshold (in general default=1) are removed. The optimal number of latent variables can be estimated by an internal cross-validation or an evaluation test sets. In order to obtain a better model, it is important to work with a well-designed training set; in other words, it has to be representative of the phenomenon under study both in terms of variability and numerousness. The test is a set of objects in which the dependent variable (Y) has to be predicted by PLS model.

Most common parameters used to evaluate PLSr or PLS-DA model performance are reported as follow:

- Q^2 index: this is a model quality index; this value measures the global contribution of the components to the predictive quality of the model. The search of the maximum value of Q^2 is equivalent to finding the most stable model.

$$Q^2 = 1 - PRESS/TSS$$

Eq: 6

Where PRESS is predictive error sum of squares, TSS is total sum of squares

- Determination Coefficient of the model (R^2): this coefficient, whose value ranges between 0 and 1, indicates the proportion of variability of the dependent variable explained by the model. The nearest to 1 R^2 is the better is the model. The main issues of R^2 is that it does not take into account the number of variables used to fit the model. Adjusted R^2 can be used instead of R^2 to overcome this limit. The number of variables used for the model development is important since the number of unnecessary variables penalizes the model; Adjusted R^2, unlike R^2, is sensitive to these penalties. Adjusted R^2 can be calculated with the following formula:

$$_{Adj}R^2 = 1- (1- R^2) \text{ x } n-1/n-p$$

Eq: 7

Where R^2 is the determination coefficient of the model, n and p are the number of observations and variables used to fit the model.

- Root Mean Squared Error (RMSE) is defined as the square root of the Mean Squared Error that measures the average of the squares of the errors or deviations, that is the difference between the predicted values and what is measured. When the error is calculated on cross validation data takes the name of root mean squared error in Cross Validation (RMSECV) while when it is calculated on prediction data root mean squared error in prediction (RMSEP)

$$\text{RMSE} = \sqrt{\frac{\sum_{I=1}^{n} wi\ (yi-\hat{y}i)^2}{W-p*}}$$

Eq: 8

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2. Experimental part

Section 2.1-Safety:

In the first section is reported the qualitative safety related to the acrylamide formed during roasted process in coffee. Exposure data have revealed that coffee is a major source of this toxicant, classified by IARC as a probable human carcinogen. Many efforts to find ways to reduce acrylamide formation during coffee processing. However, despite the mitigation strategies applied by industries, reducing acrylamide in coffee is still a challenge.

Together with the qualitative aspect, even the sensory component resulted important in description of qualitative aspect. The flavour and aroma of coffee have been studied extensively, although the underlying chemistry is not yet fully understood. The experimental part of the study was split into two main sections in which the aroma and the taste of coffee were investigated to define the chemical evolution of flavour over time when new packagings are developed, and to reduce both the waste of coffee by monitoring its shelf life, and the environmental impact of the exhausted material (mainly packaging).

Section 2.2-Aroma quality:

The aim of the project was to investigate the chemical description of the aroma of different commercial coffee blends in connection with the sensory description and the relationship to sensory acceptance by the consumer. The long-term goal was to use the results of this project to create an objective analytical tool to define the sensory acceptability of the products.

- **Section 2.2.1**

The purpose of this part was to investigate in depth the complexity of coffee flavour perception to understand the limits of the sensometric approach. A flavour-omic approach has been adopted to link chemical information from volatile and non-volatile fractions to the panel flavour evaluations. Chemical information from HS-SPME-GC-MS analysis of coffee powders was integrated with the HPLC-UV/DAD fingerprinting analyses targeted on specific wavelengths of non-volatile fractions to investigate the contribution of data fusion in flavour definition and prediction. The study is mainly focused on the *Bitter* note and then extended to the other sensory notes of interest.

- **Section 2.2.2:**

In order to define one or more chemical markers representative of the change of aroma over time, the first activities were addressed I) to define a fingerprinting of volatile compounds, able to discriminate between good and oxidised samples; II) to identify chemical markers related to product ageing; III) to determine their correlation with sensory data and IV) to apply the identified chemical markers to unknown samples randomly selected, to analyse samples already evaluated positively/negatively by the panel, and a set of different blends and packaging.

The first part, involved coffee samples characterized at the tasting by the note "aged" (oxidised) and "good" for control samples. In particular, samples of four different coffee blends were examined, in conventional commercial capsules, capsules of innovative material and ground coffee for moka in soft-pack packaging. Chemical analysis on these samples was performed through the use of analytical platforms (HS-SPME-GC-MS/FPD) that allowed obtaining a detailed chemical profile of the volatile fraction. The purpose was to evaluate any differences in the aroma profile of oxidised products, compared to good ones and to select possible chemical markers related to oxidation. The information resulting from this first section has been exploited to define the chemical fingerprints of coffee samples, to use during the second step.

- **Section 2.2.3:**

The same samples of roasted and ground (R&G) blends, in three different packaging (Eco caps in modified atmosphere and standard caps in multilayer film with aluminium) suitable for espresso brew, and soft-pack suitable for moka preparation. were analyzed every 30-days over time under two different storage conditions: at ambient conditions and under stress conditions (temperature, humidity) in numbers of 3 lots per sample type. The fingerprinting chemical markers were followed in their kinetic evolution in these samples. They were simultaneously subjected to sensory analysis in order to correlate them with the chemical data through the use of chemometric techniques to define the qualitative chemical indicators that define the acceptability (or not) of a product under stress conditions. In parallel, the kinetics of evolution of the chemical indicators related to the degradation of sensory quality identified under the stressed conditions, was followed on samples, kept at ambient conditions to check whether, if they are the same. Thus, the thresholds of ageing markers of

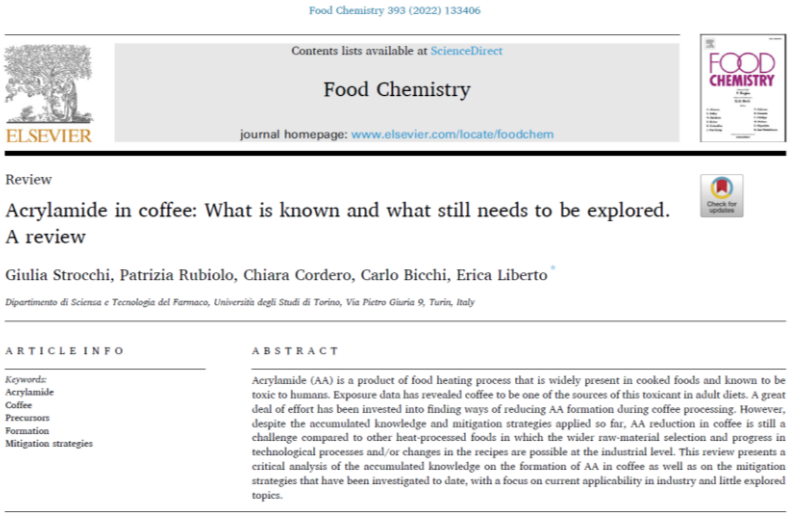
acceptability/rejection were defined. The identified chemicals were subsequently monitored on a number of representative unknown samples.

Section 2.3-Taste quality:

The non-volatile fraction was also investigated on the same set of monitored samples under accelerated conditions, with the aim of correlating the aromatic and the taste fractions, to obtain a 360° metabolomic understanding, to delineate all those factors that impact on the product quality and shelf life. This section investigated the complexity of coffee flavour, deepening studies of the non-volatile fraction, including the lipid moiety: the phenolic fraction (**section 2.3.1**), fatty acids (**section 2.3.2**) and diterpenes (**section 2.3.3**). The diterpenes, which samples set are different from the previous one (on phenolic and fatty acid fraction), have been carried out at the Department of Life Sciences & Chemistry Faculty of Health, Jacobs University, Bremen Germany under the supervision of Prof. Dr. Nikolai Kuhnert.

2.1 Safety

Acrylamide in coffee: What is known and what still needs to be explored. A review



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Review

Acrylamide in coffee: What is known and what still needs to be explored. A review

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ABSTRACT

Acrylamide (AA) is a product of food heating process that is widely present in cooked foods and known to be toxic to humans. Exposure data has revealed coffee to be one of the sources of this toxicant in adult diets. A great deal of effort has been invested into finding ways of reducing AA formation during coffee processing. However, despite the accumulated knowledge and mitigation strategies applied so far, AA reduction in coffee is still a challenge compared to other heat-processed foods in which the wider raw-material selection and progress in technological processes and/or changes in the recipes are possible at the industrial level. This review presents a critical analysis of the accumulated knowledge on the formation of AA in coffee as well as on the mitigation strategies that have been investigated to date, with a focus on current applicability in industry and little explored topics.

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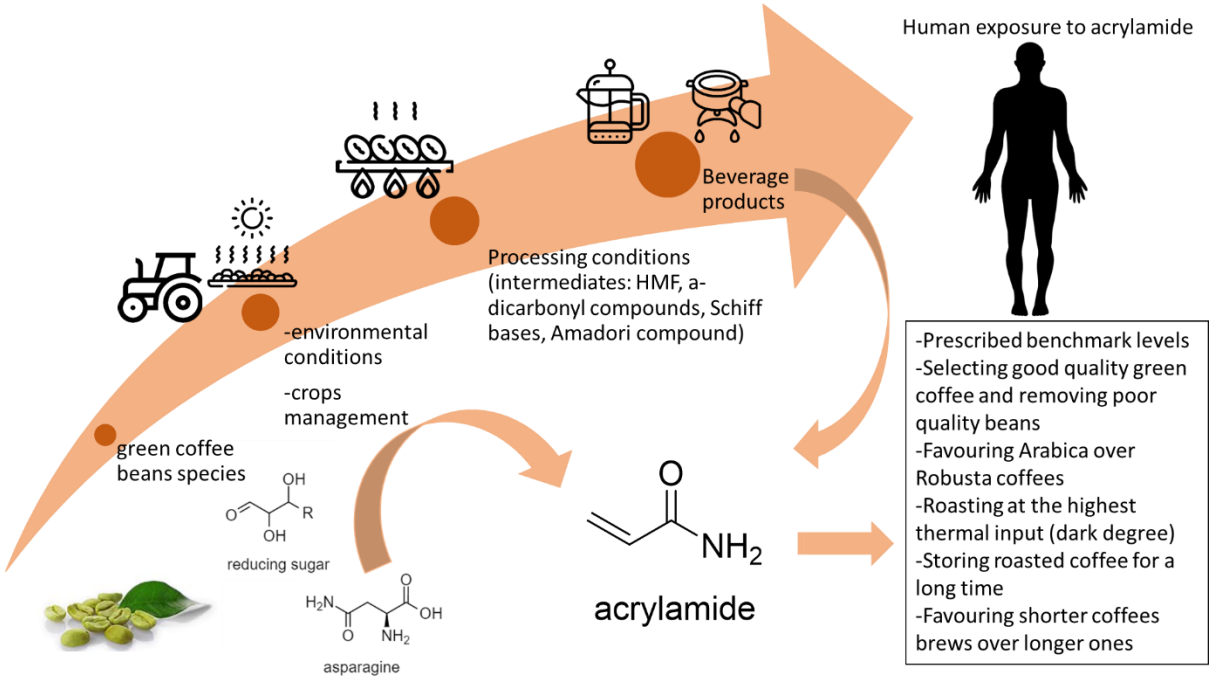
GRAPHICAL ABSTRACT



Acrylamide in coffee: what is known and what still needs to be explored. A review.

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ABSTRACT

Acrylamide (AA) is a product of food heating process that is widely present in cooked foods and known to be toxic to humans. Exposure data has revealed coffee to be one of the sources of this toxicant in adult diets. A great deal of effort has been invested into finding ways of reducing AA formation during coffee processing. However, despite the accumulated knowledge and mitigation strategies applied so far, AA reduction in coffee is still a challenge compared to other heat-processed foods in which the wider raw-material selection and progress in technological processes and/or changes in the recipes are possible at the industrial level. This review presents a critical analysis of the accumulated knowledge on the formation of AA in coffee as well as on the mitigation strategies that have been investigated to date, with a focus on current applicability in industry and little explored topics.

Keywords: Acrylamide, coffee, precursors, formation, mitigation strategies

1. INTRODUCTION

Acrylamide (AA) is a highly water-soluble organic compound. AA is currently studied mostly because of its high toxicological potential and widespread occurrence in food products (Rannou, Laroque, Renault, Prost, & Sérot, 2016). High levels of AA are found in potato chips, French fries, biscuits and roasted coffee, and it is formed in foods that are prepared at temperatures above 120 °C and possess low moisture (EFSA, 2015; EU Commission, 2017). AA has notably been classified by the International Agency for Research on Cancer as "probably carcinogenic to humans" (Group 2A) (IARC, 1994). However, in 2016, coffee drinking was evaluated by the IARC as being "not classifiable as to carcinogenicity" (Group 3) (Esposito et al., 2020; Loomis et al., 2016). The benchmark levels (μg/kg) of AA in foods are reported in EU regulation 2017/2158; in coffee they are 400 μg/kg for roasted coffee and 850 μg/kg for instant coffee (EU Commission, 2017).

A dietary-habit survey performed in over 20 countries showed that European citizens have an average daily AA intake that ranges from 0.14 to 1.31 mg/kg of body weight (bw). Similar levels were also recorded in the USA. Daily AA intake/kg bw may be especially higher in children whose relative intake, with respect to body weight, is higher, in particular, because of the concurrent consumption of baked cereals and crisp products (Semla, Goc, Martiniaková, Omelka, & Formicki, 2017). In the adult and elderly populations (20–79 years), coffee is one of the main contributors of AA intake, ranging from 9% to 29%, with that figure reaching 38–60% for baked goods and crisps, depending on the country of origin. AA concentration in coffee ranges from an average of 249 μg/kg to 710 μg/kg (average values referring to the dry powder) for roasted coffee and instant coffee respectively. As reported in the EFSA's scientific opinion on AA in food, the results were expressed in powder equivalents according to the dilution factor used to prepare the beverage. However, if we consider the respective dilution factors (from 0.035 to 0.125 for roasted coffee and 0.017 for instant coffee), some beverages obtained from roasted coffee would then contain higher AA levels than those made from instant coffee (EFSA, 2015).

Coffee is one of the most consumed beverage in the world because of its pleasant aroma, which is caused by the large range of volatiles that are produced during the roasting process (Toledo, Pezza, Pezza, & Toci, 2016). Roasting is a traditional thermal process with the primary objective not only being to achieve the desired flavour, but also to generate a dark colour and

a brittle, porous texture in the bean suitable for successive grinding and brewing. The high production temperature induces extensive chemical reactions, dehydration and profound changes in the microstructure (Folmer, 2017). At the same time, roasting leads to the development of undesired compounds of concern, such as AA and furans (Schouten, Tappi, & Romani, 2020).

Since 2002 when AA was detected in heated foods , extensive effort has been made by public research institutions and industries to investigate ways to reduce AA formation during food processing (Summa, de la Calle, Brohee, Stadler, & Anklam, 2007). However, despite the accumulated knowledge and mitigation strategies applied so far, the reduction of AA levels in coffee is still a challenge, compared to other foods (i.e. baked or fried carbohydrate-rich foods) in which wider raw-material selection and improvements in technological processes and/or changes in the recipes are possible on an industrial level. This review presents a critical analysis of the accumulated knowledge on precursors and formation pathways of AA in coffee as well as on the mitigation strategies that have been investigated to date, with particular attention being paid to current applicability in industry and the Authors' viewpoint on topics that require further exploration.

2. AA PHYSICO-CHEMICAL CHARACTERISTICS

AA is an odourless white crystalline solid with the molecular formula of C_3H_5NO and a molecular weight of 71.08 g/mol. Its IUPAC name is prop-2-enamide; and its synonyms are acrylic amide and ethylene carboxamide (Figure 1). Its main physico-chemical characteristics are: melting point: 84.5 °C; vapor pressure: 0.9 Pa (7×10^{-3} mm Hg) a 25 °C; solubility in water: 2.155 g/L, in methanol: 1.550 g/L, in ethanol: 862 g/L, in acetone: 631 g/L at 30 °C, in benzene 3.46 g/L, in chloroform: 26.6 g/L; Log Kow: −0.67; Henry's law constant at 25 °C: 1.7×10-9 atm-m^3/mol (ECHA-European Chemical Agency, 2021).

AA is stable at room temperature, but polymerizes when heated to its melting point and even when exposed to ultraviolet radiation (WHO/IPCS, 1999). AA thermally decomposes to form ammonia and carbon monoxide, carbon dioxide and nitrogen oxides (Kitahara et al., 2012; Maan et al., 2022). AA stability is quite high in aqueous solutions, but decreases under dry conditions and can be influenced by pH and the nature of the buffer (Adams, Hamdani, Lancker, Méjri, & De Kimpe, 2010). The stability of AA and its reactivity with relevant nucleophiles from various foods at elevated temperatures have been studied by Adams *et al.*

in model systems. Amino acids with nucleophilic side chains decrease the amount of free AA; cysteine (Cys) is the most reactive, while other less reactive nucleophiles, such as lysine (Lys), arginine (Arg), serine (Ser) and ascorbic acid gave similar condensation products (Adams, Hamdani, Lancker, Méjri, & De Kimpe, 2010). As an unsaturated carbonyl compound with electrophilic properties, AA can react, via Michael addition with biological nucleophilic groups including amines, carboxylates, aryl and alkyl hydroxyls, imidazoles and thiols of macromolecules (e.g. Cys residues), DNA and proteins. This reactivity is the basis of its toxicity (Nehlig & Cunha, 2020).

3. MECHANISM OF ACRYLAMIDE FORMATION

Coffee beans are subject to higher temperatures than other foods during roasting (range 220–250°C). Although the Maillard reaction is predominant over others and is responsible for the AA formation, under these harsh processing conditions, it can be expected to form via pathways beyond the commonly accepted asparagine/sugar (or carbonyl) condensation (Guenther, Anklam, Wenzl, & Stadler, 2007). The additional pathways for AA formation that have been studied and proposed are reported in Figure 1.

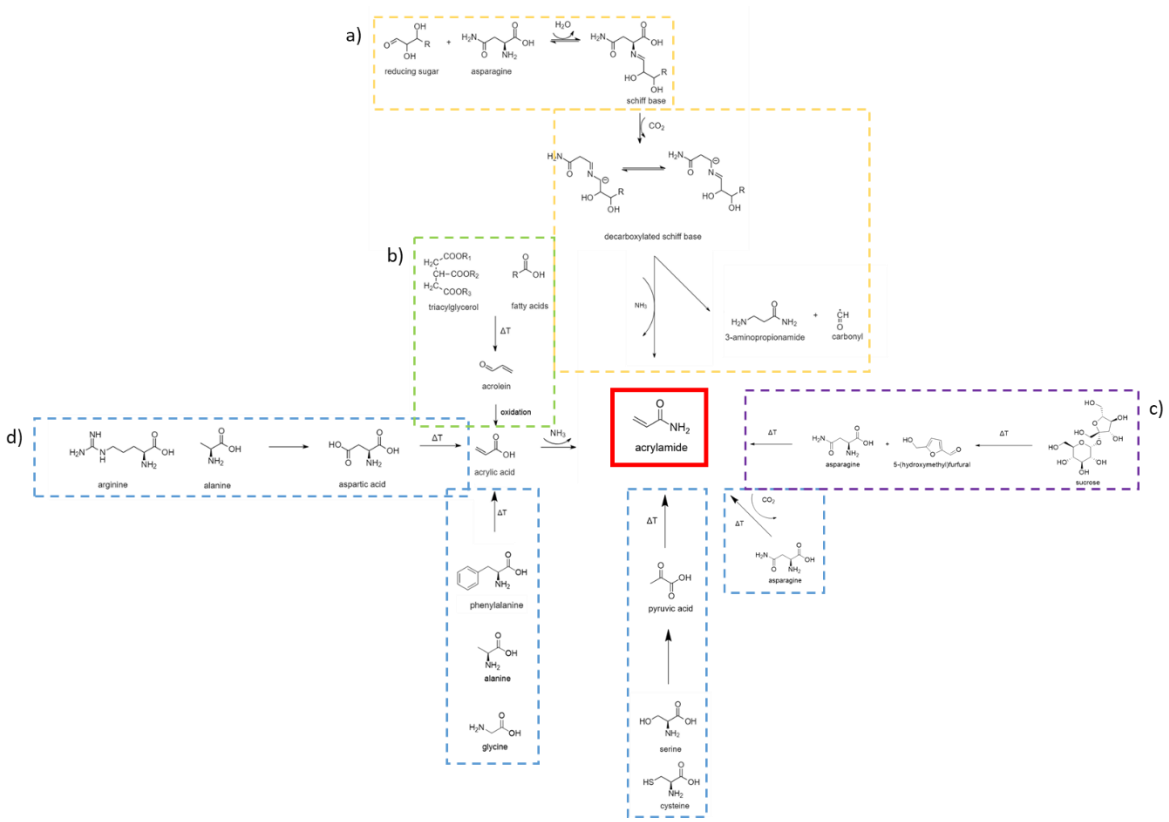

Figure 1: Formation pathways of AA: a) Maillard reaction pathway in yellow; b) via triglyceride decomposition in green; c) from 5-hydroxymethylfurfural (HMF) in violet; d) formation from pyrolysis in light blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

3.1 Formation via the Maillard reaction

Coffee beans mainly undergo the Maillard reaction during the roasting process and this promotes the formation of AA, which results from the combination of an amino residue of asparagine (Asn) and a carbonyl group from a reducing sugar (e.g. glucose) at temperatures above 120 °C (Anese, 2016; Bagdonaite, Derler, & Murkovic, 2008; Mottram, Wedzicha & Dodson, 2002). Stable isotope-labelled experiments have shown that the backbone of the AA molecule originates from Asn (Figure 1 A) (Pedreschi, Mariotti, & Granby, 2014).

In contrast to fried snacks and bakery products, green coffee apparently does not contain a source of free carbonyl compounds. However, alternative reactive carbonyls derive from linoleic acid hydroperoxide degradation or from saccharide degradation at high temperature (Belitz, Grosch, 2009). These carbonyls facilitate AA formation. Sucrose was the only sugar detected, at a concentration of approximately 8.0% in green coffee, and it tends to decompose during roasting within 15 min at 220°C (Kocadagli, Göncüoglu, Hamzalioğlu, & Gökmen, 2012).

A range of different carbonyl compounds are involved in acrylamide formation: hydroxycarbonyls, dicarbonyls (Amrein et al., 2004; Stadler et al., 2004; Zyzak et al., 2003) and alkadienals from lipid oxidation (Gökmen, Kocadağli, Göncüoğlu, & Mogol, 2012; Hidalgo, Delgado, & Zamora, 2009; Kocadagli et al., 2012; Zamora & Hidalgo, 2008) have been investigated so far. Model studies have demonstrated that α-hydroxy carbonyls are much more effective than α-dicarbonyls in converting Asn into AA as they promote the rearrangement of azomethine ylides, which are degradation products of the Schiff base (Gökmen et al., 2012; Stadler et al., 2004). The β-elimination reaction of the decarboxylated Amadori compound is the subsequent step and gives AA (Stadler et al., 2004; Zyzak et al., 2003). However, Hamzalioğlu and Gökmen have, more recently, used a multi-response kinetic modelling to show that the 3-deoxyglucosone (3-DG) was the most abundant dicarbonyl to

be formed from sucrose degradation and from the Maillard reaction during roasting that participates in producing AA (Hamzalıoğlu & Gökmen, 2020). 5-Hydroxymethylfurfural (HMF), which is the major sugar-decomposition product generated during roasting, can play a role in AA formation, and can generate more AA than glucose when heated together with Asn (Anese, 2016; Kocadagli et al., 2012). HMF is formed and accumulated during the early stages of roasting due to the simultaneous consumption of sucrose. It reaches its maximum content within 10 min of roasting at 220°C and then decreases (Figure 1C) (Gökmen et al., 2012). Cai et al. (Cai et al., 2014) have reported that the addition of chlorogenic acid (0.5 and 5 µmol/mL) to the Asn/glucose-Maillard reaction system significantly promotes AA formation, mainly by increasing HMF formation, while inhibiting its elimination. A comprehensive kinetic model, including the elementary steps for acrylamide formation, was proposed by Hamzalioğlu *et al.,* in 2020. Changes in sucrose, reducing sugars, free amino acids, Asn, AA, 3-DG, methylglyoxal, glyoxal and HMF were monitored during coffee roasting at 200, 220 and 240 °C. The results of the multi-response kinetic modelling approach indicate that sucrose degrades into glucose and a reactive fructofuranosyl cation that principally contributed to the formation of HMF, which, in turn, was found to be the most important reactive carbonyl compound in the formation of AA in coffee during roasting. Conversely, glucose mostly takes part in the formation of intermediates, glyoxal and especially 3-DG, rather than AA. Therefore, any ingredient/component that promotes HMF formation also increases AA generation. By contrast, the quinone derivative of chlorogenic acid decreases AA formation via H_2O_2 oxidation. However, this mechanism requires further investigation (Hamzalıoğlu & Gökmen, 2020).

3.2 Formation via triglyceride decomposition

In addition to carbohydrates, lipid oxidation products also participate in AA formation. In particular, di-unsaturated hydroperoxides and their degradation products such as the $\alpha, \beta, \gamma, \delta$-diunsaturated carbonyl group promote the AA formation during food heating related processes (Zamora & Hidalgo, 2008).

It is also well known that lipids (triglycerides) produce a large amount of acrolein when heated (Ehling, Hengel, & Shibamoto, 2005). Acrolein is oxidized to acrylic acid, which then reacts

with ammonia to generate AA. α-Amino acids produce ammonia via the Strecker degradation in the presence of a carbonyl compound (Figure 1B) (Stadler, Verzegnassi, Varga, Grigorov, Studer, Riediker, Schilter, 2003; Yasuhara, Tanaka, Hengel, & Shibamoto, 2003). Aspartic acid (Asp) can also release acrylic acid without involving sugars or a carbonyl source via a concerted decarboxylation/deamination pathway. In addition to Asn, other amino acids, such as L-alanine (Ala) and L-arginine (Arg), can also release acrylic acid at temperatures above 180°C (Guenther et al., 2007).

3.3 Formation via pyrolysis

Lactamide and AA can be generated in the presence of ammonia in pyrolytic reactions that involve Ser and Cys through conversion, via pyruvic acid, to lactic acid (Figure 1D) (Claus, Weisz, Schieber, & Carle, 2006).

4. FACTORS THAT AFFECT ACRYLAMIDE LEVELS

Several factors may affect AA concentrations in coffee (Figure 2A), and these are discussed in detail below.

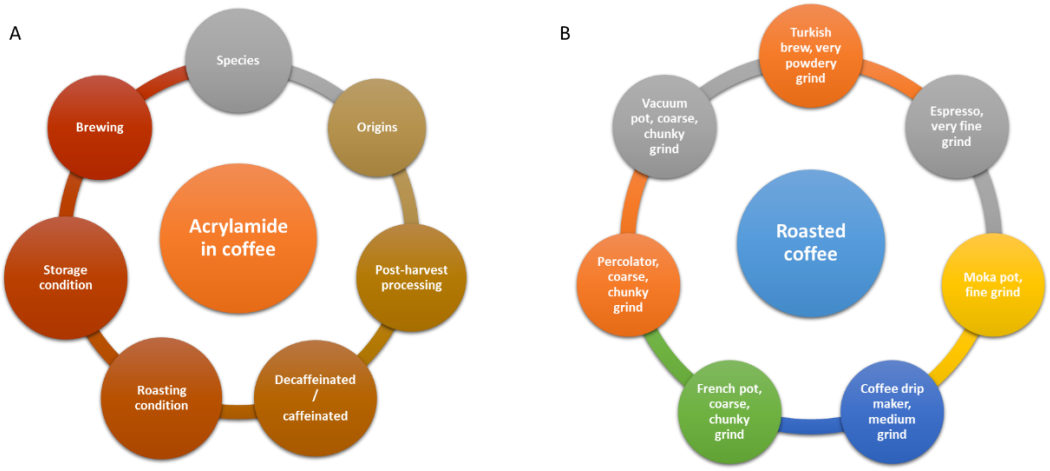

Figure 2: A. Main factors affecting acrylamide levels in coffee: coffee species (Robusta coffee contains higher levels of acrylamide than Arabica; roasting conditions (acrylamide is formed in the early stages of roasting and its content decreases with increasing temperature and roasting time); storage (acrylamide is not stable in commercial coffee stored in its original container); beverage preparation (acrylamide is extracted differently into the beverages); defective coffee beans (in particular immature ones that contain higher amounts of free asparagine). B. Examples of coffee brewing techniques and their respective grinding grades. Coffee brews are prepared using a certain volume of water (boiled,

under pressure…) and a defined amount of coffee powder. The optimal grinding degree varies with coffee brewing preparation.

4.1 Acrylamide precursors in green beans: pre- and post-harvesting

Coffee is a perennial tropical crop unlike other acrylamide-producing agricultural crops such as potatoes and cereals, which are annual crops and need to be sown or planted annually. One advantage of annual crops is that they can be more easily manipulated to reduce AA precursor formation by changing the variety or moving the production site. This is not feasible with perennial crops, such as coffee, as soil composition, temperature, altitude and water availability determine bean quality and, thereby, the quality of the coffee product. In addition, climate change and, in particular, increases in temperature can greatly influence production. Several strategies have been proposed to manage plantations, exploit ancient species and varieties, and create new hybrids are being investigated in order to counter climatic effects. However, these projects are, in principle, oriented towards the yield and flavour and less towards the impact they may also have on AA precursors.

4.1.1 *Influence of coffee species: Arabica and Robusta*

According to a research group from the Royal Botanical Gardens in Kew (Davis et al., 2019), *Coffea arabica* is a vulnerable species and at risk of extinction due to deforestation and climate change. To ensure its survival, experts suggest moving crops to higher and colder areas or upgrading irrigation systems. Unfortunately, these recommendations cannot be adopted everywhere, their application depending on origin, farm size and the nature of the land. *Coffea arabica* (Arabica) and *Coffea canephora* (Robusta) are the two leading species in the coffee market. *C. arabica* grows in a narrower range of regions, compared to *C. canephora,* as it can be cultivated in mountainous rainforests with average annual temperatures of between 18 and 21°C and rainfall of between 1100 and 2000 mm.

C. canephora mainly grows in soils that are flat or gently sloping and are well-drained.

They are characterized by different levels of amino acids, sugars and minerals, volatiles, chlorogenic acids and caffeine (Guenther et al., 2007). Many studies have reported that significantly higher amounts of AA are found in Robusta than in Arabica (Esposito et al., 2020; Lachenmeier et al., 2019). This difference seems to be associated with higher Asn content in

raw Robusta beans than in Arabica (Alves, Soares, Casal, Fernandes, & Oliveira, 2010; Bagdonaite et al., 2008; Lantz et al., 2006). In 2008, Bagdonaite *et al.*, investigated the influence of the concentrations of possible precursors in green coffee, such as amino acids, sucrose and carbohydrates, on AA formation and concluded that higher Asn content resulted in higher AA amounts. Robusta coffee was found to contain higher levels of Asn (the concentration of free Asn was 797 µg/g in Robusta and 486 µg/g in Arabica) and lower amounts of sucrose, and was confirmed to have a higher AA concentration than the investigated Arabica coffee (Bagdonaite et al., 2008; Bertuzzi, Martinelli, Mulazzi, & Rastelli, 2020). Hu *et al.* (Hu, Liu, Jiang, Zhang, & Zhang, 2021) have very recently shown that individual addition of free amino acids (i.e. Ala, Arg, Lys, Cys, Ser and Glycine (Gly), Phenylalanine (Phe), Tryptophan (Trp), and Glutamine (Gln)) in a model system solution heated in a hot-air roaster at 180°C for 5 min, promotes the AA formation. In addition, it has been observed a positive correlation between roasting time and AA amount. The authors speculated that the high level of AA at the early stages of roasting may also be due to the presence of other amino acids. Moreover, they observed that the addition of Gly and Asp can reduce AA formation, and proposed their addition during roasting. However, these findings contradict those of other authors (Guenther et al., 2007; Navarini, Terra, Colomban, Lonzarich, & Liverani, 2014; Yasuhara et al., 2003).

An investigation of the sugar fraction by Bagdonaite *et al.* (Bagdonaite et al., 2008), using a laboratory scale roaster, indicated that higher sucrose amounts lead to lower AA formation, while Stadler *et al.* (Stadler & Theurillat, 2012) reported no correlation between AA formation and reducing sugars during industrial scale roasting. Total sugars were significantly higher in the Arabica green coffee beans than in Robusta (sucrose: 7.5% in Arabica and 4.5% in Robusta (Stadler & Theurillat, 2012). Recently, Bertuzzi et al. 2020 quantified the reducing sugars during an industrial roasting process of Arabica and Robusta. The increase in reducing monosaccharides due to thermally induced hydrolysis of sucrose (i.e. 936 ± 78 mg/kg and 424 ± 69 mg/kg for fructose and glucose in Arabica, 338 ±41 and 138 ±19 mg/kg for fructose and glucose in Robusta, respectively) could explain the higher AA content in their Arabica coffees (Bertuzzi et al., 2020).

Factors, such as cultivation conditions, coffee origin and processing, can influence the content of amino acids and free reducing sugars and, thereby, the formation of AA (Bertuzzi et al., 2020; Schouten et al., 2020).

4.1.2 *Influence of coffee origins*

Coffee production is restricted to the humid tropical regions of Asia and Oceania, South America, Africa, some regions of Mexico and Central America and their respective islands (in order of productive yield). Despite the widespread of production sites, only a few studies have correlated geographical origin to AA and its precursors, while several research works have connected the different coffee species to the presence of AA, on the basis of their different chemical compositions (Alves, Soares, Casal, Fernandes, & Oliveira, 2010; Bagdonaite et al., 2008; Guenther et al., 2007; Lantz et al., 2006; Summa et al., 2007). Bagdonaite *et al.*, (Bagdonaite & Murkovic, 2004) have reported differences in AA levels in some wet-processed Arabica varieties (Columbian Excelso, Uganda Organico Biocoffee, Santos Brazil) and Robusta (Cameroon) after roasting under identical conditions. The latter was shown to contain the highest amount of AA. Among the Arabica, high quality beans (Columbian Excelso, and Uganda Organico Biocoffee) contained lower AA amounts than low quality beans (Santos Brasil). The potential effects of origin, within the same treatment and species groups (i.e. wet processed for Arabica samples and dry-processed for Robusta), can be inferred from the results of Alves *et al.*, (Alves et al., 2010). Table S1 shows the concentration of AA in the final espresso coffee product. For Robusta, higher amounts of AA were observed in coffee from African regions than from Asian samples, and there was a certain variability within the same geographical macro-area. A similar trend, although to a different extent, can be observed in Arabica samples. In 2015, Pugajeva *et al.* (Pugajeva, Jaunbergs, & Bartkevics, 2015) measured the AA content in 22 samples of roasted commercial coffee of different varieties, available in local supermarkets and labelled as monovarietal, and a variation from 166 to 503 µg/kg was found (table S1). However, the variability in their results does not allow any conclusions to be drawn as the pre-processing methods applied to the green beans were not known and their influence on the AA precursors can therefore not be evaluated. Origin and fertilization practices can influence AA precursors. In general, the effect of fertilization, climate and soil can be monitored via the state of the leaves, the growth rate of the trees, the development

of the beans and the production yield, rather than in the chemical composition of the beans (Seal et al., 2008). To the best of the authors' knowledge, no data on the impact of agricultural practices on the amount of AA precursors in beans are available. This is currently an underexplored field.

4.1.3 *Post-harvest processing and AA precursors*

Post-harvest treatments have a decisive influence on the final coffee quality and content of AA. Harvesting should take place when most of the cherries are ripe, as unripe cherries have a higher Asn content (Dias, Borém, Pereira, & Guerreiro, 2012). Cherries are harvested either by hand or mechanically (i.e. by stripping or using a vibrating ring applied to the trunk of the coffee plant) depending on the size and shape of the plantation. In general, hand picking provides harvest with riper cherries than stripping or mechanical harvesting because it makes it possible to better select the fruit, but this practice is discontinuous and costly. Cherry metabolism varies with the degree of ripeness, producing biochemical and chemical conversions that also affect the final composition of amino acids, sugars and other metabolites in the green beans, and conditioning the AA precursors (Dias, 2010; Dias et al., 2012).

After harvesting, coffee cherries must be separated from the skin and pulp, mucilage, and the parchment and then dried (Folmer, 2017). These processes allow the fruit to dry to a safe moisture content in order to inhibit the activity of bacteria and moulds.

Three main processes are possible: the dry method, the wet-process and the 'semi-washed' process. The first method, commonly named *the natural process*, consists of drying the whole fruits under the sun on raised beds or on the floor. The mucilage and skin are removed once dried. This process is mainly used for Robusta coffee.

The wet-process, also called the *washed process*, involves the fresh mature cherries being de-pulped, fermented and washed before drying. This process is mainly used for Arabica coffee.

The *semi-washed process*, also known as the honey process, involves fruit depulping and drying, and the removal of the mucilage and parchment after drying. The chemical composition changes depending on the process and provide coffees with different flavour qualities in the cup, and also have an impact on the AA precursors.

The coffee processing method does not significantly affect sucrose, the major disaccharide in green coffee beans (Kleinwächter & Selmar, 2010; Knopp, Bytof, & Selmar, 2006); the sucrose concentration, unlike that of glucose and fructose, is more significantly determined by pre-harvest, rather than post-harvest, factors. Only small amounts of glucose and fructose were detected after wet-processing, whereas their contents were significantly higher after dry-processing.

Fermentation during wet-processing results in the specific consumption (and decrease) of free sugars (glucose, fructose, arabinose, galactose and mannose). During fermentation in wet-processing, the oxygen concentration in the tank drops, due to microbial action, creating anaerobic conditions that lead to alcoholic or lactic fermentation. Conversely, in dry-processing, the coffee remains in a well-aerated environment throughout treatment, during which aerobic metabolic conditions can be maintained until the reduction of the water content deactivates the metabolic activity. The anaerobic fermentation in the fruits in wet-processed coffee leads to a greater consumption of hexoses for the generation of the same number of ATP-molecules with a major decrease of glucose and fructose (Knopp et al., 2006). Changes in amino acids occur during processing, with glutamic acid (Glu) and Asp mainly being present in the untreated beans followed by Ala and Asn in order of concentration. The wet-process led to a decrease in the concentrations of Asp, Ala and Asn, while the concentration of Glu increased. In the dry-process, the concentrations of most amino acids were either similar to those in the unfermented beans or lower. Galactose also diminishes in the dry-process (Kleinwächter & Selmar, 2010; Knopp et al., 2006). Several diverse metabolic processes occur inside coffee beans during post-harvest processing, and these can alter the chemical composition of the green beans. Drying at 40°C considerably reduces the concentrations of Asn and the other amino acids, while the steam treatment of the beans influences the free and total amino acids, and accounts for a 10% decrease in protein-bound amino acids, and a 50% loss in free amino acids (Seal et al., 2008). In 2014, Navarini (Navarini et al., 2014), demonstrated that Asp, which is present in green beans in non-negligible concentrations compared to Asn, also plays a role of similar significance to that of Asn in the formation of AA during roasting. However, Asn levels, as well as those of other amino acids, were significantly lower when beans were processed using the wet method (Dias et al., 2012). The high water content in green coffee beans renders them metabolically active. The level of moisture in green beans has been investigated by Lantz *et al.*, (Lantz et al., 2006) who

reported that changing their moisture, from 14 to 7%, did not affect AA formation in further processing steps. However, the article did not report data on the relationship between moisture content in green coffee beans and AA formation, and other studies on this topic are not available in the literature.

Last, but not least, storage and transportation should be considered in the post-harvest treatments. Beans are stored in parchment or hulled in order to allow them to reach equilibrium more quickly before shipment. Hulled beans can change their viability, which affect their composition regardless of the processing. During shipment and transportation, the beans are subjected to changes in climate zones and meteorological conditions, and often remain in containers for long periods of time during port customs clearance procedures without proper temperature and humidity control, which can potentially affect their composition and be a source of contamination (Bytof, 2021). To the best of the Authors' knowledge, studies on these steps in post-harvest treatment, in general, and on their influence on AA precursors, more specifically, are lacking in the literature.

4.1.4 *Influence of poor quality coffee beans*

The number of poor quality beans in batch acceptance and production is a central factor for evaluation in quality control. The presence of flaws and blemishes may be associated with specific problems during harvesting and post-harvest processing operations and therefore influence AA precursors. Black beans are the result of dead beans within the coffee cherries or beans that fall naturally on the ground via the action of rain or over-ripening. Immature-black beans are those that fall to the ground, remain in contact with the soil and are thus subject to fermentation (Mazzafera, 1999). A study carried out by Dias *et al.*, has shown that the peeling of immature fruits leads to a reduction in Asn levels and can therefore indirectly contribute to reducing AA formation in coffee (Dias, 2010).

4.1.5 *Decaffeination processing*

The economic impact of decaffeinated coffees is generally underestimated, but the consumers of this coffee come from a large and reliable group, including so-called millennials (the generation of young people born between the end of the 1980s and the beginning of the 2000s) who are faithful consumers by choice and not because it was "suggested by a doctor"

(Conway, 2019; Folmer, 2017). The industrial decaffeination process, involves bean pre-wetting with water, caffeine extraction and subsequent bean drying. Three methods are used to remove caffeine from the green beans: solvent extraction, water extraction and pressurized carbon dioxide. The ideal decaffeination process removes the caffeine from the bean cells without any other alteration to the bean. The industrial decaffeination process of green coffee beans does not significantly affect the final AA content, (Alves et al., 2010; Bagdonaite et al., 2008; Bertuzzi, Rastelli, Mulazzi, & Pietri, 2017), probably because the process does not influence the content of AA precursors.

5. ROASTING

The process of roasting is a fundamental and key step in converting green beans into flavourful roasted coffee with physical properties for good quality in the cup, and sensory properties such as colour, aroma and taste. However, the process and roasting parameters, such as temperature and time, also affect AA levels. Coffee behaves differently than other AA-producing foods. While AA content typically rises with colour or browning degree, due to its origin as a Maillard reaction product, it decreases from light to very dark roasts (Lachenmeier et al., 2019; Summa et al., 2007). AA formation is higher in the early stages of roasting, as has been shown by Bagdonaite (Bagdonaite et al., 2008) who found that, when applying different roasting times (from 5 to 15 min) and temperatures (from 220 to 260°C), the highest concentrations of AA were obtained at low temperatures (220 °C) and short roasting times (5 min). The amount of AA during roasting exponentially increases initially, reaches a maximum and then rapidly decreases. Under more intense roasting conditions, AA was degraded until it could no longer be detected, while Asn and the other precursors decreased mainly because of reactions induced by the thermal process. In 2007, Summa *et al.* (Summa et al., 2007) reported lower AA concentrations in Arabica than in Robusta, when roasted in a hot air roaster at 236 °C to a medium degree. AA occurrence was extremely variable and strictly correlated to both the roasting parameters and the coffee species and, thereby, to the composition of the blends. Few studies have been conducted on the relationship between the amount of AA and coffee origins. Lantz (Lantz et al., 2006) analysed a significant number of green beans (17 Arabica and 6 Robusta), that were roasted to a medium degree in a rotating fluidized bed roaster for 2.5 min to light colour, and they concluded that the main factor affecting the level of AA is the ratio between the two species

in the blends, with Robusta producing higher AA levels on average. Time and roasting are the most significant parameters, with both shorter and lighter roasting giving higher AA levels.

Lantz and co-workers also studied the kinetics of the formation of AA in coffee samples over 90–720 s of roasting to different roasting degrees (measured by light reflectance, LRU index) in three roasters: A) a fluidized bed roaster with mechanically supported coffee beans movement, and a green coffee batch size of 2 kg (Probat RT 3SY/Emmerich/Germany); B) a rotating fluidized bed roaster with heat transfer by convection, and a batch size of 2 kg (Neuhaus Neotec RFB6/Reinbek/Germany); and C) a drum roaster with heat transfer mainly by conductivity, and a batch size of 0.5 kg (Probat PRG500/Emmerich/Germany). The authors concluded that the maximum level of AA, independently on the roaster, is formed early during the heating process and then decreases with increasing roasting time and degree (403 µg/kg of AA at LRU > 95 (very light) after 135-150s, while it is absent at LRU < 65 (very dark) after 670 – 870 s) (Lantz et al., 2006).

Studies using deuterium-labeled AA that was spiked into green coffee beans confirmed that the amount of AA increases exponentially at the onset of roasting, reaching an apparent maximum of 2000 µg/kg, and then decreases rapidly as the rate of degradation exceeds the rate of formation (Alves et al., 2010). Very high levels of AA were detected in the lightest roasted coffee samples, with maximums of 1240 and 2190 µg/kg, for Arabica and Robusta, respectively. The concentration of the undesired molecule decreased proportionally, in the two species, with the increase of roasting degree in both ground coffee and espresso brews. Table 1 lists the studies on the impact of roasting conditions on AA levels in roasted coffee, as reported in the literature.

Table 1: Studies on the impact of roasting conditions on acrylamide levels in roasted coffee.

AA Robusta levels	AA Arabica Roasting levels	Roasting	Roasters	References
>3500 µg/ kg	<500 µg/kg	250 ◦C at 7,5min	Laboratory roaster	(Bagdonaite & Murkovic, 2004)
378 µg/kg	251 µg/kg	Roasting for 2.5 min to reach a medium roasting degree (T not reported)	Fluidized bed roaster Rotating fluidized roaster Drum roaster	(Lantz et al., 2006)
>250 µg/kg	>200 µg/kg	236 ◦C in 7,2 min (Robusta) 236 ◦C in 6,2 min (Arabica)	Laboratory roaster	(Summa, de la Calle, Brohee, Stadler, & Anklam, 2007)
708 µg/kg	374 µg/kg	Roasting at 240 ◦C for 7.5 min	Oven	(Bagdonaite, Derler, & Murkovic, 2008)
1.71–2.92 µg per cup (30 mL) Espresso	0.87–1.52 µg per cup (30 mL) Espresso	Roasting at 210 ◦C for 10 min	Probat roaster	(Alves, Soares, Casal, Fernandes, & Oliveira, 2010)
	468 µg/Kg	At 220 ◦C in 5 min	Oven	(G¨okmen, Kocadağli, G¨oncüoğlu, & Mogol, 2012)
	1100 µg/kg	167 ◦C at 22 min	Probat roaster	(Madihah, Zaibunnisa, Norashikin, Rozita, & Misnawi, 2013)
37.6 µg/kg		203 ◦C	Pilot roaster	(Budryn, Nebesny, & Oracz, 2015)
	130–470 µg/kg	Six different roasting profiles	Laboratory roaster	(Lachenmeier et al., 2019)
795 µg/kg	1045 µg/kg	175–177 ◦C at 10 min	Horizontal rotating durum machine	(Bertuzzi, Martinelli, Mulazzi, & Rastelli, 2020)
495 µg/kg	348 µg/kg	Light roasting	Probatino rotary drum roaster	(Esposito et al., 2020)

136 µg/Kg	109 µg/kg	191 ◦C in 18 min (Robusta) 191 ◦C in 15 min (Arabica)	Pilot plant roaster	(Schouten et al., 2021)

Kocadagli (Kocadagli et al., 2012) discussed the kinetics of the formation of AA from HMF, which reduces with increasing roasting degree, and Lachenmeier *et al.* (Lachenmeier et al., 2019) confirmed this behaviour for AA only. They explained these results using different roasting conditions: i) Kocadagli: oven at 220°C for 5-10-20-30-60 min; ii) Lachenmeier: laboratory roaster using six different roasting profiles, namely coffee roasting (fast and slow drying), espresso roasting (fast and slow drying), Scandinavian roasting (very light roasting) and Neapolitan roasting (very black roasting).

However, the roasting process is often carried out in small-scale roasters at fixed temperatures. Bertuzzi *et al.*, (Bertuzzi et al., 2020) investigated trends in AA content using an industrial coffee roasting process from 90°C to 215°C for 16 min. Quite surprisingly, the authors found the maximum AA level in Arabica. They hypothesized that this may be due to the higher concentration of sucrose and reducing sugars in Arabica compared to Robusta. During roasting, reducing sugars tend to initially increase because of the thermal hydrolysis of sucrose, and then to decrease due to AA formation after 10 minutes (Bertuzzi et al., 2020). Most studies focus on the dominant AA formation during the first period of roasting and its decrease with the intensification of the thermal process. Conversely, few studies have reported data on AA evolution over prolonged roasting times. Pastoriza *et al.*, (Pastoriza, Rufián-Henares, & Morales, 2012) suggested that the decrease in AA may be due to its chemical interaction with melanoidins, whose concentration increases with roasting time and that seem to act as modulators of AA levels. AA continuously decreases at 180°C from 6 minutes of roasting, compared to control samples, probably because of its thermal decomposition. The AA decrease was found to be dose-response and related to the reaction time and initial amount of melanoidins in the media. By contrast, pH (from 3.5 to 7.0) did not have a significant effect on AA reactivity with melanoidins. AA reduction was hypothesized to be due to its reaction with the nucleophilic amino groups of amino acids from the protein backbone of melanoidins, via Michael addition, although the exact mechanism is still unknown. The addition of soluble melanoidins to the brew seem to modulate the content of

AA. Badoud *et al.*, (Badoud et al., 2020) investigated the routes of AA degradation with ^{14}C-labeled and stable isotope ^{13}C-labeled materials, and found that approximately 30% of AA was lost to volatilization, and 70% remained in the matrix, of which only 50% was in the free soluble form.

The importance of the roasting process on flavour and colour, and the relatively narrow range for commercial products make AA mitigation in coffee particularly complex.

Indeed, although darker roasting is a potential option to reduce AA, it generates other undesirable compounds, i.e. furans, furfuryl alcohol and HMF, and which definitively affect the final taste.

5.1 Storage of roasted coffee

Several studies have demonstrated that AA is not stable during the storage of packed roasted coffee; with stability depending on time, temperature and the atmosphere inside the package.

Delatour *et al.*, (Delatour, Périsset, Goldmann, Riediker, & Stadler, 2004) reported a reduction in AA, from 771 to 256 µg/kg and from 203 to 147 µg/kg, for soluble and roasted coffee, respectively. The soluble coffee powder was stored at room temperature and in its original tightly closed container for 12 months while the roasted coffee was stored under these same conditions for a period of 7 months. Andrzejewski *et al.*, (Andrzejewski, Roach, Gay, & Musser, 2004) have observed significant losses of AA (40–65%) after 6 months during the secondary shelf-life of coffee, i.e. when the package is opened for home consumption. The content of AA was also measured at -40°C to check whether this loss was related to the temperature. Results indicated that the AA amount at -40°C did not change for 8 months when the same sample of roasted and ground coffee was stored in its original open container, and suggested that AA loss over time only occurs in ground coffee with open containers stored at room temperature. The storage of roasted coffee in open container obviously heavily affects its flavour producing a staling effect and speeds up oxidation processes (Manzocco, Calligaris, Anese, & Nicoli, 2016).

Hoenicke *et al*. (Hoenicke & Gatermann, 2005), however, reported a smaller AA reduction from 305 to 210 µg/kg for roasted and ground coffee and from 285 to 200 µg/kg for roasted beans, after 3 months of storage at 10 – 12°C in sealed vacuum-packs. Under the same

conditions, AA was shown to be stable in soluble coffee and in the extracts of coffee substitutes.

AA decrease has been related to temperature. In 2006, Lantz (Lantz et al., 2006) found that there is a clear proportional and temperature dependent decrease in AA levels in vacuum-packed ground and roasted coffees that were stored for 12 months at temperatures between -18 and 37°C. As expected, the most significant AA reduction and rate were registered in the samples stored at the highest temperature (37°C), with this process following second order reaction kinetics. In 2008, Baum (Baum et al., 2008) carried out studies with ^{14}C-labeled AA as a radiotracer on roasted and ground coffee to define the fate of AA that was lost during storage. Coffee samples were spiked with the ^{14}C AA and stored for 48 weeks at room temperature and at 37°C, and the ^{14}C AA was measured in the coffee brew, filter residue and volatiles. Total radioactivity decreased in the brew over storage and, in particular, at 37°C, and increased in the filter residue concomitantly. No formation of volatile ^{14}C-AA-related compounds was detected during storage and coffee brewing. Approximately 90% of the radiolabelled AA in the filter residue (spent R&G coffee) remained tightly bound to the matrix. Michalak *et al.* (Michalak, Gujska, Czarnowska, Klepacka, & Nowak, 2016) also confirmed the results of Delatour *et al.,* (Delatour et al., 2004) as they reported AA reductions of 33% and 28% in instant coffee and coffee substitutes respectively, in storage at 25°C after 12 months, and a less significant decrease at 4°C.

Hoenicke *et al.* suggested, in 2005, that AA losses over time probably occur because of reactions with other components in coffee beans and powders. Reactions with compounds containing SH groups may have a significant impact on AA reduction during storage. In general, the high reactivity of AA with nucleophilic components, such as the sulfhydryl, amino and hydroxyl groups of peptides, proteins and melanoidins, might be responsible for its reduction in stored coffee. AA is therefore rather stable in foods such as cereal-based products because they do not contain sulfur derivatives (Hoenicke & Gatermann, 2005; Michalak et al., 2016). However, experiments with ^{14}C-labeled AA as a radiotracer have shown that furanthiol, which is an abundant aroma component in roasted coffee, was not involved in the formation of covalent AA adducts and thus does not substantially contribute to decreases in AA during storage. Table 2 lists the studies that are available in the literature on

AA decreases in roasted coffees and coffee products during storage under different conditions.

Table 2: Studies on the decrease of acrylamide in roasted coffee and coffee products during storage under different conditions.

Roasted coffee type	Packaging	Storage condition	AA loss	References
Ground	Opened original package	Room T at 6 months	40–65%	(Andrzejewski, Roach, Gay, & Musser, 2004)
Ground and soluble	Opened original package	Room T at 7 and 12 months respectively	28% and 66% respectively	(Delatour, P´erisset, Goldmann, Riediker, & Stadler, 2004)
Beans and ground	Closed vacuum package	10–12 ◦C at 3 months	30% and 31% respectively	(Hoenicke & Gatermann, 2005)
Ground	Closed vacuum package	37 ◦C at 12 months	88%	(Lantz et al., 2006)
Soluble and coffee substitutes	Closed package	25 ◦C at 12 months	33% and 28% respectively	(Michalak, Gujska, Czarnowska, Klepacka, & Nowak, 2016)

6. BREW PREPARATION

While the majority of published studies focus on the assessment of AA content in coffee beans, some researchers have also investigated the amount of AA that is effectively ingested by consumers in their coffee brews (Alves et al., 2010; Andrzejewski et al., 2004; Bagdonaite et al., 2008). AA intake through coffee beverages depends on consumption habits (type, strength and volume of beverage, and intake frequency), which are influenced by the cultural and personal preferences of consumers. Coffee is ground into powder, with the objective of increasing the surface of the interface between the water and the solid to accelerate the transfer of soluble substances into the brew (Soares, Alves, & Oliveira, 2015). AA is highly soluble in water and is thus easily transferred from the coffee powder to the beverage. Three main processes are used to prepare coffee brews (Figure 2B), decoction (boiled, Turkish,

vacuum and percolation), infusion (filter or coffee drip and Neapolitan), and *pressure* (press-pot or French press, moka and espresso). Most of these methods can be identified by their geographical denomination rather than the description of the method itself and are linked to local traditions. Moreover, instant coffee or soluble coffee powder, is also included in this section since it is one of the most widely consumed beverages and shares the extraction of bean components, with the only difference being that this occurs in the technological industrial brewing step before the soluble coffee powder is obtained.

During brew preparation, AA extraction can be affected by factors such as the temperature of the water, the time that the water is in contact with the ground coffee and the applied pressure. In any case, AA is almost completely extracted, in proportions of around 92 to 99% because of its high polarity and water solubility (Alves et al., 2010; Andrzejewski et al., 2004; Bertuzzi et al., 2017). AA levels between 6 and 16 μg/L were found in brewed coffee prepared using an electric drip coffee maker by measuring the variation when the brew was heated in the coffeepot over time. The results showed that AA is quite stable in brewed coffee, since no significant decreases in its levels were observed after 5 hours of heating (Andrzejewski et al., 2004). Similar results were also found by Alves (1.7 to 75 μg/L), Sirot (37 μg/L), and Mesías (7.7 to 40 μg/L) (Alves et al., 2010; Mesías & Morales, 2016; Sirot, Hommet, Tard, & Leblanc, 2012). In 2006, Lantz *et al.* reported that espresso-coffee brewing only partially extracts AA from ground coffee due to the short contact time with water, unlike with other coffee brewing methods, such as the plunger pot and filtered coffee (Lantz et al., 2006). Alves *et al.*, (Alves et al., 2010) found that the AA extraction rate using the percolation method was very similar for both Arabica and Robusta, and that the increase in the water volume that percolates through the coffee cake is responsible for higher AA extraction, ranging from 59 to 98% for Robusta, and from 62% to 99% for Arabica, with volumes of extract ranging from 20 (Italian "ristretto" coffee) to 70 mL (Italian "lungo" coffee). Although the short contact time results in incomplete AA extraction during espresso coffee preparation, the high coffee/water ratio leads to higher AA concentration than in other coffee brews (Mesías & Morales, 2016; Soares et al., 2015). The ever-increasing success of coffee capsules has brought attention to AA contents in the resulting brews. Alves, however, did not find significant differences between espresso caps and conventional espresso (33.4–55.3 μg/L). Furthermore, they found similar AA contents in decaffeinated coffee (24.8–49.5 μg/L), confirming that the decaffeination process does not influence acrylamide precursors (Alves et al., 2010).

Başaran *et al.*, (Başaran, Aydın, & Kaban, 2020) analysed 41 commercial coffees that were obtained from local markets and coffee shops, and found that instant coffee contained higher levels of AA, than traditional Turkish coffee and ready-to-drink (brewed) coffee. The reason for the high amount of AA in instant coffees may be due to their industrial processing, as they are brewed with pressurized liquid water at approximately 175°C. Further evaporation processes, including freeze-drying and spray-drying, concentrate the coffee components including AA (Mussatto, Machado, Martins, & Teixeira, 2011).

Kang *et al.*, (Kang, Lee, Davaatseren, & Chung, 2020) investigated the presence of AA in cold and hot brews; cold brews were prepared at 5°C and 20°C for 12 h using steeping and dripping, whereas hot brews were obtained at 80°C and 95°C for 5 min using the pour-over method. Cold brews showed higher levels of AA than hot brews, probably because of the relatively longer contact time with water. The brewing time and, thereby, the water/coffee ratio, the blend composition and roasting degree all significantly influence the level of AA in the final beverage. AA intake through coffee brews therefore essentially depends on consumption habits (type, strength and volume of beverage, together with intake frequency), which vary with cultural and consumer preferences. Table 3 reports literature studies on the impact of brewing techniques and conditions on AA levels in final coffee beverages.

Table 3 Impact of brewing techniques and conditions on acrylamide levels in final coffee beverage.

Coffee Brew	Acrylamide levels in beverages (µg/ L)	References
French press cafetiere	9	(Granby & Fagt, 2004)
Filter coffee	6–16 8 50	(Andrzejewski et al., 2004; Granby & Fagt, 2004; S¸enyuva & G¨okmen, 2005)
Turkish coffee	29–75	(S¸enyuva & G¨okmen, 2005)
Instant coffees	42–338	(S¸enyuva & G¨okmen, 2005)
Espresso coffee	11.4–36.2	(Soares, Cunha, & Fernandes, 2006)
Moka coffee	16.6	(Sagratini et al., 2007)
Espresso coffee Decaffeinated coffee	33.4–55.3 24.8–49.5	(Alves et al., 2010)
Coffee brews from vending machine	7.7 to 40	(Mesías & Morales, 2016)
Instant coffee, Traditional turkish coffee Ready-to-drink coffee	16.5 to 79.5 5.3–54.8 5.9 to 38.8	(Bas¸aran, Aydın, & Kaban

7. ACRYLAMIDE MITIGATION STRATEGIES

To reduce AA intake, the food industry has tried to change processes and/or product parameters without compromising taste, texture and appearance of their products (Food and Drink Europe, 2019; Pedreschi et al., 2014; Schouten et al., 2021, 2020). Many mitigation techniques can be adopted, at different steps during coffee processing (Figure 3).

Figure 3: Options for reducing acrylamide amount in final coffee beverages.

7.1 Enzymatic treatment of green beans

The formation of AA in coffee can be limited by two enzymatic treatments: i) with asparaginase, which catalyses the hydrolysis of Asn into Asp and ammonia via the hydrolysis of the Asn side-chain amide group (Corrêa et al., 2021); and ii) with acrylamidase, which can convert AA into acrylic acid (Cha, 2013).

As free Asn is a limiting factor for AA formation in coffee, some authors have studied the possibility of limiting this component in green coffee using asparaginase, and thereby reducing AA formation during roasting (Mottram et al., 2002). A patented enzymatic treatment WO/2004/037007, that is based on the asparaginase method was revealed by The Procter & Gamble Company as a means to reduce the AA content in roasted coffee. However, the complexity of the preliminary treatments that must be performed on the green coffee beans to ensure an effective interaction between the enzyme solutions and the Asn contained in the beans is a significant drawback. Hendriksen *et al.* (Hendriksen, 2013) evaluated the

effect of different doses of asparaginase on the reduction of Asn levels in green coffee. The results indicated that treating green coffee beans with low doses of asparaginase (2000–6000 ASNU) produced a 70–80% decrease in Asn and a 55–74% decrease in AA after roasting. A major obstacle here is ensuring the homogeneous distribution of the active enzyme over the entire substrate.

A number of techniques improve the contact between the enzyme and coffee substrate. Pre-treatment can facilitate the extraction and contact between Asn and asparaginase, promoting its migration into the beans. Dria *et al.*, (Dria et al., 2007) listed a series of pre-treatments for this, including drying, hydrating, rinsing with or without mechanical action, pressurizing, steaming, blanching, heating, reduced-pressure processing and particle-size reduction. These processes were very often not verified, even for sensory impact, and are not applicable at the industrial level (Anese, 2016). In addition, Navarini *et al.* in 2014 patented a method to reduce AA enzymatically in a water extract of green beans. The enzymes used in this method were asparaginase and aspartase in solution. The authors found that Asn and Asp are present in similar concentrations in the extract and that Asp contributes to the formation of AA, although in lower amounts compared to Asn. After enzymatic treatment, the water extract was re-incorporated into the green beans before roasting. This treatment gave an AA reduction of about 70%, without affecting the sensory properties of the final brew (Navarini et al., 2014). This hypothesis, although of interest because in addition to the AA reduction do not seem to affect coffee sensory properties, has not been demonstrated in peer reviewed article(s). It is also contrasted by several authors who report that the effect of Asp on AA formation is negligible, and who strongly support the correlation between Asn and AA (Belitz et al., 2009; Dias et al., 2012; Guenther et al., 2007; Schouten et al., 2020). In 2019, Porto *et al.* (Porto, Freitas-Silva, Souza, & Gottschalk, 2019) treated Arabica and Robusta coffee beans with asparaginase, and obtained Asn reductions of approximately 60% and 35%, respectively. The beans were pre-treated for 30-45 minutes with steam to open the pores and favour the enzymatic process. In the same way, Corrêa *et al.*, (Corrêa et al., 2021) have shown that the pre-treatment of Arabica coffee beans with steam improved the results of asparaginase treatment, with an AA reduction close to 59%, compared to the control sample, and 77% compared to the blank sample.

7.2 Mitigation using roasting strategies

Some authors have proposed optimizing the roasting process to mitigate AA content, with the aim of finding the best conditions to obtain both the desired roasting degree and lower AA concentrations. Madihah *et al.* (Madihah, 2013) optimized the roasting time and temperature conditions for Arabica coffee beans, and found the optimal conditions to be 168°C for 22 min. Under these roasting conditions, a low amount of AA was formed (1110 µg/kg) with a score of the overall sensory evaluation of the brews of 7.5 out of 10 points.

Esposito *et al.* optimized roasting conditions on an industrial scale for Arabica and Robusta in order to fulfil the requirements of taste and aroma, as well as to reduce the concurrent AA formation. They found that, with the proper set up of roasting conditions, AA concentration can be reduced in Robusta samples by between 20% and 90%. However, the roasting degree was measured colorimetrically without any sensory evaluation of the final products (Esposito et al., 2020).

Alternative roasting technologies have been attempted. Theurillat *et al.* (Theurillat, Leloup, Liardon, Heijmans, & Bussmann, 2006) evaluated the use of a steam/pressure roasting pilot plant unit. Results showed that the steam treatment carried out on green and roasted coffee did not significantly influence the final AA content in either of the final roasted samples.

Anese *et al.*, (Anese et al., 2014) subjected coffee beans to a medium-roasting process under reduced pressure conditions leading to a reduction in AA levels of 50% compared to conventionally roasted coffee, and a minimal impact on sensory properties. The low-pressure conditions generated inside the roaster, which exerted a stripping effect, preventing AA from being accumulated. Nevertheless, this AA-mitigation strategy is probably not of general interest as coffee roasted to a medium degree is almost only consumed in the American and Northern European markets.

Budryn *et al.*,(Budryn, Nebesny, & Oracz, 2015) studied AA formation upon the roasting of Robusta samples with air at different speeds, humidity, time and temperature. These roasting conditions resulted in lower AA formation when air velocity was decreased at temperature in the range of 190–216 °C and air humidity was increased at higher temperatures (e.g., at 216 °C). A relatively low AA level (0.0376 µg/g) was found in coffee samples roasted at 203°C, although polyphenols underwent moderate deterioration.

Guenther *et al.* found that saturated steam roasting can reduce AA content by up to 10%. However, the process had a negative impact on the taste and aroma of the coffee (Guenther et al., 2007).

Rattanarat *et al.*, (Rattanarat, Chindapan, & Devahastin, 2021) studied the effect of superheated steam (SHS) roasting on the formation and reduction of AA, and, interestingly, found that SHS roasting resulted in lower AA content in medium- (∼16%) and dark-roasted (∼25%) beans at 250°C. Nevertheless SHS, used as an alternative to roasting, impacted upon the flavour of (Robusta) coffee, producing brews with higher sweetness and citrus-like acidity (Chindapan, Soydok, & Devahastin, 2019).

7.3 AA removal from roasted coffee beans and brews

All methods and proposed technologies should, of course, also be tested for their impact on the sensory quality of the final product and feasibility from an industrial point of view before being adopted. Banchero *et al.,* (Banchero, Pellegrino, & Manna, 2013) proposed the use of supercritical CO_2 to remove AA from roasted coffee. The efficiency of AA removal ranged from 8% to 45% at an extraction time of 525 min, and increased to 79% after 22 hours. Changes in pressure did not affect the results, but temperature was the variable that drove the extraction process. Furthermore, the addition of ethanol (up to 9.5% w/w) changed the polarity of the supercritical solvent mixture, resulting in an increase in extraction performance. The most effective operative conditions were found to be 100°C, 200 bar and 9.5% w/w ethanol.

Cha (Cha, 2013) reported a technique that can remove AA from brews using bacterial enzymes at relatively high temperatures. Extracts from *Ralstonia eutropha* AUM-01 and a thermophilic strain, *Geobacillus thermoglucosidasius* AUT-01, were used to remove 50% of AA from coffee brews. In 2016, Anese *et al.* (Anese, 2016), proposed using acrylamidase to hydrolyse AA to acrylic acid, which is less toxic than AA, but corrosive for the skin and mucosa, and ammonia. However, this process may affect the brew's sensory profile. Akillioglu *et al.* (Akillioglu & Gökmen, 2014) proposed a mitigation method for AA in instant coffee that is based on baker's yeast (S*accharomyces cerevisiae*, 1–2%, w/v) mixed with sucrose (0–10, w/v). The mixture was fermented at 30°C for 48h with an AA concentration decrease of about 70%. The results revealed that both the sucrose and yeast concentrations affected the AA mitigation during

fermentation and that its reduction was due to the effect of metabolic conversion via yeast metabolism.

Using immobilized enzymes is a further possibility for minimizing AA content. Bedade *et al.,* (Bedade, Sutar, & Singhal, 2019) have proposed immobilizing bacterial acrylamidase from *Cupriavidus oxalaticus* ICTDB921 on chitosan-coated alginate beads. The immobilized acrylamidase has an optimal pH/temperature of 8.5/65 °C, showed improved pH/thermal and shelf stability and retained 80% activity after four cycles. They applied it to instant coffee with complete AA degradation after 60 min of treatment, starting from an initial concentration of 100–500 µg/L. The authors successfully tested the immobilized acrylamidase in both batch and continuous operations on a packed column for the effective AA removal from a roasted instant coffee solution, although some limitations in continuous operation, which were linked to column performance, were found.

8. CONCLUSIONS

The intake of AA from coffee and coffee products has been widely discussed in the literature. However, not many studies on possible AA mitigation are available. A number of strategies and approaches (Figure 3) that the coffee industry may use to mitigate AA levels in their final products are currently available (Food and Drink Europe, 2019), they include:

- selecting good quality green coffee and removing poor quality beans
- favouring Arabica over Robusta coffees
- roasting at the highest thermal input (dark degree)
- storing roasted coffee for a long time
- favouring shorter coffees brews over longer ones.

In particular, several articles have reported that darker roasted coffees are characterized by lower AA contents than light and medium ones, due to AA degradation during processing. Nevertheless, the reduction of AA in darker roasted coffee may not be a generally applicable solution as this type of coffee is mainly appreciated from the Southern European consumers, in contrast to Northern European and American consumers who prefer lighter roasted products (Schouten et al., 2020). In addition, stronger roasting can increase the formation of other toxic substances (i.e. furans).

The applicability of the asparaginase enzyme in the treatment of green coffee is limited due to the poor permeability of the green beans and the additional processing steps required (steam treatment and soaking in a water bath) for enzyme effectiveness. Moreover, this treatment influences the sensory properties of coffee and therefore cannot be expected to become a generalized AA mitigation process in coffee production. However, this technique should be evaluated on a case-by-case basis according to the origin of the green coffee, the amount of enzyme to be used and the desired quality of the final product.

Although some innovative strategies for AA reduction have been proposed and may be of interest, including roasting in modified environments, vacuum or superheated vapor, and the use of bacterial enzymes to remove AA from brews, they still need to be tested at an industrial level. Moreover, can lead to changes in aroma composition, not only affecting the quality of the product, but also its acceptance by consumers. However, most research on AA mitigation fails to report completely exhaustive information and some of them are also contradictory (i.e. Bertuzzi et al., 2020). In particular, there is a significant lack of knowledge on the effect of agricultural practices and geographical origins on AA precursors. Finally, most processes are studied in laboratory/pilot plants and the scaling-up conditions and sustainability of these processes are still to be investigated.

It is therefore necessary that studies be expanded on all aspects and that the link between origin and AA quantity is investigated. For instance, the effect of climate change and how it impacts agronomical and primary processing practices and AA precursors requires attention. In this respect, varietal improvement might also be guided by potential reductions in AA formation, while maintaining the sensory quality of the product. In conclusion, further studies are needed to find appropriate and practical solutions for AA mitigation in coffee and to study the health implications of AA in complex mixtures, such as coffee brews. A recent review by Nehlig and Cunha (Nehlig & Cunha, 2020) highlighted how most toxicological studies are carried out on pure AA and on animals, while studies that directly evaluate the effects that AA in foods have on human health have not provided direct evidence of carcinogenic effects. In addition, the risk to human health from AA depends on the conditions of exposure, i.e., the kinetics of adsorption, distribution and excretion in the human body, while this kinetic-dynamic profile is also related to the other constituents of coffee and more in general to the human diets. The mitigation strategies proposed so far to meet the EU precautionary principle on food safety, are devoted to taking appropriate measures to reduce the presence of AA to

as low as reasonably achievable (ALARA). This view also needs to take into account other factors, such as potential risks from other contaminants and/or synergy or competition with other components in the brew, the sensory properties and quality of the final product, and the feasibility of any process, in terms of both application at industrial level and costs.

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LIST OF ACRONYMS

AA	Acrylamide
Ala	Alanine
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
Cys	Cysteine
3-DG	3-Deoxyglucosone
Gln	Glutamine
Glu	Glutamic acid
Gly	Glycine
HMF	5-Hydroxymethylfurfural
LRU	Light reflectance units
Lys	Lysine
Phe	Phenylalanine
R&G coffee	Roast and Ground coffee
Ser	Serine
SHS	Saturated steam
Trp	Tryptophan

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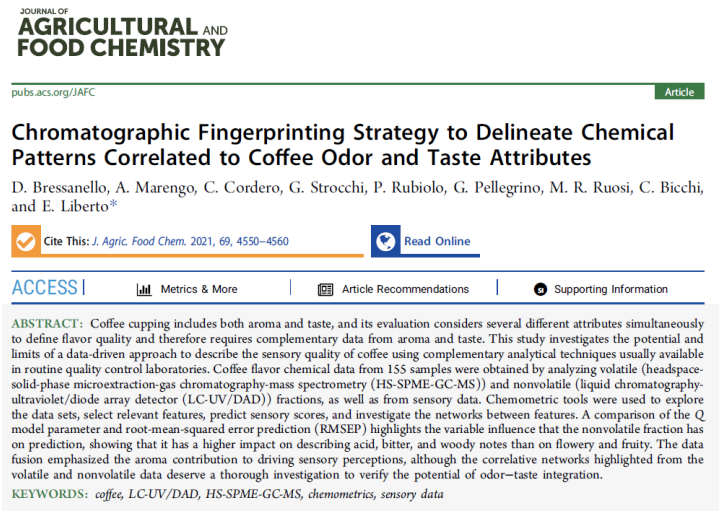
SUPPLEMENTARY

Table S1: Monovarietal commercial roasted samples of different origin available in local supermarkets adapted from Alves et al., 2010 and Pugajeva et al., 2015.

Pugajeva et al., 2015			**Alves et al. 2010**			
Origin/brand	**Variety**	**Acrylamide content (µg/Kg)**	**Origin**	**Variety**	**Color**	**Acrylamide content (µg/L)**
Costa Rica, Tarrazu	Arabica	185	Hawaii	Arabica	132	29.1±0.8
Santo Domingo, Organico	Arabica	166	Costa Rica	Arabica	127	38.0±0.3
India monsooned Malabur	Arabica	453	Jamaica	Arabica	126	33.3±0.2
Nicaragua Maragogype	Arabica	260	Colombia	Arabica	122	32.7±0.2
Colombia	Arabica/dark	182	Ethiopia	Arabica	128	44.6±1.5
Colombia	Arabica/light	179	Honduras (1)	Arabica	123	49.4±0.5
Brazil Santos	Arabica/dark	218	Honduras (2)	Arabica	130	50.8±1.2
Brazil Santos	Arabica/light	196	Brazil	Arabica	138	32.8±0.3
India Plantation	Arabica/light	299	India (1)	Robusta	118	56.9±1.4
India Plantation	Arabica/dark	272	India (2)	Robusta	133	84.1±3.4
Blend Triage Arabica	Arabica	408	Uganda (1)	Robusta	122	75.5±0.8
Ethiopian Sidamo	Arabica	267	Uganda (2)	Robusta	122	73.5±1.4
Vietnam	Robusta	361	Cameroon (1)	Robusta	124	80.5±1.5
Indonesia Java	Robusta	503	Cameroon (2)	Robusta	132	89.4±3.7
Vietnam wet polished	Robusta	283	Ivory Coast	Robusta	131	97.3±3.9
			Indonesia	Robusta	125	58.0±0.2

2.2 Aroma quality

2.2.1 Chromatographic Fingerprinting Strategy to Delineate Chemical Patterns Correlated to Coffee Odour and Taste Attributes



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Chromatographic Fingerprinting Strategy to Delineate Chemical Patterns Correlated to Coffee Odor and Taste Attributes

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ABSTRACT: Coffee cupping includes both aroma and taste, and its evaluation considers several different attributes simultaneously to define flavor quality and therefore requires complementary data from aroma and taste. This study investigates the potential and limits of a data-driven approach to describe the sensory quality of coffee using complementary analytical techniques usually available in routine quality control laboratories. Coffee flavor chemical data from 155 samples were obtained by analyzing volatile (headspace-solid-phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS)) and nonvolatile (liquid chromatography-ultraviolet/diode array detector (LC-UV/DAD)) fractions, as well as from sensory data. Chemometric tools were used to explore the data sets, select relevant features, predict sensory scores, and investigate the networks between features. A comparison of the Q model parameter and root-mean-squared error prediction (RMSEP) highlights the variable influence that the nonvolatile fraction has on prediction, showing that it has a higher impact on describing acid, bitter, and woody notes than on flowery and fruity. The data fusion emphasized the aroma contribution to driving sensory perceptions, although the correlative networks highlighted from the volatile and nonvolatile data deserve a thorough investigation to verify the potential of odor−taste integration.

KEYWORDS: *coffee, LC-UV/DAD, HS-SPME-GC-MS, chemometrics, sensory data*

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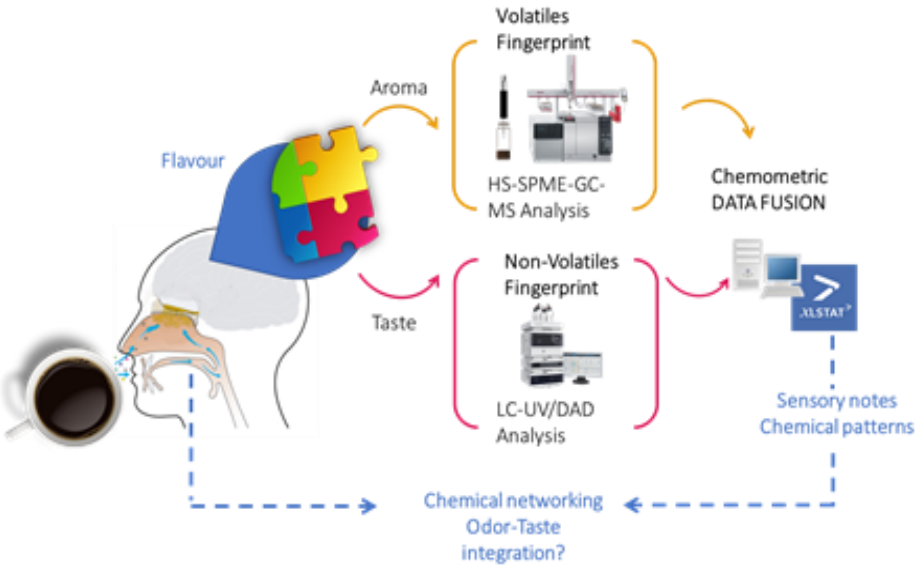
GRAPHICAL ABSTRACT



Chromatographic Fingerprinting Strategy to Delineate Chemical Patterns Correlated to Coffee Odor and Taste attributes

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ABSTRACT

Coffee cupping includes both aroma and taste, and its evaluation considers several different attributes simultaneously to define flavor quality therefore requiring complementary data from aroma and taste. This study investigates the potential and limits of a data-driven approach to describe the sensory quality of coffee using complementary analytical techniques usually available in routinely quality control laboratory. Coffee flavor chemical data from 155 samples were obtained by analyzing volatile (HS-SPME-GC-MS), and non-volatile (HPLC-UV/DAD) fractions, as well as from sensory data. Chemometric tools were used to explore the data sets, select relevant features, predict sensory scores and investigate the networks between features. A comparison of the Q model parameter and RMSEP highlights the variable influence that the non-volatile fraction has on prediction, showing that it has a higher impact on describing *Acid*, *Bitter* and *Woody* notes than on Flowery and Fruity. The data fusion emphasized the aroma contribution to driving sensory perceptions, although the correlative networks highlighted from the volatile and non-volatile data deserves a thorough investigation to verify the potential of odor-taste integration.

Keywords: Coffee, HPLC-UV, HS-SPME-GC-MS, chemometrics, sensory data

INTRODUCTION

The characteristics and sensory properties of coffee flavor are unique and of high appeal for consumers.[1] The pleasure system includes different brain areas that are linked to emotional, memory-related, motivational and linguistic aspects of food evaluation that are, in turn, mediated by several sensory modalities and sub-modalities that contribute to flavor perception.[1–3]

Flavor perception is therefore a complex sensation given by the interaction between aroma, perceived through the sense of smell [orthonasally and retronasally (food aroma)] and taste, perceived at the level of the oral cavity.[3,4] However, the sense of smell often dominates flavor perception, and aroma active compounds can modulate taste intensity.[1,3,5] Cross-modal interactions are therefore fundamental to delineating the hedonic profile of a food, and this has become a strategic route for industry in designing new food products and healthier formulations (e.g. with less salt or sugars).[6–11] On the other hand, taste-active compounds may influence the release of volatiles and impact upon their actual concentration in the headspace.[6]

The evaluation of coffee flavor is a key step of the production chain; from raw-material selection to the creation of new valuable blends. Coffee sensory quality is nowadays rated by cupping protocols that intrinsically satisfy the multimodal-perception concept. The sensory panel therefore plays a fundamental role in this respect.[12] The cupping protocol is however time-consuming, requires properly trained and aligned professional panelists, and may suffer from subjectivity. The ever-increasing consumption of coffee highlights the need for analytical techniques at supporting sensory panel evaluation in routine quality controls (QC) or formulation and design of new blends. Suitable instrumental methods should therefore: i) provide reliable information on coffee aroma and taste, including their possible interactions; ii) support a consistent prediction of key-sensory attributes in line with sensory panel outcomes; and iii) inform, at a molecular level, on the presence of key-analytes patterns within a quality control laboratory routine.

Several analytical approaches and/or integrated strategies attempted, over the years, to combine the chemical composition of a product with its flavor profile[13–22]; however, to date, they have not replaced a sensory panel evaluation, especially in regulatory contexts where sensory quality concur to define labelling (e.g., extra-virgin olive oil[22,23]) or a commercial value (e.g., coffee[13,15,17,18,21,24–26]).

The limited application of instrumental tools in the industrial context might be explained taking in consideration the breath and reliability – and thereby rate of success - of several approaches

modelled on set of samples with low/limited representativeness or trained by *a priori* defined lists of targeted chemical variables[15,19,24]. Issues related to their applicability might therefore be related to the attempt of reducing the extremely complex phenomenon of sensory perception, triggered by multiple ligands (volatiles and non-volatiles) and modulated by their cross-modal interactions, to a few correlations (*e.g.,* reductionist approach[27]) or to the adoption of spectroscopic/spectrometric technologies that have limited or not univocal "molecular resolution". A further point is the natural and reasonable skepticism of quality control decision makers to abandon, or replace, a normalized/established protocol in favor of alternative procedures.

In this context, modern omics disciplines dealing with food (sensomics[28], flavoromics[29] and food metabolomics[30]) can be of help suggesting more systemic approaches to the chemical interpretation of complex biological phenomena by untargeted investigations (*e.g.,* integrationist approach[27]). Many research provides proof-of-evidence on the potential of applying omics workflows and concepts to identify "features patterns" (i.e. patterns of potential informative components) with high correlation to a biological output.[31] Very recently, Nicolotti et al. [32] conceptually validated an "artificial intelligence smelling machine", an analytical workflow based on sensomics, that attempts to simulate human olfaction by accurately define key-odorants patterns responsible of the aroma of a food.

Moreover, machine learning applied to fingerprinting and/or profiling technologies highlighted strong relationships and networking between aroma and flavor. In a meta-analysis study on various food aroma and flavor, Dunkel *et al.*[28] evidenced several groups of odorants, validated by sensomics, with specific associations to odor notes, and showed that the networking of odor notes might open up possibilities in the exploration of these associations. Tromelin *et al.* [33] found potential similarities and links between odorant and odor spaces using a multivariate-driven approach on a large odorant database, while Wang *et al.*[34] confirmed that odorant and non-odorant compounds interact in the expression of a perceived sensory attribute. Very recently, Guichard *et al.*[35] investigated odor-taste networks in commercial multi-fruit juices using cheminformatics, and showed that network visualization link between odor (green, grass, vegetal) and taste (bitterness) descriptors had strong associations. Barba *et al.*[36] demonstrated that odorants enhancing targeted taste perception might be exploited to modulate overall taste profile in foods and beverages.

In this complex and intriguing scenario, starting by preliminary results obtained by applying *omics* principles to the modelling of specific coffee aroma notes[15], this study is a step forward in evaluating

chromatographic fingerprints of volatile and non-volatile components as diagnostic signatures with strong correlation with selected taste and aroma attributes, i.e. *bitterness*, *acidity*, *flowery*, *fruity*, *woody*, *and spicy*.

Moreover, fingerprinting is combined to machine learning, by partial least squares (PLS) algorithms, and extended to a comprehensive data matrix obtained by combining together peak features information deriving from volatiles and non-volatiles. PLS drives features selection toward those informative patterns capable of predicting sensory attributes and explaining correlations between them. Analytical platforms for fingerprinting were selected in light of routine control laboratories requirements for high batch-to-batch reproducibility, separation efficiency and confirmatory potentials. Selected coffee powders were analyzed for their volatile fingerprints by headspace solid phase micro extraction followed by gas chromatography mass spectrometric detection (HS-SPME-GC-MS), and for their non-volatile fingerprints from the corresponding brews analyzed by liquid chromatography with UV/DAD detection (LC-UV/DAD) to simulate the main phases of the cupping protocol according to the Specialty Coffee Association (SCA)[37] that evaluates both smell and taste, and here used to describe samples sensory notes.

MATERIALS AND METHODS

Samples and chemicals

Samples, consisting of roasted and ground coffees to suit a coffee-filter machine, were kindly supplied over a period of 24 months by Lavazza Spa (Turin, Italy). The grinder was a Superjolly grinder with a stepless micrometrical grinding adjustment by Mazzer (Venice, Italy), the particle size average was: 425 µm ± 75 µm and percentages dust (% of particles below 100 µm): 13% ± 3%.

Mono-origin samples from different countries were selected for their distinctive and peculiar sensory notes, they accounted for a total of 155 samples belonging to *Coffea Arabica* L. (Arabica,

n= 85) and *Coffea canephora* Pierre ex- A. Froehner (Robusta, n= 70) species. Table S1 reports details on all analyzed samples. The roasting was carried out on a laboratory Probat BRZ2 drum roaster (Emmerich am Rhein, Germany) by applying optimized protocols. A 150 g of coffee beans were roasted at 200°C for 8-12 min until reaching a color of 55°Nh (Neuhaus degrees) in line with the international standardization protocol for cupping (SCAA protocol).[37] Coffee color was carefully measured by ground-bean light reflectance, with a single-beam Neuhaus Neotec Color Test II

instrument (Genderkesee, Germany) at a wavelength of 900 nm. Samples were roasted no more than 24 hours prior to cupping and left for at least 8 hours to stabilize as indicated by the protocol.[37]

The coffee brew for cupping and analysis was prepared from 18 g of coffee powder and 300 mL of water at 88-94°C with a commercially available coffee filter machine Xlong TSK-197A (Lavazza Spa, Turin, Italy). Two milliliters of brew were then filtered using a 0.2 µm 13 mm nylon membrane syringe filter (Agilent, Little Falls, DE, USA) and 20 µL were directly injected for LC-UV/DAD analysis.

LC-grade acetonitrile (LC-MS grade) and formic acid (>98% purity) were obtained from Merck while de-ionized water (18.2 MΩ cm) was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA).

Cryptochlorogenic acid; 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid were obtained from Phytolab (Vestenbergsgreuth, Germany). Chlorogenic acid, neochlorogenic acid, 3,4-dicaffeoylquinic acid, trigonelline and caffeine were obtained from Sigma Aldrich (Bellefonte, USA).

Normal alkanes, ISTD (*n*-C13), dibutyl phthalate and reference compounds for identity confirmation of volatiles reported in Table S2 (a) were all from Merk (Milan, Italy).

Sensory analysis of coffee samples

Cup quality was assessed for several flavor attributes: *Acid*, *Bitter*, *Flowery*, *Fruity*, *Spicy,* and *Woody* by an external trained panel of six assessors. The intensities of each attribute were evaluated simultaneously on a scale from 0 to 10. The sensory data provided by the external panel had already been verified at the origin by the ANOVA analysis with a *post-hoc* test. Average scores from the panel were used for the investigated attributes.

Coffee sensory properties were evaluated both by sniffing the powder and the brew obtained using the filter method, and by tasting aspiring the beverage into the mouth.[37] This multistep protocol allows panelists to evaluate different attributes, with some being more closely linked to aroma (sensory notes like *flowery*, *fruity*, *woody* and *spicy*) and others more closely to taste (*acidity* and *bitterness*).

Volatile fingerprints: sampling and analysis conditions

The fingerprint corresponding to the volatile fraction of coffee, including also aroma active compounds, was obtained on dry roasted and ground coffee powders by HS-SPME followed by GC-MS analysis. SPME sampling was performed by a Combi-PAL AOC 5000 (Shimadzu, Milan, Italy) with

a Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) fiber of d_f 65 µm and 1 cm length from Merck (Bellefonte, PA, USA). Fiber selection was based on the results about profiles representativeness obtained in a previous study[38] while its conditioning was performed as recommended by the manufacturer.

Coffee samples (1.50 g of fine and homogeneous powder) were accurately weighed in headspace vials (20 mL) and immediately sealed after the operation. Headspace sampling was performed for 40 minutes at 50°C vibrated at a constant speed. The internal standard was pre-loaded onto the fiber by sampling 5 µL of a 1000 mg/L solution of *n*-C13 in dibutyl phthalate (DBP) placed in a 20 mL headspace vial kept for 20 min at 50°C at a constant speed. After sampling, the analytes were recovered via the thermal desorption of the fiber, for 5 min at 250°C, into the GC injector. All samples were analyzed in triplicate.

Analysis was performed with a Shimadzu QP2010 GC–MS system equipped with Shimadzu GC-MS Solution 2.51 software (Shimadzu, Milan, Italy). Chromatographic conditions: injector temperature: 250°C; injection mode: splitless; carrier gas: helium at a flow rate of 1 mL/min. Capillary column: SGE SolGelwax (100% polyethylene glycol) 30 m x 0.25 mm d_c x 0.25 µm d_f (Trajan Scientific and Medical, Melbourne, Australia). Temperature program, from 40°C (1 min) to 200°C at 3°C/min, then to 250°C (5 min) at 10°C/min. MS conditions: ionization mode: EI (70 eV); temperatures: ion source at 200°C; transfer line at 250°C. Scan range: 35-350 *m/z*; scan speed 666 amu/sec.

Analytes identification was performed using linear retention indices (I^T_S) and EI-MS spectrum that were either compared to those of authentic standards, to those collected in-house or in commercial libraries (Wiley 7N and NIST 14 Mass Spectral Data).

Non-volatile fingerprint: analysis conditions

The non-volatile fraction was analyzed using a LC-UV/DAD system, Model 1200 Agilent, Little Falls, DE, USA), equipped with a Spectra System UV Diode Array Detector 1100 series (Agilent, Little Falls, DE, USA). Data acquisition and data handling were performed by Chemstation LC 3D software Rev.3.03 01-SR1 (Agilent, Little Falls, USA). The LC column was a Platinum EPS C18 (250 × 4.6 mm, 80A, 4 µm) (Alltech, Deerfield, USA).

LC operative conditions: injection volume 20 µL; mobile phase: A: water/formic acid (999:1, v/v) B: acetonitrile/formic acid (999:1, v/v); flow rate, 1.0 mL/min. The gradient program was as follows: 15% B for 7 min, 15-55% B in 20 min, 55-100% B in 25 min, 100% B for 2 min. Before re-injection,

the LC system was stabilized for at least 5 min. The UV/DAD acquired within the wavelength range 210-600 nm and at a 2.5 spectra/sec. Acquisition wavelengths were 276 and 325 nm.

Compounds identity confirmation and putative identifications were carried out on a LC-MS/MS system consisting of a Shimadzu Nexera X2 unit equipped with a photodiode detector SPD-M20A connected, in series, to a triple quadrupole Shimadzu LCMS-8040 MS system equipped with an electrospray ionization (ESI) source (Shimadzu, Dusseldorf Germany).

The separation column was an Ascentis Express C18 (15 cm x 2.1 mm, 2.7µm) from Supelco (Bellefonte, USA). Operative conditions: injection volume, 5 µl; mobile phases, A: water/formic acid (999:1, v/v), B: acetonitrile/formic acid (999:1, v/v); flow rate, 0.4 mL/min. Mobile-phase program: 15% B for 7 min, 15-55% B in 3 min, 55-100% B in 1.5 min, 100% B for 1 min, total pre-running and post-running time 23 min. UV/DAD detection for profiles monitoring was set within the wavelength range 220-450 nm. MS operative conditions were as follows: heat-block temperature: 200°C; desolvation line (DL) temperature: 250°C; nebulizer gas flow rate: 3 L/min; drying gas flow rate: 15 L/min. Mass spectra were acquired both in positive and negative full-scan modes over the 100-1000 *m/z* range at an event time of 0.5 sec. Product Ion Scan mode (collision energy: - 35.0 V for ESI+ and 35.0 V for ESI-, event time: 0.2 sec) was applied to compounds for which a correspondence between the pseudomolecular ions [M+H]+ in ESI+ and [M-H]- in ESI- had been confirmed.

Data Processing

Data sets (Sensory scores, GC-MS and LC-UV/DAD fingerprints) were explored by Principal Component Analysis (PCA) followed by Multiple Factorial Analysis (MFA). This statistical tool enables to investigate the relationships between chromatographic fingerprints from the two analytical platforms and to compare them with the sensory data. Features selection for each data set, related to the sensory note, was performed using VIP>1 (variable importance in projection) from Partial Least Square-Discriminant Analysis (PLS-DA) on samples that were suitable to minimize and maximize the sensory notes expression. This procedure was used to select peak features that were either used afterword to model, by PLS regression, single perception modalities (*i.e.*, aroma or taste) or flavor *in toto* by combining the two data set (i.e., volatiles and non-volatiles fingerprints. Data elaboration was performed using XLSTAT statistical and data analysis solution software (version 2020.1.3 - copyright Addinsoft 2020).

Raw analytical data representing the chromatographic fingerprints of volatiles and non-volatiles were pre-processed, as shown in the work-flow of Figure 1, via the temporal alignment of

chromatograms and background noise subtraction. This pre-processing was made with Pirouette software ver. 4.5 (Infometrix, Inc., Bothell, WA, USA). Raw signals (GC and LC) were converted into an array X ($I \times J$) where I corresponds to detector intensities and J to corresponding retention times. Replicated analyses were treated independently. Therefore, each analytical platform provided a tensor that included raw signals arrays for all the analyzed samples.[39,40] The output was a table listing, in the columns, all detected features together corresponding response and rows reporting sample analytical replicates. A response threshold was set to filter out noise peaks, it corresponded to 3 times the standard deviation of signal-to-noise ratio (S/N) values sampled at different time points with both techniques. The S/N for GC-MS was set at 15 and for LC-UV/DAD at 8 and 10 respectively for 276 and 325 nm.

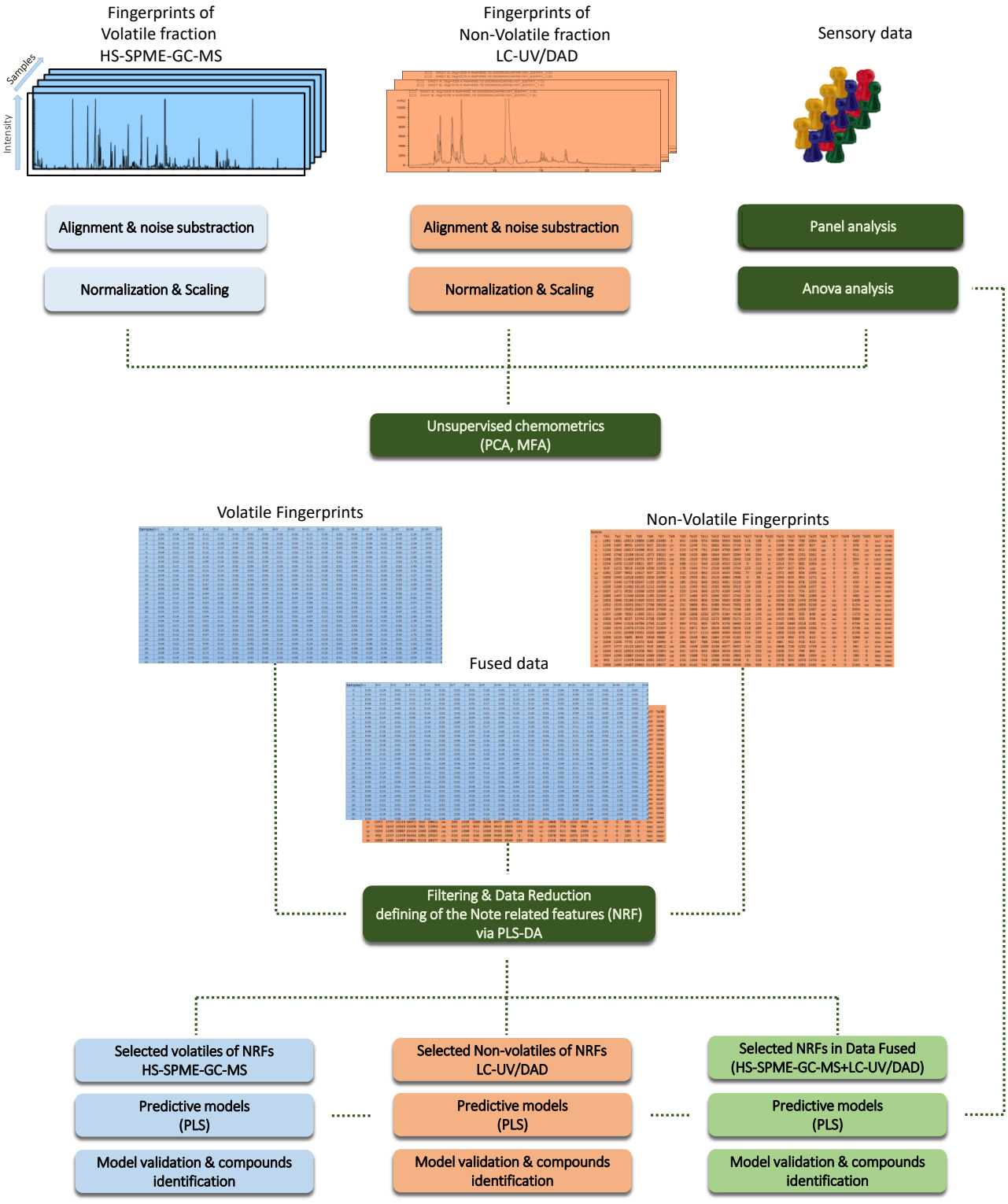

Figure 1: Work-flows of chemical and sensory data treatment.

Data then underwent unsupervised multivariate analysis to highlight, if present, diagnostic patterns, and were next treated with supervised methods (PLS-DA) to select features that were most related

to each sensory attribute. Selected features were then used to evaluate the ability to predict sensory scores for the investigated attributes using PLS models.[41] The instrumental data from both the volatile and non-volatile fractions were first independently elaborated, and then combined/fused in a single data matrix to investigate their contribution and/or ability in predicting selected sensory attributes.

The models based on HS-SPME-GC-MS fingerprint, on HPLC-UV/DAD fingerprint and those obtained by elaborating the fused data, were evaluated and compared on the basis of their model quality index (Q^2), Coefficient of Determination (R^2) and the Root Mean Squared Error Cross-Validation (RMSECV) and Prediction (RMSEP). The model quality index (Q^2) measures the global goodness-of-fit and the predictive quality of the analytes used in the model (volatiles, non-volatiles and data fusion). The maximum value of Q^2 is equivalent to the most stable model. The Coefficient of Determination of the model (R^2) indicates the proportion of variability in the dependent variable (sensory score) explained by the model, and ranges between 0 and 1; the closer R^2 is to 1, the better the model. The main issue with R^2 is that it does not take into account the number of variables used to fit the model. This limit has been overcome by the Adjusted R^2. The number of variables used to develop the model is important since the number of unnecessary variables penalizes the model; unlike R^2, Adjusted R^2 is sensitive to these penalties. Adjusted R^2 can be calculated using the following equation:

$$_{\text{Adj}}R^2 = 1 - (1 - R^2) \times \frac{n-1}{n-p}$$

where R^2 is the determination coefficient of the model, n and p are the numbers of observations and variables used to fit the model, respectively. The differences between the predicted values and those measured is given by the Root Mean Squared Error (RMSE), which determines the average of the squares of the errors or deviations. The error calculated in the cross-validated data is known as root mean squared error in Cross Validation (RMSECV), while the value calculated in the prediction data is the root mean squared error in prediction (RMSEP).

$$RMSEP = \sqrt{\frac{\sum_{I=1}^{n}(y_i - \hat{y}_{i/i})^2}{n}}$$

y_i is the experimental response and $\hat{y}_{i/i}$ is the response predicted by the regression model, where i/i indicates that the response is predicted by a model that was estimated when the $i-th$ sample was left out from the training set. [42]

RESULTS AND DISCUSSION

This section mainly focuses on the chromatographic fingerprinting approach and on its correlation to sensory data.

Exploring data matrices

Data exploration was, at first, performed using Principal Component Analysis (PCA) on volatiles and non-volatile fingerprint features, treated independently. Each sample (observation) was described by all of the features detected above the fixed S/N threshold (variables).

Results are illustrated in Figure 2A and 2B, which show the distribution of the samples over the two PCs, that were able to cover more than 50% of the total variance of the data matrix [68% for volatiles (Figure 2A) and 53% for non-volatiles (Figure2B)]. In both cases, good separation can be observed between the Arabica (Blue) and Robusta (Green) samples, suggesting that both chemical fractions provide a similar contribution to sample discrimination.[38]

Multiple Factor Analysis (MFA) was then used to compare the three different data matrices: chemical fingerprints (volatiles and non-volatiles) and the sensory data related to the seven sensory notes considered (*i.e.*, *Acid*, *Bitter*, *Flowery*, *Fruity*, *Woody* and *Spicy*).

MFA proceeds in two steps: i) first it computes a PCA of each data table, and 'normalizes' each data table by dividing all elements by the first singular value obtained from its PCA; ii) secondly, all normalized data tables are aggregated into a single data table that is then analyzed via a (non-normalized) PCA, which provides a set of factor scores for observations and loadings for the variables.[43] The results, displayed by filtered group (by origins) for better visualization, are reported in Figure 2C, which shows how volatiles (conventionally indicated in the figure as Ar) and non-volatiles (conventionally indicated in the figure as Ta) for the different samples have quite similar

branches. RV correlation coefficients, shown in Table 1, indicate to what extent the distribution of the tables/variables are related two-by-two, reflecting the amount of variance shared from the tables. The more correlated the variables are, the higher the RV coefficient is (variation range 0-1). The mutual correlation between volatiles and non-volatiles was 0.921, between sensory data and non-volatiles it was 0.505, while for sensory data and volatiles it was 0.549 (Table 1).

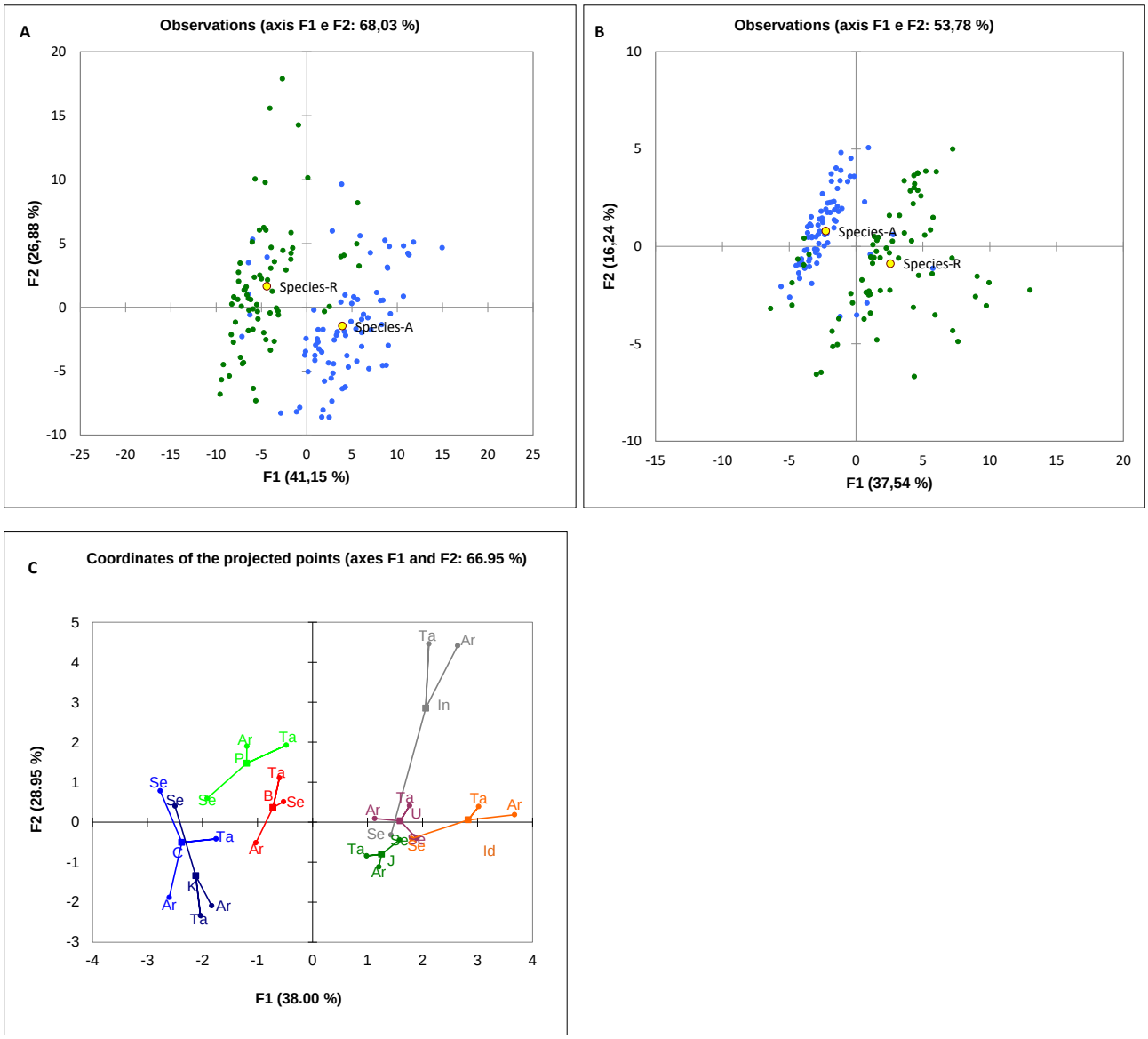

Figure 2: The score plots obtained from the PCA of Volatiles (A) and Non-volatiles (B) data respectively and (C) Coordinates of projection points from Multiple factor analysis (MFA). Legend (C): B, C, J, U, In, Id, K, P indicate coffee origins; Se: sensory data; Ar: aroma data (volatile fraction by HS-SPME-GC-MS); Ta: taste data (non-volatile fraction by LC-UV/DAD).

Table 1: Multiple factor analysis results and RV correlation coefficients.

	Volatiles (Ar)	**Non-volatiles (Ta)**	**Sensory (Se)**	**MFA**
Volatiles (Ar)	1.000	0.921	0.549	0.951
Non-volatiles (Ta)	0.921	1.000	0.505	0.934
Sensory (Se)	0.549	0.505	1.000	0.748
MFA	0.951	0.934	0.748	1.000

These data suggest a possible relationship between chemical fingerprints, although the correlation is not particularly high. Results confirm what already reported in the literature; i) the volatiles have an important role in the definition of the coffee sensory profile; and ii) volatiles, including aroma active compounds, better correlate with sensory features than non-volatiles, including taste active compounds.[3,5,44]

The correlation values (MFA values) reported in Table 1 indicate that the multicollinearity between the information provided by the chemical fingerprints and the sensory analysis is weak, while suggesting that they both contribute to the definition of the overall coffee flavor and that no aspect can fully be ignored.

The next sections focus on the workflow that was adopted to develop a predictive model for the *Bitter* note. In particular, the information provided by volatiles and non-volatiles fingerprints independently or combined together will be explored. The *Bitter* note was also taken as a test bench because of its relevance in the hedonic profile of the coffee brew. The adopted strategy was then also applied to all other sensory notes considered.

Bitter flavor evaluation: chemical components-selection strategy

Bitter and acid notes are typically perceived as taste attributes. *Bitter* was here chosen as model note to explore how the volatile, non-volatile fraction or combined data, might correctly describe the bitter-score in prediction. The objective is to understand whether a traditional taste attribute

can be (more) correctly described by combining chemical data from both taste-active and odor-active features.

The features that were highly correlated to the expression of high and low *Bitter* notes were first selected by PLS-DA. This step was applied to volatile and non-volatile data sets separately, and later, to the fused data matrix. Figure 3 shows the chromatographic fingerprints resulting from GC-MS (volatiles Figure 3A) and LC-UV/DAD (non-volatiles Figure 3B). The grey lines indicate the retention times of the most relevant features designated by PLS-DA.

The values for the Variable Impact on Projections (VIP) were used as a filter parameter, as VIP coefficients reflect the relative importance of each X variable in the prediction model. A cut-off of 1 and a non-zero standard deviation (SD) were used to select features. Figures S1A and S1B in supporting information show the results for volatiles, while Figures S1C and S1D indicate those for non-volatiles, treated independently.

The prediction models were then developed by applying a PLS regression algorithm to the selected *Bitter*-related features.

150 samples were used to build up the regression model, 20 of them were randomly employed as a validation set, and 30 were excluded from the training set and adopted as an external test set. A comparison of the PLS regression model parameters is reported in Table 2. The results unexpectedly suggest that the data from the volatiles and from the fused data matrix (volatiles + non-volatiles) show a similar behavior in the description of the *Bitter* note. Although the fingerprinting of non-volatiles was obtained by applying selected wavelengths characteristic of bitter-related chemicals (*i.e.* caffeine, trigonelline and chlorogenic acid derivatives), it provided less information than volatiles alone (see both the Q^2 and coefficient of determination (R^2) values in Table 2). This is probably due both to other inferences in the description of this note, and to the partial (not comprehensive) fingerprinting of the non-volatile fraction by LC-UV.

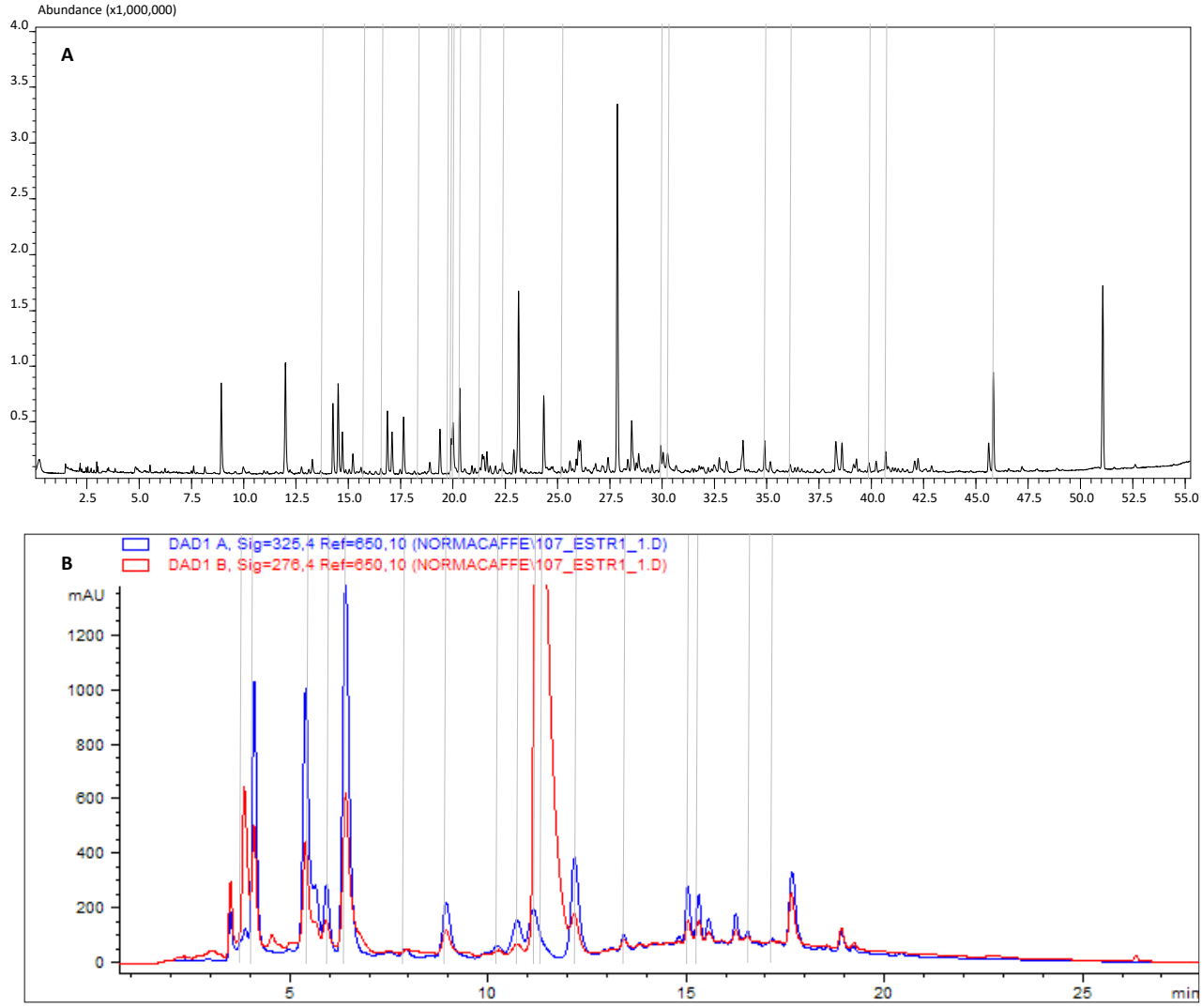

Figure 3: HS-SPME-GC-MS of coffee powder (A) and an LC-UV/DAD coffee brew (the wavelengths were set at 276 and 325 nm) (B), fingerprints of a coffee sample with high bitter notes. Grey bars show features that were related to the bitter note and subjected to identification with I^Ts, MS commercial libraries and/or pure standards, or that were putatively-identified by LC-MS.

Table 2: Comparison of the parameters of the PLS-regression models, in validation and prediction, that were obtained using aroma and taste, singularly, and data fusion (Volatiles + Non-Volatiles) for the six investigated notes. Models are built with specific selected features that were derived from PLS-DA analysis carried out on each sensory attribute following the strategy described in relative sections.

PLS model performance

Bitter	**Volatiles**	**Non-volatiles**	**Fused data**
n°variables	22	14	39
Q^2	**0.742**	**0.666**	**0.692**
R^2	0.892	0.810	0.888
RMSECV	0.579	0.659	0.575
RMSEP	**1.073**	**0.929**	**1.120**
Acid			
n°variables	22	10	26
Q^2	0.723	0.450	0.703
R^2	0.829	0.636	0.825
RMSECV	0.594	0.854	0.605
RMSEP	0.898	1.069	0.875
Flowery			
n°variables	20	14	27
Q^2	0.223	0.199	0.099
R^2	0.585	0.498	0.597
RMSECV	0.806	1.042	0.847
RMSEP	0.972	1.067	1.020
Fruity			
n°variables	19	16	39
Q^2	0.158	0.184	0.033
R^2	0.607	0.508	0.786
RMSECV	0.814	0.922	0.619
RMSEP	0.615	0.610	0.876

Spicy			
n°variables	22	16	32
Q2	0.320	0.331	0.458
R2	0.709	0.720	0.823
RMSECV	1.063	1.051	0.821
RMSEP	1.066	0.971	1.217
Woody			
n°variables	23	9	37
Q2	0.708	0.472	0.706
R2	0.879	0.714	0.885
RMSECV	0.798	1.228	0.782
RMSEP	0.920	0.948	1.129

[a] Models are built with specifically selected features that were derived from PLS-DA analysis carried out on each sensory attribute following the strategy described in relative sections.

Bitter-related components in the volatile fraction: informative volatiles and aroma-active compounds

The volatile fraction provided information that is useful to characterize the bitter-note signature in the analyzed samples. Further MS and retention data investigation into *Bitter*-related features led to the identification of several aroma-active compounds, including: pyrazines (1-methylethenylpyrazine (Ar6), 5-Methyl-6,7-dihydro-5H-cyclopentapyrazine (Ar24), 2-*n*-propyl pyrazine (Ar28), 2,3-dimethylpyrazine (Ar32), 2-methyl-5H-6,7-dihydrocyclopentapyrazine (Ar25), 2,3-dimethylpyrazine (Ar32), 2-ethyl-3,5-dimethylpyrazine[§] (Ar39)); phenols (4-ethylguaiacol[§] (Ar46), 4-vinylguaiacol[§] (Ar48), guaiacol[§] (Ar66)); 2-phenylethanol (Ar55); 1-H-pyrrole (Ar8); 2-furanmethanethiol[§] (Ar17); furfuryl methyl sulfide (Ar63); and furfuryl pyrrole (Ar65). Table S2 lists the identified *Bitter*-related volatiles together with their odor quality, experimental and tabulated I^Ts, MS similarity match with the reference spectra from commercial databases and/or pure standards. Several analytes (§) are coffee key-aroma compounds, as indicated by Blank *et al.* [45]. Interestingly, these compounds are described as earthy, roasty, burnt and phenolic, but none of

them was directly related to bitterness. Nevertheless, Barba *et al.* [36] suggested that 8% of the panelists associated furfural with the description *Bitter* taste, although it is conventionally reported as *bready* and *caramellic*. Moreover, in a study on fruit juices, Guichard *et al.*[35] observed that an enhancing effect on *Bitter* perception was triggered by a correlative pattern of non-*Bitter*-eliciting odorants, such as ethyl-2-methyl and 2-ethylbutanoate, ɣ-decalactone, furfural, allo-ocimene, butyl-acetate, β-myrcene and pentanoic acid. These authors stated that odorants that enhance a target-taste perception may be exploited to modulate the overall taste perception in foods and beverages.

Bitter-related chemicals in the non-volatile fraction: informative analytes and taste-active compounds

The non-volatile fingerprints were elaborated using the same strategy as for the volatiles (see section above). Model performance is reported in Table 2, and confirmed the existing positive correlation between the *Bitter* note and some chemical features detected by LC-UV/DAD. *Bitter*-related chemicals were then identified (or putatively identified) using *post-hoc* LC-MS/MS analysis.[46–48]

Peaks with a maximum of absorption at 325 nm are characterized by pseudomolecular ions at 337 m/z and 335 m/z, in the ESI^+ and ESI^- acquisition modes, respectively, with diagnostic fragments at 163 m/z (ESI^+) and 161 m/z (ESI^-) that correspond to the caffeic acid moiety with a loss of a water molecule, and that can be putatively identified as caffeoylquinic lactones. Similarly, peaks with characteristic UV-absorption maxima at 323 nm and 310 nm can tentatively be attributed to feruloylquinic acid isomers and coumaroylquinic acid, respectively. This is confirmed by the presence of pseudomolecular ions at 369 m/z (ESI^+) and 367 m/z (ESI^-) for feruloylquinic acid, and at 339 m/z (ESI^+) and 337 m/z (ESI^-) for coumaroylquinic acid and other diagnostic fragments, as reported by Martini *et al.* 2017[49] (Figure S2).

Table S3, in supporting information, lists the identified bitter-related non-volatiles together with their retention times, λ-max, molecular weight, molecular ions and MS/MS data.

Some of the most relevant components are two feruloylquinic acid isomers (FQA – Ta6, Ta18), three caffeoylquinic lactone isomers[§] (CQL – Ta11, Ta10, Ta13), one feruloylquinic acid isomer[§] (FQA–Ta10), 3,4 and 4,5 dicaffeoylquinic acid (diCQA-Ta21, Ta25) and caffeine[§] (Ta14) (Figure 3B and Table S3 in supporting information). Most of these (§) were already associated to the *Bitter* note by Hofmann *et al.*[47,50,51], although, rather surprisingly, here caffeoylquinic acid isomers (CQAs-Ta3, 5,

7) and trigonelline (Ta2) were not strongly correlated to *Bitter*. Moreover, although to a different extent, the heat map in Figure 4 shows that most of the identified taste active compounds were positively correlated with volatiles related to *Bitter* (Ar6, 8, 17, 24-25, 32, 39, 44-48, 55, in light green). In this figure, the Pearson's correlation matrix between volatiles (Ar) and non-volatiles (Ta) at 5% of confidence level is visualized in a green-to-red color scale, with the colors ranging from light green ρ=+1 to red ρ=-1. Most of the targeted compounds in Table S3 increase with the same trend in the most bitter samples, albeit with different magnitudes.

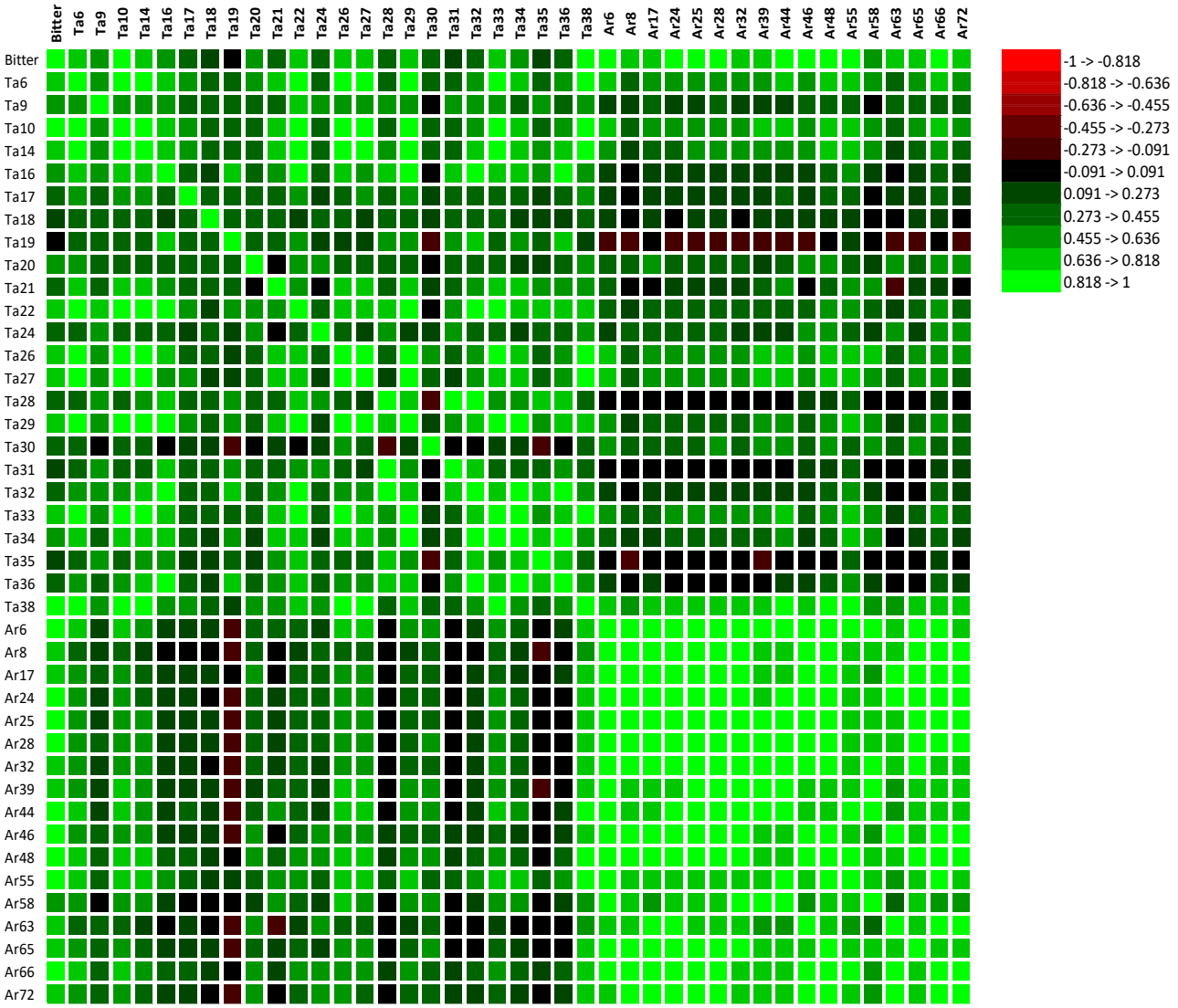

Figure 4 Pearson's correlation matrix of volatile (Ar) and non-volatile (Ta) fractions represented by a heat map, color scale from light green ρ=+1 to red ρ=-1, confidence level 5%.

Bitter evaluation: performance evaluation of data-fusion strategy

This paragraph investigates the possible gain in explanatory and predictive power when chemical information from volatiles and non-volatiles are combined together in developing models. GC and LC analyses are partly complementary, in terms of compounds analyzed, and the combination of the data sets may be more informative by revealing, for instance, possible associations between volatiles and non-volatiles (Figure 3).

As flavor perception derives from the interaction between aroma and taste, the combination of the information provided by the two different fractions was expected to increase the performance of the predictive model. The regression model to predict the scores of the *Bitter* sensory attribute was built from the fused data matrix (GC and LC data), without a preliminary selection of variables from the PLS-DA of the single approaches, and by re-submitting the fused data set to the work-flow established for each single analytical technique. The performance of the fused data matrix regression model was in line with those obtained using fingerprint data from volatile and non-volatile models (Table 2). Data fusion did not improve the overall prediction quality of the model (Q^2 in bold in Table 2), the error in the cross-validation set (RMSECV), nor the prediction of the external test set (RMSEP). Results showed that the model had better prediction quality than the non-volatile fraction alone, but worse than that of the volatile fraction alone. The non-volatile fraction did not add information to better understand the perception while volatiles alone had better performances even to model a taste perception. Although the coffee non-volatile fraction analyzed is not fully representative, nevertheless the considered non-volatile markers are well-established sensory quality marker in routine controls. [46,47,50,51,52] The volatile fraction possibly have an actual influence in driving the description of this sensory attribute.[53,54] This possibility, together with the correlation pathways between volatiles and non-volatiles, deserves much more in-depth investigation.

Investigation of the integrated approach into all sensory notes

The data-elaboration workflow, validated on the *Bitter* note, was also used to investigate the other flavor attributes, and to understand to which extent the two chemical fractions (single or combined) play a role in sensory-quality description. Table 2 summarizes the performance of the models in predicting sensory scores.

The Q^2 values clearly indicate that the non-volatile fraction had a differential impact on the prediction models. Non-volatiles showed better performance for *Acid*, *Spicy* and *Woody* notes than

for *Flowery* and *Fruity* notes. This trend was also confirmed by the R^2 values, which were higher in *Acid*, *Spicy* and *Woody* notes.

The behavior of RMSECV slightly differed; these values were in compliance with the previous observations on *Bitter* only, for the *Acid* note, and less evident for the others. The non-volatile fraction was expected to have a lower impact on *Fruity* and *Flowery* notes since these notes are considered to be closer to aroma attributes, and the associations with components detected using the adopted analytical method have not yet been found.[55]

As a general consideration, most of the notes (with the exception of *Spicy*) showed better performance when the predictive model was based on volatile features (Table 2), suggesting a better agreement between HS-SPME-GC-MS data and sensory scores within the investigated sample set.

The results of the fused data bring to three different scenarios:

- *Acid* and *Woody* notes: the models on fused data showed acceptable performance (Q^2 around 0.7, R^2>0.8 and a RMSECV lower than 1). Their overlap with the results of the PLS models, which were developed using volatiles data alone, suggests that the non-volatiles provides a negligible contribution into the flavor note description.
- *Flowery* and *Fruity* notes: the performance of the models based on fused data were worse than that based solely on volatiles. As expected, here volatiles provide meaningful and consistent information than the fused-data sets. Non-volatiles increase the noise and act as confounding elements.
- *Spicy* note: the performance of the fused data model is slightly better than that obtained from the single fractions. The most significant improvements were observed in the Q^2 and RMSECV values. This result is supported by the fact that some key spicy volatile compounds (mainly phenolics, such as guaiacoles) originated from the thermal degradation of chlorogenic acids (i.e. those detected in HPLC analyses)[56] monitored on the LC-UV/DAD fingerprints.
- The low model stability registered for *Flowery*, *Fruity* and *Spicy* notes might be linked to the unbalanced distribution of the samples within the training set; the number of samples with low scores for these notes was much higher than that for samples with high scores. This unbalanced sample distribution makes the use of PLS algorithm challenging, and requires suitable algorithms to better follow data evolution. The optimization of regression, via a non-

parametric algorithm on volatile data, might improve prediction ability of the models for these sensory attributes.[57]

These results show that the screening carried out with two different analytical platforms routinely used in quality control laboratories have a complementary role but with different relevance in describing coffee sensory quality. While MFA suggests the existence of a certain orthogonality between volatile and non-volatile data, the regression models highlight the key role played by the volatile fraction, and therefore of the aroma, in the sample sensory characterization. The performance of the PLS models, built up with the fused fingerprints, is comparable to that obtained from HS-SPME-GC-MS. These correlative results were already sensorially confirmed by some authors, who have reported that flavor perception, in all its aspects, is mostly linked to aroma composition and impact.[1,3,5] These observations, together with the good results obtained in the definition of *Acid* and *Bitter* notes (considered as "typical taste notes") from the volatile data, make it possible to hypothesize that the analysis of the volatile fraction may be sufficiently representative to delineate coffee flavor, and provide reliable chemical fingerprints that can be associated to some sensory notes, including those typical of taste. Moreover, the correlative results highlighted from the volatile non-volatile fused data deserves a thorough investigation to verify the potential of odor-taste integration.

The reported correlative patterns indicate that the integrated approach can successfully be used as a complement to sensory analysis, in particular to design coffees with specific flavor profiles.

As a general consideration, the success in the development of these methods requires a high consistency and alignment of the sensory panel in products evaluation, since subjectivity in data collection can influence the development of the mathematical model for scores prediction. However, the natural variability of the coffee matrix and its complexity makes difficult to achieve a good representativeness for all commercial coffees treated at the industrial level.

To make more robust and reliable instrumental tools for sensory prediction, a huge amount of data, both from sensory profiling and chemical fingerprinting, are necessary. Modern artificial intelligence algorithms might be of help to simulate human skills but training data should match with the actual complexity of the phenomenon of multimodal flavor perception.

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SUPPLEMENTARY

Table S1: List of samples analyzed with the species to which they belong, the origins, togheter with the total Robusta and Arabica samples and of the total number of samples per country of origin.

Sample name	Species	Origins
1	R	INDONESIA
2	R	INDIA
3	R	INDONESIA
4	R	TANZANIA
5	A	BRAZIL
6	R	BRAZIL
7	R	INDONESIA
8	A	BRAZIL
9	R	VIETNAM
10	R	UGANDA
11	R	INDONESIA
12	R	INDIA
13	R	INDONESIA
14	R	INDIA
15	R	TANZANIA
16	A	BRAZIL
17	R	BRAZIL
18	R	INDONESIA
19	A	BRAZIL
20	R	VIETNAM
21	R	VIETNAM
22	R	UGANDA

Sample name	Species	Origins
50	R	INDIA
51	R	VIETNAM
52	R	TANZANIA
53	R	TANZANIA
54	R	UGANDA
55	R	BRAZIL
56	A	INDONESIA
57	A	ETHIOPIA
58	A	PAPUA NEW GUINEA
59	A	COLOMBIA
60	A	BRAZIL
61	R	BRAZIL
62	R	GUATEMALA
63	R	INDIA
64	R	TANZANIA
65	A	BRAZIL
66	A	COLOMBIA
67	A	COLOMBIA
68	A	GUATEMALA
69	A	BRAZIL
70	R	BRAZIL
71	R	INDIA

Sample name	Species	Origins
101	A	BRAZIL
102	R	UGANDA
103	R	VIETNAM
104	R	MEXICO
105	A	KENYA
106	A	COLOMBIA
107	A	COLOMBIA
108	A	BRAZIL
109	A	BRAZIL
110	R	INDIA
111	R	INDONESIA
112	A	VIETNAM
113	A	HONDURAS
114	A	NICARAGUA
115	A	ETHIOPIA
116	A	BRAZIL
117	R	UGANDA
118	R	VIETNAM
119	R	TANZANIA
120	A	COLOMBIA
121	A	PAPUA NEW GUINEA
122	A	HONDURAS

Sample name	Species	Origins
152	R	LAOS
153	R	INDIA
154	R	INDONESIA
155	R	VIETNAM
Total Robusta	70	
Total Arabica	85	
Total per Origins		
BRAZIL	35	
BURUNDI	1	
COLOMBIA	11	
COSTA RICA	2	
ETHIOPIA	9	
GUATEMALA	4	
GUATEMALA	4	
HONDURAS	2	
INDIA	17	
INDONESIA	16	
KENYA	2	
LAOS	2	
MEXICO	2	
NICARAGUA	1	

Sample name	Species	Origins	Sample name	Species	Origins	Sample name	Species	Origins
23	R	INDONESIA	72	R	VIETNAM	123	A	ETHIOPIA
24	R	INDIA	73	A	VIETNAM	124	R	TANZANIA
25	R	INDONESIA	74	A	BRAZIL	125	R	INDONESIA
26	R	INDIA	75	A	BRAZIL	126	A	ETHIOPIA
27	R	TANZANIA	76	A	BRAZIL	127	A	ETHIOPIA
28	A	BRAZIL	77	A	BRAZIL	128	A	INDIA
29	R	BRAZIL	78	R	VIETNAM	129	A	UGANDA
30	R	INDONESIA	79	R	INDONESIA	130	A	TANZANIA
31	A	BRAZIL	80	R	TANZANIA	131	A	ETHIOPIA
32	R	VIETNAM	81	A	UGANDA	132	R	INDIA
33	R	VIETNAM	82	A	COLOMBIA	133	R	TANZANIA
34	R	UGANDA	83	A	COLOMBIA	134	A	PERU
35	A	COLOMBIA	84	A	BRAZIL	135	A	PERU
36	A	COLOMBIA	85	A	BRAZIL	136	R	INDONESIA
37	A	TANZANIA	86	R	VIETNAM	137	R	INDIA
38	A	PAPUA NEW GUINEA	87	R	VIETNAM	138	A	PERU
39	A	RWANDA	88	A	INDIA	139	A	PERU
40	A	TANZANIA	89	A	KENYA	140	A	MEXICO
41	A	BRAZIL	90	A	ETHIOPIA	141	R	INDIA
42	A	BRAZIL	91	A	COLOMBIA	142	A	PERU
43	A	BRAZIL	92	A	PERU	143	A	GUATEMALA
44	A	BRAZIL	93	A	BURUNDI	144	A	TANZANIA
45	A	BRAZIL	94	A	BRAZIL	145	A	ETHIOPIA
46	A	BRAZIL	95	R	INDIA	146	R	TANZANIA
47	R	INDIA	96	A	INDONESIA	147	A	ETHIOPIA
48	R	GUATEMALA	97	A	LAOS	148	A	TANZANIA
49	R	VIETNAM	98	A	BRAZIL	149	A	TANZANIA
50	R	INDIA	99	A	BRAZIL	150	A	COSTA RICA
51	R	VIETNAM	100	A	BRAZIL	151	A	COSTA RICA

Total per Origins	
PERU	6
TANZANIA	17
UGANDA	8
VIETNAM	16

*Table S2: Lists of identified Bitter-related volatiles together with their odor quality, the experimental and tabulated ITs and MS similarity match with the reference spectra. §Coffee key-aroma compounds by Blank et al.[45] Identification criterion: (a) authentic reference compound and/or (b) I^T ± 20 and spectral similarity "tentative identification". ^From NIST 14 Mass Spectral Library; & from ref. [56] and *http://www.thegoodscentscompany.com/*

#	Compound Name	Odor Description	Identification	Calc. I^Ts	Lit. I^Ts	% Mass spectral similarity match^
Ar58	Ethylpyrazine	*Nutty-roaste**	a,b	1329	1343	96
Ar32	2,3-Dimethylpyrazine	*Nutty, coffee, peanut butter, walnut **	a,b	1341	1354	94
Ar28	2-*n*-Propylpyrazine	*Green vegetable*&	b	1413	1425	91
Ar17	2-Furanmethanethiol	*Roasted, Burnt, coffee-like**	a,b	1432	1440	97
Ar39	2-Ethyl-3,5-dimethylpyrazine§	*Earthy, Bake*&	a,b	1441	1449	93
Ar63	Furfurylmethylsulfide	*Coffee-like odor**	a,b	1483	1496	90
Ar44	3,5-Diethyl-2-methylpyrazine	*Coffee-like**	b	1489	1491	86
Ar8	1-H-Pyrrole	*Sweet, ethereal**	a,b	1513	1525	92
Ar6	1-Methylethenylpyrazine	*Roasted, Nutty*&	b	1590	1560	86
Ar24	5-Methyl-6,7-Dihydro-5H-cyclopentapyrazine	*Nutty, Sweet**	a,b	1633	1633	87
Ar25	2-Methyl-5H-6,7-dihydrocyclopentapyrazine	*Nutty, Sweet*&	b	1708	1719	88
Ar65	Furfurylpyrrole	*Geen, hay-like**	a,b	1853	1866	97
Ar66	Guaiacol§	*Smoky/sweet**	a,b	1860	1860	96
Ar55	2-Phenethyl alcohol	*Floral, woody, honey-like**	a,b	1902	1912	85
Ar72	Phenol	*Plastic**	a,b	1997	2000	87
Ar46	4-Ethylguaiacol§	*Spicy*&	a,b	2021	2037	91
Ar48	4-Vinylguaiacol§	*Clove-like**	a,b	2185	2193	95

Table S3: List of identified and putatively-identified compounds in the coffee brews. Each compound is quoted using its relative retention time, UV spectrum, pseudomolecular ions and molecular weight fragments as obtained by the Product Ion Scan mode (PIS) and Identification Confidence value (IC). The Identification Confidence value is in agreement with CAWG (2007) guidelines and indicates (Sumner et al., 2007)[48]: Level 1: Identified compound (a minimum of two independent orthogonal data (such as retention time and mass spectrum) compared directly to an authentic reference standard); Level 2: Putatively annotated compound (similarity between chromatographic and spectral data and the published data) (Frank & Zehentbauer, 2006; Perrone et al., 2008)[46,47]; Level 3: Putatively characterized class of compounds.

#	Compound Name	RT (min)	λmax (nm)	Mol. weight (g/mol)	[M–H]+ m/z	[M–H]– m/z	MS^2 + m/z	MS^2 - m/z	IC
Ta2	Trigonellin	0.76	263	137	138				1
Ta3	3-O-Caffeoylquinic acid	1.12	323/233	354	355	353			1
Ta7	5-O-Caffeoylquinic acid	1.40	322/239	354	355	353			1
Ta5	4-O-Caffeoylquinic acid	1.47	324/235	354	355	353			1
Ta6	Feruloylquinic acid 1	1.58	323	368	369	367	177/149/145/117	134/149/193	3
Ta14	Caffein	1.58	270	194	195				1
Ta11	Caffeoylquinic lactone 1	1.97	325/296	336	337	335	163/145/117	161/133	3
Ta10	Feruloylquinic acid2	2.14	323	368	369	367	177/149/145/117	134/149/193	3
Ta15	Coumaroylquinic acid	2.14	310	338	339	337	147/119	173/119/191	3
Ta18	Feruloylquinic acid 3	2.54	323	368	369	367	177/149/145/117	134/149/193	3
Ta12	Caffeoylquinic lactone 2	2.89	326	336	337	335	163//145/117	161/133	3
Ta13	Caffeoylquinic lactone 3	3.18	326	336	337	335	163//145/117	161/133	3
Ta20	Caffeoylquinic lactone 4	3.47	326	336	337	335	163/145/117	161/133	3
Ta21	3,4 diCaffeoylquinic acid	8.13	324	516	517	515			1
Ta22	3,5 diCaffeoylquinic acid	8.97	322/299	516	517	515			1
Ta25	4,5 diCaffeoylquinic acid	9.63	325	516	517	515			1

Figure S1: PLS-DA sample scores of two Bitter categories (high and low Bitter) (A), and VIP projection on component 1 (B), together with standard deviation and cut-off line. A and B refer to the volatile fraction (Ar), while C and D to the non-volatiles (Ta).

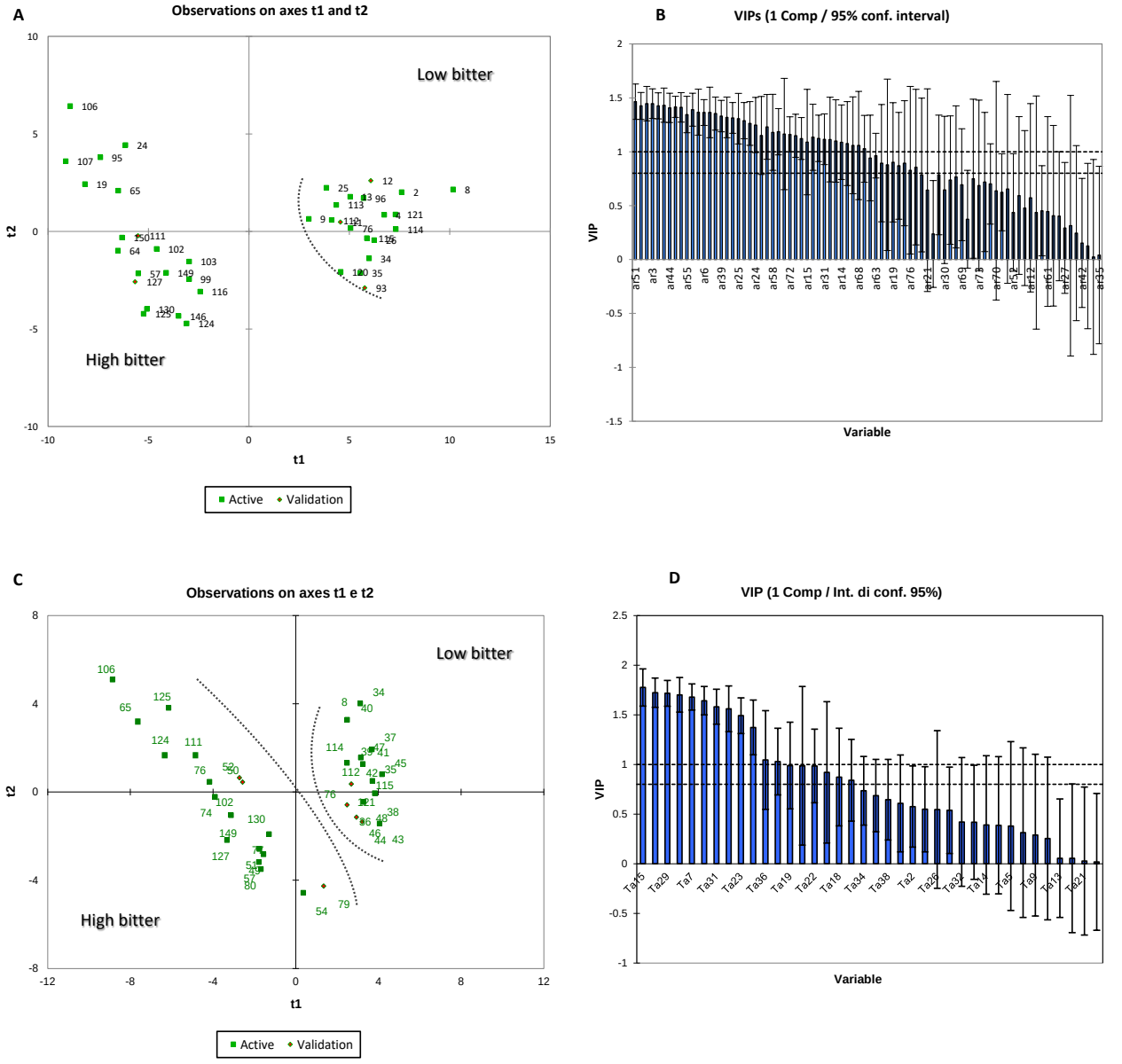
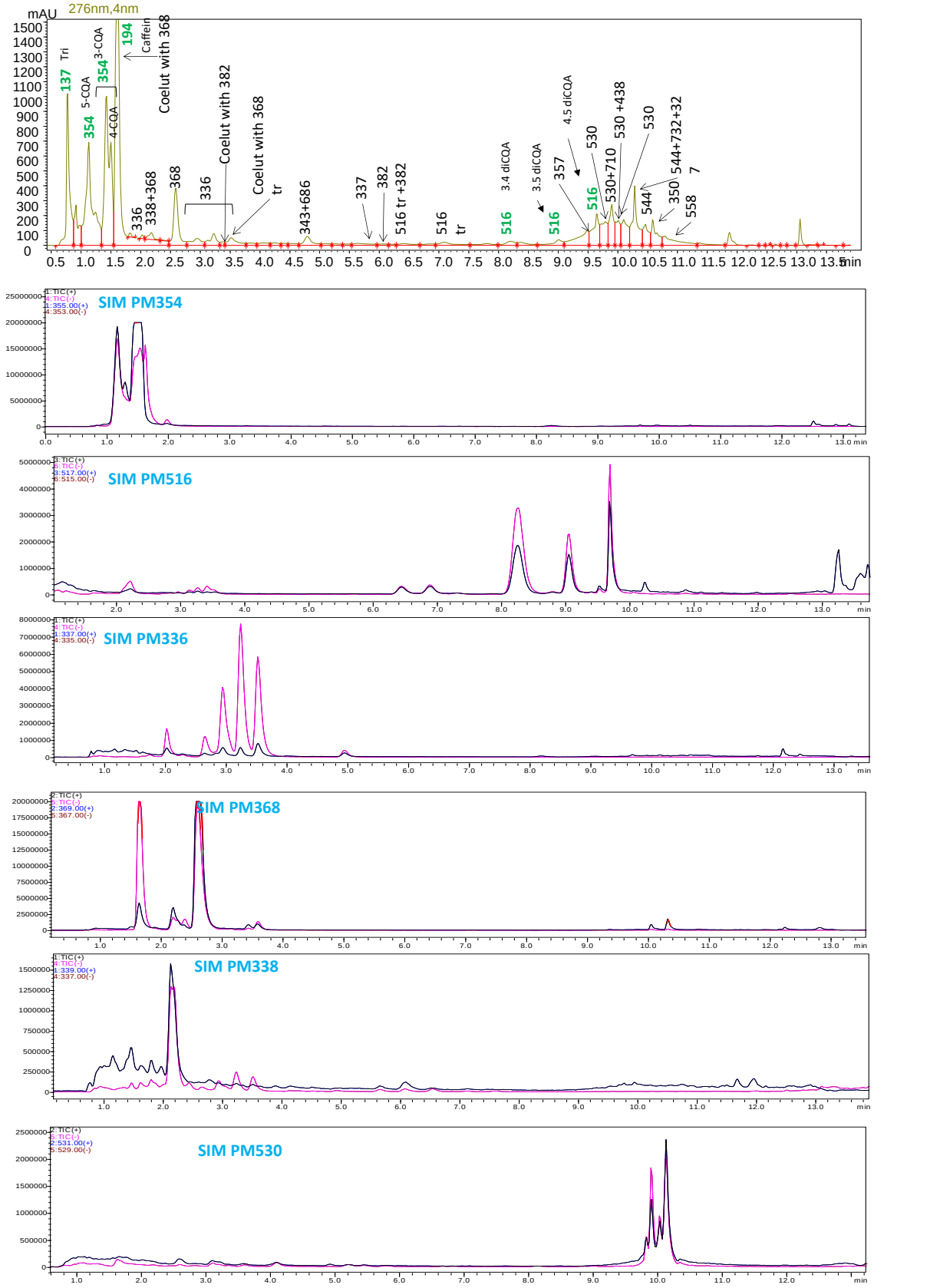


Figure S2: LC-DAD-MS profile of a coffee brew at λ = 276 nm with MRM of specific ions. Compounds are reported in Table S3.



2.2.2 Potential Aroma Chemical Fingerprint of Oxidised Coffee Note by HS-SPME-GC-MS and Machine Learning

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Potential Aroma Chemical Fingerprint of Oxidised Coffee Note by HS-SPME-GC-MS and Machine Learning

Giulia Strocchi [1], Eloisa Bagnulo [1], Manuela R. Ruosi [2], Giulia Ravaioli [2], Francesca Trapani [2], Carlo Bicchi [1], Gloria Pellegrino [2] and Erica Liberto [1,*]

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Abstract: This study examines the volatilome of good and oxidised coffee samples from two commercial coffee species (i.e., *Coffea arabica* (arabica) and *Coffea canephora* (robusta)) in different packagings (i.e., standard with aluminium barrier and Eco-caps) to define a fingerprint potentially describing their oxidised note, independently of origin and packaging. The study was carried out using HS-SPME-GC-MS/FPD in conjunction with a machine learning data processing. PCA and PLS-DA were used to extrapolate 25 volatiles (out of 147) indicative of oxidised coffees, and their behaviour was compared with literature data and critically discussed. An increase in four volatiles was observed in all oxidised samples tested, albeit to varying degrees depending on the blend and packaging: acetic and propionic acids (*pungent, acidic, rancid*), 1-H-pyrrole-2-carboxaldehyde (*musty*), and 5-(hydroxymethyl)-dihydro-2(3H)-furanone.

Keywords: coffee volatilome; oxidative note; aroma fingerprint; HS-SPME-GC-MS/FPD; machine learning

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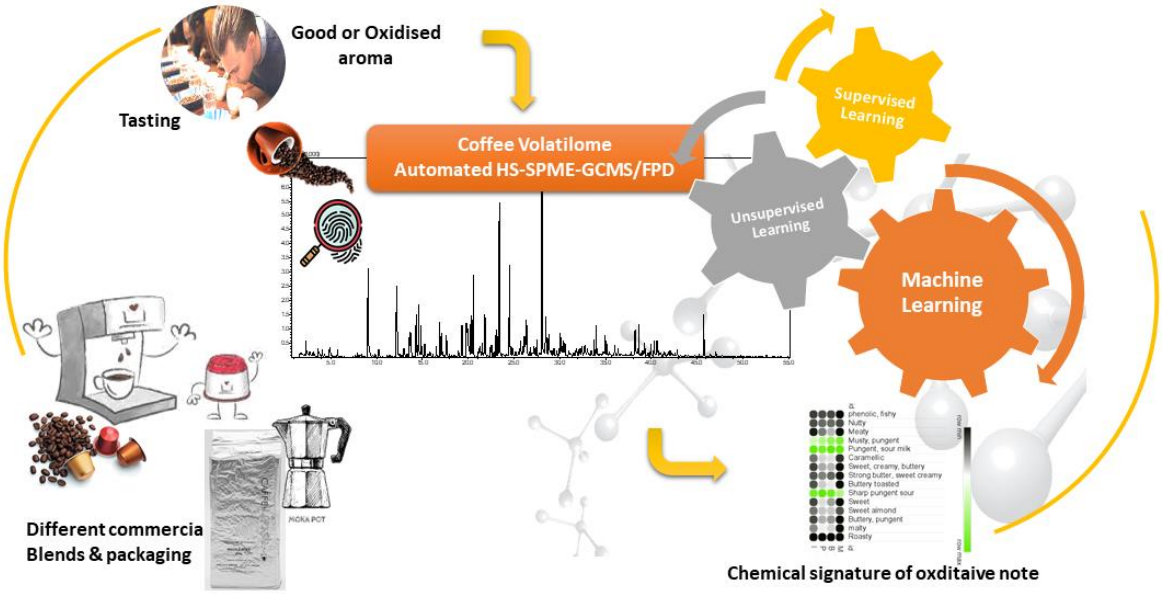
GRAPHICAL ABSTRACT



Potential Aroma Chemical Fingerprint Of Oxidised Coffee Note by HS-SPME-GC-MS and machine learning.

Giulia Strocchi[1], Eloisa Bagnulo[1], Manuela R. Ruosi[2], Giulia Ravaioli[2], Francesca Trapani[2], Carlo Bicchi[1], Gloria Pellegrino[2], Erica Liberto[1]

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ABSTRACT

This study examines the volatilome of good and oxidised coffee samples from the two commercial coffee species (i.e. Coffea arabica (Arabica) and Coffea canephora, (Robusta)) in different packaging (i.e. standard with aluminium barrier and Eco-caps) to define a fingerprint potentially describing their oxidised note, independently on origin and packaging. The study has been carried out using HS-SPME-GC-MS/FPD in conjunction with a machine learning data processing. PCA and PLS-DA have been used to extrapolate 25 volatiles (out of 147) indicative of oxidised coffees, and their behaviour has been compared to literature data and critically discussed. An increase in four volatiles was observed in all oxidised samples tested, albeit to varying degrees depending on the blend and packaging: acetic and propionic acids (pungent, acidic, rancid), 1-H-pyrrole-2-carboxaldehyde (musty) and 5-(hydroxymethyl)-dihydro-2(3H)-furanone.

Keywords: Coffee volatilome, oxidative note, aroma fingerprint, HS-SPME-GC-MS/FPD, machine learning.

1. INTRODUCTION

The quality of the coffee is related to the aroma and flavor developed because of the chemical reactions that take place during roasting and on the basis of the precursors present in the bean, which are due to various terroir and processing factors. The pleasant aroma of a freshly opened packet of roasted coffee is an additional indicator of quality [1].

Coffee is considered a stable product with a long shelf-life, although it is still an active "chemical reactor" after roasting, as the components resulting from the pyrolysis of the non-volatile precursors, the degradation of lipids and sugars and the Maillard reaction(s) are very reactive.

During storage, chemical and physical changes can affect the quality of roasted coffee. The changes in sensory features are generally due to the loss of key aroma compounds and to the appearance of oxidation products that can cause unpleasant flavours [2–4]. The loss of aroma freshness during storage, known as "staling", i.e. “..a sweet but unlovely flavour and aroma of roasted coffee which reflects the oxidization of many of the pleasant volatiles and the loss of others; a change in the flavour and the acid constituents causing a partial bland tone..” [5], mainly depends on temperature, humidity, presence of oxygen and barrier capacity of the packaging [3,6–9].

Although sensory analysis performed by a professional panel is the reference system for assessing coffee quality, several studies have been conducted with the aim of combining sensory and chemical analysis to define the freshness of a product through an objective analytical approach [10–13]. The available studies have principally identified a number of chemical markers associated with ageing, but most of them have focused on the loss of the main key odorants rather than the formation of new compounds [2,14–19].

In particular, low-molecular-weight, sulphur-containing compounds, such as methanethiol (sulphurous, putrid) and 2-furfurylthiol (roasty, coffee-like) [2,19], Strecker aldehydes and α-dicarbonyl compounds, such as propanal (ethereal pungent, earthy), 2-methyl propanal, 2- and 3-methyl butanal (malty), 2,3-butanedione and 2,3-pentanedione (buttery, pungent) [3,8,17,20] have been indicated as possible reasons for the loss of freshness in the aroma Table S1.

Although several volatile markers have been proposed as a means of monitoring changes in coffee freshness, the adoption of their absolute amounts can be questioned, as they are influenced by their initial concentrations, which in turn depend on variables such as the blend, degree of roasting, grinding and other factors [16,21]. On the other hand, the use of indices calculated using the ratio between the quantities of selected diagnostic components in the headspace is a more robust technique that better reflects the changes taking place in the headspace. Several indices have been proposed as indicators of coffee freshness and thus as possible quality indicators Table 1 lists some of the quality indicators (volatiles and indices) proposed so far correlate with the decrease in freshness of the aroma. For a more comprehensive list of quality indicators related to aroma decline over time and under the conditions studied, see Table S1. The 2-methylfuran/2-butanone (M/B) ratio is one of the best known. Its decrease over time can be correlated with the appearance of a sweet, but not pleasant, aroma [3,15,16,21].

Other proposed indices of coffee freshness are those involving sulphur compounds, however, only the 2-furfurylthiol/hexanal (FFT/HE) and, recently, dimethyl disulphide/methanethiol (DMDS/MeSH) indices were found to be useful as indicators for staling over long-term storage [20].

Despite their number, the studies in this field have always focused on one or two compounds in the expression of coffee freshness loss, rather than on the synergism between the components responsible for the oxidised perception of the overall coffee volatilome [22]. Furthermore, due to the complexity and dynamics of the chemistry involved, coffee degradation studies have mainly been conducted on a single species, package or condition [22]. Systematic studies in this direction on different species, packaging and materials have been done only in recent years [7,20,23]. Therefore, defining the oxidation chemical footprint of coffee can be an objective valuable tool to be used in screening as a support to the sensory panel in testing new and more sustainable packaging. In these perspectives, machine learning tools plays a key role to extract and describe relevant information encrypted in complex data by using different algorithms and visual tools [24–28].

This study investigates the volatilome of good quality coffee (from now “good” for short) and oxidised coffee (i.e. Coffea arabica (Arabica) and Coffea canephora, (Robusta)) in different packaging (i.e. standard with aluminium barrier and Eco-caps) by combining HS-SPME-GC-

MS/FPD with machine learning to define a potential fingerprint describing the oxidised note of roasted coffee.

Table 1: Indices of coffee ageing from a literature survey.

Compounds ratio (Indices)	**Trend over time**	**Reference**
Acetone / 2,3-butanedione	increase	[16,21]
2-Butanone / 2-methylfuran	increase	[20]
2-Methylfuran/ methanol (M/M)	increase	[3,5] [16] [21]
2,3-Butanedione / 2-methylfuran	increase	[16,20-21]
Dimethyl disulphide / methanethiol	increase	[20]
2-Butanone / methanethiol	increase	[20]
Acetone / propanal	increase	[16-21]
Thiophene / propanal	increase	[16,21]
Thiophene / 2,3-butanedione	increase	[16,21]
2-Methyl butanal / 2,3-butandione	increase	[16]
2-Methyl butanal / propanal	increase	[16]
2-Methylfuran / 2-butanone (M/B)	decrease	[3,16] [21]
2-Methylfuran / 2,3-butanedione (M/BD)	decrease	[3,16]
Methanethiol / hexanal (MT/HE)	decrease	[3]
2,3-Butanedione / 2-methylfuran	decrease	[21]

2. MATERIALS AND METHODS

2.1 Reference standards and solvents

Reference compounds for key-odourant identity confirmation were either obtained from the library of standards of the authors' laboratory or purchased from Merck (Mi-lan, Italy). They are listed in Table S2 in the supplementary material and marked with an asterisk. The homologous series of n-alkanes (from n-C9 to n-C25) for Linear Reten-tion Index (ITS) determination and solvents (cyclohexane and dibutyl phtalate), all HPLC-grade, were all obtained from Merck (Milan, Italy).

2.2 Coffee samples

Samples of the different blends and packaging of commercial roasted coffee were sup-plied by Luigi Lavazza S.p.A. (Turin, Italy).

Samples included thirty R&G (roasted and ground) coffees for moka preparation from three lots packed under vacuum in a multilayer film with an aluminium barrier (M samples). M samples consist of Brazilian Arabica and natural Robusta with balanced and full-bodied. A set of Eco caps in modified atmosphere for espresso coffee (5 caps) from different lots of different blends named B and P (100% Arabica of different ori-gins) and I (50/50 Arabica and Robusta) for a total of 30 samples were also included. I samples are top quality Arabica from South America, and Robusta from Africa and Southeast Asia with spicy notes. B is a mix from Central and South America and washed Arabica from organic cultivation. P is a blend from Brazilian, Asian and Cen-tral-South American Arabica, with slight caramel and chocolate notes.

Part of the coffee samples was stored at room temperature; the other part was subjected to accelerated ageing under stressful storage conditions in an oven at 37°C and 50% relative humidity. The samples were classified as good (G) and oxidised (OX) by a trained industrial sensory panel. The oxidised note was defined as the intensity of the smell/aroma attributable to rancid notes, walnut oil, peanut shell, old dried fruit, and "old" coffee, also often referred to as a cardboard note, e.g. damp, or closed/stale pizza box directly/indirectly perceived by the olfactory organ. The reference standard to de-fine the oxidative note was a moka coffee that had been kept open for 4 months at room temperature.

2.3 Sampling conditions

Automated HS-SPME sampling was run on a combi-PAL AOC 5000 Autoinjector as-sembled on a GC-MS system (Shimadzu - Milan, Italy).

SPME fibres, Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) df 65 µm - 1 cm, were obtained from Supelco (Bellefonte, PA, USA). Fibres were conditioned before use as recommended by the manufacturer.

The internal standard IS (n-C13) for peak response normalization was pre-loaded onto the SPME fibre by exposing the SPME fibre to the headspace of a 5 µL IS standard stock solution in dibutyl phtalate (1000 mg/L) for 20 minutes at 50°C [30–32].

2.4 GC-MS/FPD instrument set-up

The GC-MS system consisted of a Shimadzu QP-2010 (Shimadzu - Milan, Italy) oper-ating in EI mode at 70 eV. The GC transfer line was set at 260°C, ion source tempera-ture 200°C. The scan range was set to m/z 35-350 with a scan speed of 666 amu/s. A SolGel-Wax column (100% polyethylene glycol) (30 m × 0.25 mm dc, 0.25 µm df) from Trajan (Melbourne, Australia) was used. The carrier gas was helium, which was used at a constant flow rate of 1 mL/min. The oven temperature program was: 40°C (1min) to 200°C at 3°C/min, then to 250°C (3min) at 10°C/min. FPD detector: 260 °C. All other analysis conditions were those reported in the GC-MS paragraph.

Injections for linear retention index (ITS) determination were carried out using the com-bi-PAL AOC 5000 auto sampler: injection mode split; split ratio 1:20; volume 1 µL; and, temperature 250°C. Fibre thermal desorption was performed in splitless mode.

Data were acquired with a GCMS-LabSolution version 4.3. (Shimadzu-Milan, Italy).

Aroma components sampled from the coffee headspace were either identified by com-paring their calculated ITs and mass spectra to those of authentic samples or, tenta-tively, to those collected in in-house and/or commercial libraries (Wiley 7N and Nist 14 Mass Spectral Data) or reported in the literature. Table S2 reports the compounds identified with their experimental and literature ITs, mass spectral similarity and, where available with reference standards evidenced with an asterisk.

2.5 Statistical analysis

Principal component analysis (PCA), Partial Least Square discriminant analysis (PLS-DA), the Kruskall-Wallis test and Violin plots were performed using XLSTAT software ver. 2021.2.1 (Addinsoft, New York, NY USA). PCA was used to visualize in-formation and sample clusters as a function of combinations of variables, while PLS-DA was used to extrapolate the variables of importance in discriminating the two classes of samples (good/oxidised). The Kruskall-Wallis test was used to evaluate the significance of the selected analytes. The heat map was created by gene-e (https://software.broadinstitute.org/GENE-E/).

3. RESULTS AND DISCUSSION

The following sections address: (a) the chromatographic profiling of the volatile frac-tion of R&G coffee samples and the differences between the good and oxidised samples; b) the extraction of the informative volatiles describing oxidised coffees; c) a compari-son with literature data on the loss of coffee freshness and staling, focusing on potent odorants;d) the determination of potential markers of oxidised coffee regardless of packaging and blend.

3.1 Chemical profiling of good and oxidised samples

Although coffee is a stable product compared to other perishable foods, fresh aromas can be quickly lost over time. After roasting, coffee continues to be an active chemical reactor due to compounds formed from: i) the pyrolysis of non-volatile precursors (i.e. pyridine derivatives from trigonelline); ii) the degradation of lipids and sugars (i.e. acrolein, dihydrofuranones, cyclopenten/exenolones, and pyrones); and, iii) the Mail-lard reaction (i.e. deoxyosone derivatives, 5-hydroxymethylfurfural, Strecker aldehydes etc.) that are still highly reactive. Its stability over time can therefore also be influenced by the physical form of coffee (grains or powder), the barrier provided by the packag-ing and external factors such as temperature, humidity, light and the presence of oxy-gen. Appropriate profiling of the volatile fraction enables diagnostic and unbiased mapping of its volatilome [22]. In this study, volatile-fraction sampling conditions and tools were set to achieve sufficient sensitivity to recover most of the volatiles describing the main aroma notes, while maintaining the complexity, and thus the informative power, of the coffee volatilome [31–33]. The analysed coffee samples were described by the 147 volatiles listed in Table S2. Figure 1 is an illustrative pattern of the chemical signatures of the good and oxidised moka samples (M) visualized with a heat map of the normalized volatile responses versus the IS (n-C13). Samples are clustered using ascendant hierarchical clustering based on Euclidean distances using the average link-age agglomerative method. The samples in the heat map are clustered into two groups: good (MG) and oxidised samples (MOX). A comparison of the two groups allow us to distinguish the pool of components with lower abundance (in orange), i.e. those that are lost or degraded with the oxidation of coffee, from the components that are more abundant in the MOX samples (in brown), indicating their possible formation or in-crease with oxidation.

The principal component analysis (PCA) of all samples in Figure 2 a displays two groups that are separated by the first PC component (F1). The clusters partially overlap because they include different blends, packaging (Eco and standard pack for M) and samples for different

brewing modes (moka and caps for espresso coffee). Looking at the most standardised samples, i.e. moka (Figure 2 b), the clustering of oxidised and good samples is well defined for the first PC with an explained variance (expl.var.) of 77.50% compared to 50.30% of F1 in Figure 2 a; this is nevertheless a positive result in-dicating that the effect of ageing and oxidation is also when looking at different coffees and packs. The PCA carried out only on the cap samples shows the differences between the blends (Figure 2 c); blends P and B are 100% Arabica while blend I is a 50/50 Ara-bica and Robusta mix. They also vary in the roasting processes to obtain the desired aroma for the different commercial blends.

3.2 Extraction of the informative volatiles of the oxidised note

Supervised PLS-DA machine learning was applied to the whole sample set to extract informative volatiles that describe the oxidised coffee note. The samples was splitted in a training set (n=45) and a validation set (n=15). The training set model was cross-validated by Jackknife (CV=5) and the model re-computing after removing each group from the training set, one by one. The cumulative Q^2 statistics was 0.886 on the first 2 components with a classification rate of 93.33%.

Table 2 lists the 71 significant volatiles, out 147, with a VIP (variable importance in projection) > 1 in describing the oxidised samples. VIP scores estimate the importance of variables in the projection used in the PLS-DA model and are often used to select variables; when a variable presents a VIP that is close to or greater than 1, it can be considered important in the given model. Table 2 also reports the VIP standard devia-tions and boundaries at a 95% confidence limit, the Pearson correlation coefficient (r) and descriptions of odour quality.

Most volatiles present high r, and volatiles with a r>0.7 are strongly correlated with oxidation. The components with 0.5<r>0.7 are moderately correlated and are more linked to the packaging. These data show that several volatiles are more abundant in oxidised samples and some, in bold, are common to all packaging. Although coffee aroma is the result of synergistic effect between several volatiles, some of the substances that correlate better with oxidation have been described as having an unpleasant odour.

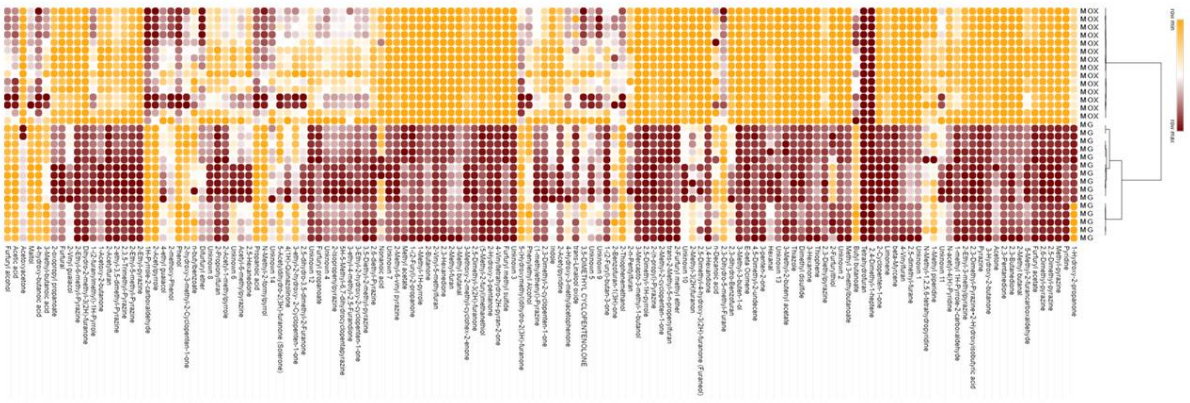

Figure 1 Heat map of the chemical normalized IS fingerprints of the good and oxidised moka samples (M). Samples are clustered using ascendant hierarchical clustering based on Euclidean distances using the average linkage agglomerative method. MG (Moka good coffee) and MOX (Moka oxidised coffees).

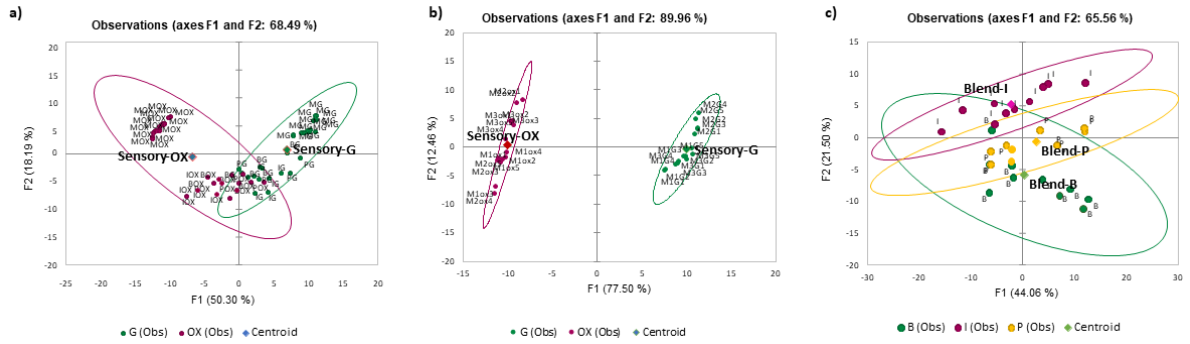

Figure 2: PCA of all samples investigated: a) good and oxidised samples, 2b) Moka good and oxidised samples, c) blends in Eco-caps.

*Table 2: VIP from PLS-DA (variable important in the projection) > 1 in describing oxidised samples, volatile standard deviation and boundaries at a 95% confidence limit, Pearson correlation coefficient (r) and the description of the odour quality together with the compound CV% in the different blends/packaging. Volatiles that decrease or increase in amount in all blends/packaging are indicated in bold, known volatiles from the literature that are related to staling are indicated in italics, while those that display different trends in comparison to literature data are indicated by *.*

Variable	VIP	Standard Deviation	Lower Bound (95%)	Upper Bound (95%)	Pearson Corr Coeff (r)	Odour Description	M	B	P	I
3-Penten-2-one	1.4394	0.2165	1.0151	1.8637	**−0.9097**	**Sharp and acetone-like and fruity, phenolic and fishy**	**−100.00**	**−79.31**	**−77.15**	**−82.32**
2-Methyl-6-vinyl pyrazine	1.3987	0.1979	1.0108	1.7866	**−0.8840**	**Nutty**	**−73.05**	**−68.67**	**−66.57**	**−76.99**
3-Mercapto-3-methyl-1-butanol	1.3850	0.1752	1.0415	1.7284	**−0.8753**	**Meaty**	**−100.00**	**−43.63**	**−61.47**	**−94.05**
1H-Pyrrole-2-carboxaldehyde	1.3830	0.1574	1.0746	1.6914	**0.8741**	**Musty, corny, pungent**	**133.27**	**59.47**	**46.09**	**20.86**
Propanoic acid *	1.3826	0.1738	1.0420	1.7231	**0.8738**	**Pungent, sour milk**	**164.69**	**103.41**	**94.90**	**112.32**
2-Cyclopenten-1-one	1.3818	0.2082	0.9737	1.7898	**−0.8733**	**-**	**−80.82**	**−73.27**	**−72.19**	**−80.48**
2,5-Dimethyl-3(2H)-furanone *	1.3688	0.1657	1.0439	1.6936	**−0.8651**	**Caramellic**	**−83.62**	**−31.17**	**−31.05**	**−64.88**
4,5-Dimethyl-2-undecene	1.3621	0.1363	1.0949	1.6293	−0.8609	-	−100.00	−13.40	−30.64	−10.97
2,6-Dimethyl-pyrazine	1.3532	0.1186	1.1207	1.5857	−0.6007	chocolate	−83.13	−14.27	−14.93	−35.93
Unknown 7 (m/z 94 Ti; 78; 137)	1.3471	0.1335	1.0854	1.6088	**−0.8514**	**-**	**−93.79**	**−76.21**	**−63.80**	**−93.55**
2,5-Dimethyl-pyrazine	1.3441	0.1150	1.1187	1.5695	−0.8495	Nutty, peanut, musty, earthy	−81.37	−15.17	−12.90	−36.53
trans-2-Methyl-5-*n*-propenylfuran	1.3415	0.1059	1.1339	1.5491	−0.8479	-	−100.00	−6.93	−18.27	−27.64
(5-Methyl-2-furyl)methanethiol	1.3332	0.1208	1.0965	1.5700	−0.6007	Sulphurous roasted coffee	−93.81	9.07	−9.72	−58.88
2-Ethyl-pyrazine	1.3214	0.0768	1.1709	1.4719	−0.6007	Nutty	−86.00	−10.17	−6.47	−36.85
2,3-Dimethyl-pyrazine + 2-Hydroxyisobutyric acid	1.3167	0.0667	1.1860	1.4474	−0.8322	Nutty	−81.23	−21.05	−19.29	−35.15
3-Methyl-3-buten-1-ol	1.3157	0.1056	1.1086	1.5227	−0.8315	Sweet fruity	−100.00	2.27	−2.83	−45.65
β-Myrcene	1.3087	0.0987	1.1152	1.5022	−0.8271	Spicy	−93.49	−17.86	−39.94	−25.97
Unknown 2 (m/z 110 Ti; 67; 95; 110)	1.3063	0.0870	1.1357	1.4769	−0.8256	-	−100.00	-6.66	−10.90	−42.11
2-Hydroxy-3-pentanone	1.2972	0.0778	1.1447	1.4496	−0.8198	Truffle	−79.27	19.01	17.10	−19.21
1-(2-Furyl)-2-propanone	1.2963	0.0965	1.1071	1.4855	−0.8193	Caramellic fruity, spicy radish	−94.75	18.43	−2.16	−51.57
2,3-Hexanedione	1.2963	0.1139	1.0731	1.5195	**−0.8193**	**Buttery**	**−100.00**	**−44.12**	**−45.32**	**−81.16**
Methyl-pyrazine	1.2889	0.0497	1.1915	1.3864	−0.8146	Nutty	−88.20	1.20	8.18	−26.97
E-β Ocimene	1.2842	0.1056	1.0772	1.4911	−0.8116	Sweet herbal	−78.07	−18.08	−38.52	−25.83
2,5-Dimethyl-1H-pyrrole	1.2815	0.1200	1.0463	1.5167	**−0.8100**	**-**	**−100.00**	**−95.09**	**−91.86**	**−100.00**
2-Vinyl-5-methylfuran	1.2800	0.0953	1.0933	1.4667	**−0.8090**	**-**	**−99.62**	**−32.72**	**−33.07**	**−81.11**
2,3-Butanedione *	1.2706	0.1258	1.0240	1.5172	**−0.8030**	**Strong butter, sweet creamy, pungent caramel**	**−97.22**	**−70.20**	**−59.22**	**−74.93**
3-Methoxy-2-methyl-cyclohex-2-enone	1.2702	0.1347	1.0063	1.5341	−0.8028	-	−69.30	−13.64	−22.16	−42.05
4-Vinyltetrahydro-2H-pyran-2-one	1.2644	0.1275	1.0146	1.5142	**−0.7991**	**-**	**−98.02**	**−94.02**	**−82.92**	**−97.51**
3,4-Hexandione	1.2582	0.0860	1.0897	1.4267	**−0.7952**	**Buttery toasted, almond, nutty, caramellic**	**−100.00**	**−28.39**	**−33.45**	**−75.84**
Unknown 13 (m/z 57 Ti; 99; 149)	1.2499	0.0914	1.0708	1.4290	−0.7899	-	−100.00	−12.07	−21.41	−54.20
2-Ethyl-6-methyl-pyrazine	1.2489	0.0366	1.1771	1.3207	−0.7893	Nutty	−70.21	−19.38	−18.96	−26.23
5-(Hydroxymethyl)-dihydro-2(3H)-furanone	1.2486	0.1323	0.9893	1.5080	**0.7892**	**-**	**227.90**	**71.38**	**25.43**	**47.26**
N-Methyl-2-formylpyrrol	1.2421	0.1153	1.0162	1.4680	0.7850	Musty	59.35	34.05	34.82	−10.28
3-Hexanone	1.2420	0.0511	1.1418	1.3421	−0.7849	Fruity	−100.00	16.55	−2.18	−22.28
1-Methyl-1H-pyrrole	1.2304	0.0708	1.0916	1.3692	−0.7777	Powerful smoky woody	−99.27	−37.86	−15.68	−86.77
2-Furfuryl methyl ether	1.2298	0.0555	1.1210	1.3387	−0.7773	Roasted coffee	−100.00	4.02	−1.64	−25.91
Acetoxyacetone	1.2255	0.0553	1.1172	1.3338	−0.7745	Fruity buttery, dairy nutty	−56.87	4.86	7.71	−43.39
Methyl 3-methylbutanoate	1.2248	0.0373	1.1518	1.2978	−0.7741	Strong apple fruity	−100.00	−0.85	−8.82	−22.56
Unknown 3 (m/z 43 Ti; 71; 86)	1.2217	0.0650	1.0943	1.3491	−0.7705	-	−84.10	2.44	−3.15	−35.78
Dihydro-2-methyl-3(2H)-furanone	1.2191	0.0509	1.1194	1.3189	−0.7705	Bready	−96.01	18.00	19.12	−27.59
2-Ethyl-5-methyl-Pyrazine	1.2163	0.0542	1.1100	1.3226	−0.7687	Coffee bean	−65.21	−18.77	−18.27	−26.70
Acetic acid *	1.2122	0.1590	0.9005	1.5238	**0.7661**	**Sharp pungent sour vinegar**	**81.92**	**89.61**	**115.20**	**93.88**
2-Methyl-2-cyclopenten-1-one	1.1964	0.0822	1.0354	1.3574	−0.7562	-	−80.09	7.49	10.57	−29.86
Unknown 12 (m/z 81 Ti; 53; 161)	1.1908	0.0638	1.0657	1.3159	**−0.7526**	**-**	**−49.90**	**−31.58**	**−32.12**	**−75.80**
2-Acetylpyridine	1.1886	0.0713	1.0488	1.3284	−0.7512	Popcorn	−47.45	−0.70	−10.46	−32.11
2-Thiophenemethanol	1.1884	0.1027	0.9872	1.3897	0.7511	Savory roasted coffee	60.09	16.15	7.91	−24.96
3-Methyl-2-butenyl acetate	1.1855	0.0694	1.0496	1.3215	0.7511	Sweet fresh banana	−100.00	−16.60	−11.96	22.33
2-Acetylfuran	1.1743	0.1149	0.9491	1.3995	−0.7422	Sweet balsam, almond, cocoa, caramel coffee	−53.67	27.11	16.78	−17.42
Pyrazine	1.1702	0.1051	0.9642	1.3762	−0.7396	Pungent sweet corn like roasted hazelnut barley, floral	−93.84	18.49	37.49	-25.82
2,5-Dimethyl-4-hydroxy-3(2H)-furanone (Furaneol) *	1.1615	0.0986	0.9684	1.3547	**−0.7341**	**Sweet cotton candy caramel**	**−100.00**	**−51.51**	**−27.84**	**−79.86**
Unknown 1 (m/z 57 Ti; 43; 86)	1.1510	0.0689	1.0159	1.2861	−0.7275	-	−100.00	32.65	31.86	−15.13
Furfuryl methyl sulphide	1.1471	0.1065	0.9384	1.3558	−0.7250	Onion, garlic, sulphury	−100.00	4.89	−20.66	−58.08
Furfuryl acetate	1.1453	0.0570	1.0335	1.2571	−0.7239	Garlic, pungent vegetable, onion	−58.77	10.41	−6.46	−34.68
2,3-dihydro-benzofuran	1.1398	0.0967	0.9503	1.3292	**−0.7204**	**-**	**−69.61**	**−40.42**	**−55.80**	**−75.81**
2-Ethyl-3-methylpyrazine	1.1257	0.1581	0.8159	1.4356	−0.7115	Raw potato	−64.47	−19.53	−16.08	−20.59
4-Vinylfuran	1.1244	0.0512	1.0241	1.2247	**−0.7106**	**-**	**−100.00**	**−27.21**	**−29.47**	**−86.08**
3-Hydroxy-2-butanone	1.1204	0.1519	0.8227	1.4180	−0.7081	Sweet buttery creamy	−77.64	48.61	50.30	1.99
2,5-Dimethyl-furane	1.1092	0.0570	0.9975	1.2208	−0.7010	Meaty	−100.00	6.62	14.55	−36.44
***trans*-Linalool oxide**	1.1006	0.1842	0.7395	1.4617	**−0.6956**	**-**	**−20.50**	**−31.54**	**−43.72**	**−36.60**
1-Methyl 1H-Pyrrole-2-carboxaldehyde	1.0968	0.0968	0.9070	1.2866	−0.6932	-	−36.14	4.63	−1.09	−25.06
Limonene	1.0918	0.1373	0.8227	1.3609	−0.6900	Terpenic	−54.76	19.10	−45.30	−9.57
Pyridine	1.0895	0.1543	0.7871	1.3919	−0.6886	Fishy	−91.20	0.29	9.05	−29.83
Furfural *	1.0775	0.0508	0.9780	1.1770	**−0.6810**	**Sweet woody almond fragrant baked bread**	**−68.79**	**−38.45**	**−35.34**	**−64.13**
2-Propionylfuran	1.0759	0.1379	0.8056	1.3462	−0.6800	Fruity	−40.96	12.05	−2.07	−22.46
Butyl butanoate	1.0738	0.1853	0.7106	1.4370	0.6787	Sweet, fruity, fresh, diffusive, and ripe	178.85	38.13	−48.61	82.24
Thiazole	1.0715	0.2109	0.6580	1.4849	−0.6772	Fishy	−100.00	28.64	36.27	−4.72
N-acetyl-4(H)-pyridine	1.0695	0.1148	0.8444	1.2946	−0.6759	Burnt	−53.74	−16.85	−15.76	−48.11
Acetone	1.0676	0.2008	0.6740	1.4611	−0.6747	Ethereal, apple, pear	−97.27	21.50	98.39	−44.69
2-Butanone	1.0594	0.1815	0.7036	1.4151	−0.6583	Ethereal	−98.10	53.26	111.98	−11.23
2,3-Pentanedione *	**1.0515**	**0.0663**	**0.9216**	**1.1814**	**−0.6646**	**Buttery, sweet, nutty, pungent**	**−99.44**	**−54.20**	**−43.11**	**−79.92**
2-Methyl-furan	1.0416	0.0949	0.8556	1.2277	0.6054	Chocolate	−99.55	19.61	61.60	−52.88
3-Methyl butanal *	0.9974	0.0876	0.8257	1.1692	**−0.6304**	**Malty, cocoa, fruity**	**−99.37**	**−34.48**	**−21.75**	**−57.25**
2,3,5-Trimethyl-pyrazine	0.9929	0.2666	0.4703	1.5155	−0.6275	Nutty, cocoa, earthy	−50.68	−2.96	-3.57	−11.81
2,3-Dimethyl-2-cyclopenten-1-one	0.9904	0.2087	0.5813	1.3995	−0.6259	-	−33.81	−7.82	−14.35	−35.06
2-Furfurylthiol *	0.9713	0.0378	0.8973	1.0453	**−0.6139**	**Roasty (coffeelike)**	**−100.00**	**−100.00**	**−100.00**	**−100.00**
2-Methyl butanal *	0.9627	0.1193	0.7288	1.1966	−0.6084	Green, malty, buttery	−99.57	6.25	22.49	−11.14
1-Acetoxy-2-butanone	0.9613	0.1127	0.7404	1.1822	−0.6076	-	−43.87	−1.30	−7.75	−41.96
Indole	0.9600	0.1225	0.7200	1.2001	−0.6068	-	−52.19	−12.20	−20.45	−57.89

This result means that several components change independently of the packaging and blends, although to different extents. Figure S1 shows the percentage coefficients of variation (CV%) of the normalized responses of the good versus oxidised samples in the different packages. The figure shows variations above 20%, which is the relative standard deviation of the analytical method (RSD% > 20), whose limits are indicated in the figure by the orange horizontal dashed lines. The CV% was calculated using equation 1:

CV%= - [(good norm. response – oxidised norm. response)/ good norm. response]*100 (eq.1)

Moka samples (Figure S1 a) show a significant reduction in aroma components com-pared to the capsules (Figure S1 b, c and d), especially in the volatile components asso-ciated with freshness notes, such as sulphurous compounds (thiols), pyrazines, pyrroles and alcohols; and this fact could be related to the higher surface exposure of the matrix to potential oxidation and humidity effects [34]. On the other hand, the composition of the volatile fraction of coffee in capsules varies less, probably because it is packaged under a modified atmosphere.

3.3 Comparison of the informative volatiles in oxidised coffees and literature data on markers of coffee aging

Several studies have investigated the change in coffee aroma over time, and most have focused on the loss of freshness (i.e. staling) and/or have monitored the evolution of aroma over a short period [2–4,8,16–18,21,35], with only a few investigating long-term effects [7,20]. Table S1 lists the volatiles that have been reported in previous studies as being linked to the deterioration of roasted coffee aroma.

Few volatiles characterising the oxidised coffees in our samples have already been de-scribed in the literature as ageing markers. The components that showed similar trends in the aged samples to those previously reported are: 2/3-methylbutanal (malty); 2,3-butandione (buttery, pungent); 2,3-pentandione (buttery, pungent); 2-furfurylthiol (roasty, coffee- like); 2,5-dimethyl-3(2H)-furanone (caramellic), 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol) (sweet, candy) (those with a VIP higher than 1 are highlighted with an asterisk in Table 2).

In this study, a number of components known to be related to ageing behaved differ-ently in oxidised samples than described in the literature (i.e. 2-butanone, 2-methylfuran, hexanal,

2-acetylfuran, dihydro-2-methyl-3(2H)-furanone) (highlighted in italics in Table 2) or do not vary significantly (CV % below 20%) in all samples stud-ied (i.e. 1-methyl-1H-pyrrole, N-acetyl-4(H)-pyridine). The latter observation, in par-ticular, may be related to the different blends and packaging, here considered different from the literature data, indicating that staling or ageing indices depend on species, blend and packaging [2,11,17,20].

For example, 2-butanone (ethereal) was observed to be present in lower amounts in oxi-dised samples M and I, as reported by Glöss and Marin who noted a reduction over time [3,20,35], and on the opposite in B and P samples.

Hexanal (fatty, sweaty) is a known by-product and marker for the degradation of lino-leic acid by autoxidation, which increased over time (Table S1) [3,14,36]. Surprisingly, it is present in lower amounts in the oxidised coffees than in the good coffees in our ex-periments (Figure 3). This can be explained by its high reactivity and the strongly oxi-dative environment, which leads to the formation of hexanoic acid (sour fatty sweat). 2-acetylfuran (sweet balsam, caramel) decreased in amount in the M (100% Arabica for moka) and I samples (a blend containing Robusta in Eco caps), while, on the other hand, it increased in B and P (100% Arabica in Eco caps) (Figure 3 and Table 2). The behaviour of 2-acetylfuran in I samples confirms the previous results of Cincotta et al., which cover 6 months of storage [7]. These authors also report that 2-acetylpyrrole (musty, nutty) decreases in blends containing Robusta, while, in this study, it increased in aged samples (Figure 3 and Table S2). 4-Vinylguaiacol (phenolic, smoky) was indi-cated as a staling component by Kallio and Holscher [15,16] as it decreases with time, as it does in all oxidised coffees in these experiments although its VIP is below 1 (i.e. 0.8919) and (r) of -0.5637 (Figure 3 and Table S2). This behaviour, however, is not con-firmed in Robusta samples in caps after 6-months storage [7] (Table S1).

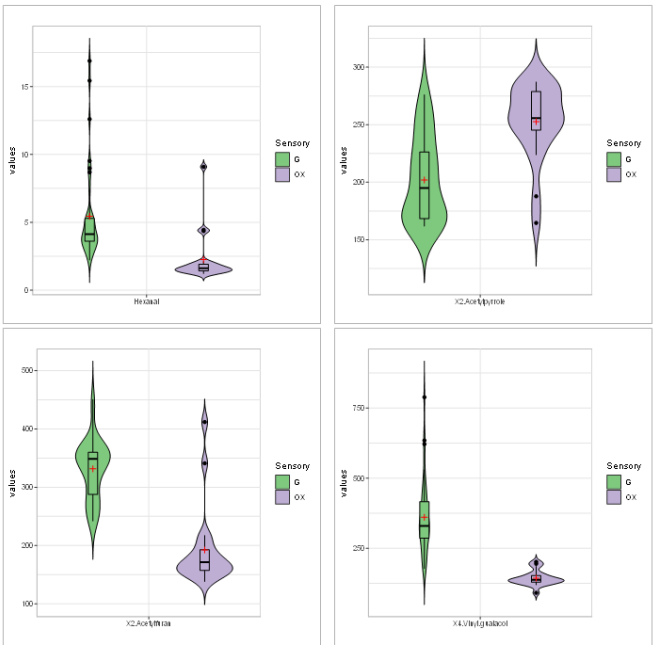

Figure 3: Violin plots of the trends of some markers from the literature in analysed samples.

Furfurylthiol (FFT- roasty, coffee-like) is known as a potent coffee odorant and freshness marker [19,33,36]. It is not included in the selected VIP because it is outside the fixed VIP cut-off. The FFT has a VIP of 0.9713, a moderate correlation with the oxidation process (r = 0.6323) and decreases dramatically in oxidised samples from the first stag-es of storage. The decrease in FFT may also be due to evaporation, adsorption onto the solid matrix and internal reactions. FFT, and all thiols in general, are highly reactive nucleophiles that can degrade in the presence of hydroperoxides, oxidise (e.g. MeSH to DMDS) and react with phenolic compounds [37]. In particular, Hofmann et al., showed that FFT decreases because it becomes trapped by low-molecular-weight melanoidins, unlike what happens with mercaptan-aldehydes [38–40]. More recently, Glöss et al.,[20] have identified the ratio between DMDS (sulphur, cabbage) and MeSH (sulphur, cabbage) as a freshness index for coffee, and underlined that DMDS increases because of the oxidation of MeSH. This index was recognized by monitoring the transformation of coffee aroma quality over time at room temperature. This condition is not often investigated over a longer period of time, since the temporal evolution of coffee aroma, e.g. to evaluate its shelf-life, is usually determined under accelerated conditions due to the stability of coffee over time. In the present study, this behaviour was observed in oxidised Eco-caps coffees, while the index was not measurable in M samples, since DMDS and DMTS (dimethyl trisulphide) (onion-like) were not detected. On

the other hand, dimethyl sulphone (sul-phurous burnt), which is a MeSH derivative that is related to a strong oxidation, in-creased threefold (Table 3) [36].

Table 3: Comparison some sulphur markers from the literature and the DMDS/MeSH ratio in good and oxidised samples. §Under limit of detection.

	Dimethylsulphone		**Dimethytrisulphide**		**Dimethyldisulphide**		**Methanthiol**		**Ratio DMDS/MESH**	
	Good	**Ox**	**Good**	**Ox**	**Good**	**Ox**	**Good**	**Ox**	**Good**	**Ox**
M	0.05	0.15	0.05	0.00	3.53	n.d.§	0.13	0.06	26.45	n.d. §
B	0.07	0.03	0.07	0.03	2.65	5.61	0.19	0.08	14.01	74.25
P	0.07	0.07	0.10	0.02	3.18	19.09	0.16	0.12	19.74	153.94
I	0.06	0.10	0.10	0.04	3.50	12.44	0.11	0.12	30.85	100.02

3.4 Potential markers of the oxidised coffee note

Twenty-five highly significant volatiles describing oxidised coffees with a similar trend in all blends and packages studied, and with a CV % of at least 20, were identified as potential markers for oxidised coffees. They are listed in bold in Table 2.

Most of them are present in oxidised samples in lower amounts, some are already known to decrease over time (in italics in Table 2) and others behave differently than in previous studies. These compounds are highly reactive heterocycles, especially in the presence of humidity and oxygen, which could explain their decrease in oxidised cof-fees [36]. Four volatile compounds increase in all samples examined, albeit to varying degrees depending on the mixture and packaging: acetic and propionic acids (pungent, sour, rancid); 1-H-pyrrole-2-carboxaldehyde (musty); and, 5-(Hydroxymethyl)-dihydro-2(3H)-furanone.

Short-chain fatty acids are formed in roasted coffee by the breakdown of polysaccha-rides during roasting. Their continuous increase during storage could be due to the chemical cleavage of triglycerides (TGA), which contributes to the change in sensory properties [9].

The typical oxidised note perceived by the olfactory organ is due to the altered balance of the volatiles in oxidised samples and can be associated with rancid, walnut oil, pea-nut shell, old dried fruit and “old" coffee notes. The coffee volatilome includes both odourant and non-odourant compounds, the latter of which contribute to a synergistic effect in aroma

perception [41–43]. It has highly informative power in describing the potential evolution of aroma over time [7,15,21], and is a diagnostic tool for the “iden-titation” of blends/origins and the detection of applied technological processes [44–47].

4. CONCLUSIONS

Twenty-five target components of the coffee volatilome were identified as markers of coffee oxidation because they showed the same behaviour and statistical significance in all samples studied, regardless of packaging and blending. Together, they play a syner-gistic role in the detection of oxidised coffee and can be considered as the fingerprint of the oxidised note. Four volatiles associated with ageing increased in all the packages studied: acetic and propionic acids (pungent, sour, rancid), 1-H-pyrrole-2-carboxaldehyde (musty) and 5-(hydroxymethyl)-dihydro-2(3H)-furanone, while the other 21 decreased in oxidised coffees.

The literature survey suggests several compounds as markers for the decrease in aroma quality of R&G coffee during storage, but they are not standardised. However, most of the proposed markers and indices seem to be more closely related to coffee aroma freshness, in particular: 2 and 3-methylbutanal (malty), 2-propanal (ethereal pungent, earthy), 2,3-butan and pentandione (buttery, pungent), dimethyl sulphide (cabbage-like), DMDS (sulphur, cabbage), MeSH (sulphur, cabbage), 2-FFT (roasty, coffee- like), and the ratios of 2-methylfuran/2-butanone, 2-methylfuran/2,3-butanedione, and MeSH/hexanal, the decrease of which was associated with coffee staling, or DMDS/MeSH, which increased with storage.

The composition of the volatilome also depends on packaging and blend. A comparison of these results with those in the literature shows that some markers are in common and present in lower amounts in oxidised coffees, these include 2,3-butanedione, 2,3-pentandione, 2-furfurylthiol, acetic acid (pungent, sour) and furaneol (sweet, candy), and the indices 2-methylfuran/2-butanone and 2-butanone/2-methylfuran. Conversely, other components seem to be specific to the packaging and/or blend, including 2-methylfuran, 2-methylbutanal, 2-acetylfuran, 2,5-dimethylfuran (meaty), 2-methyl-2-cyclopenten-1-one.

These data provide information on the oxidised note and show that artificial intelligence can be used successfully to instrumentally define the change in the quality of the coffee aroma over time.

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SUPPLEMENTARY

Figure S1: CV% graph visualization of the standardized responses of the chromatographic profiles together with the CV% cut-off>20 (orange range).

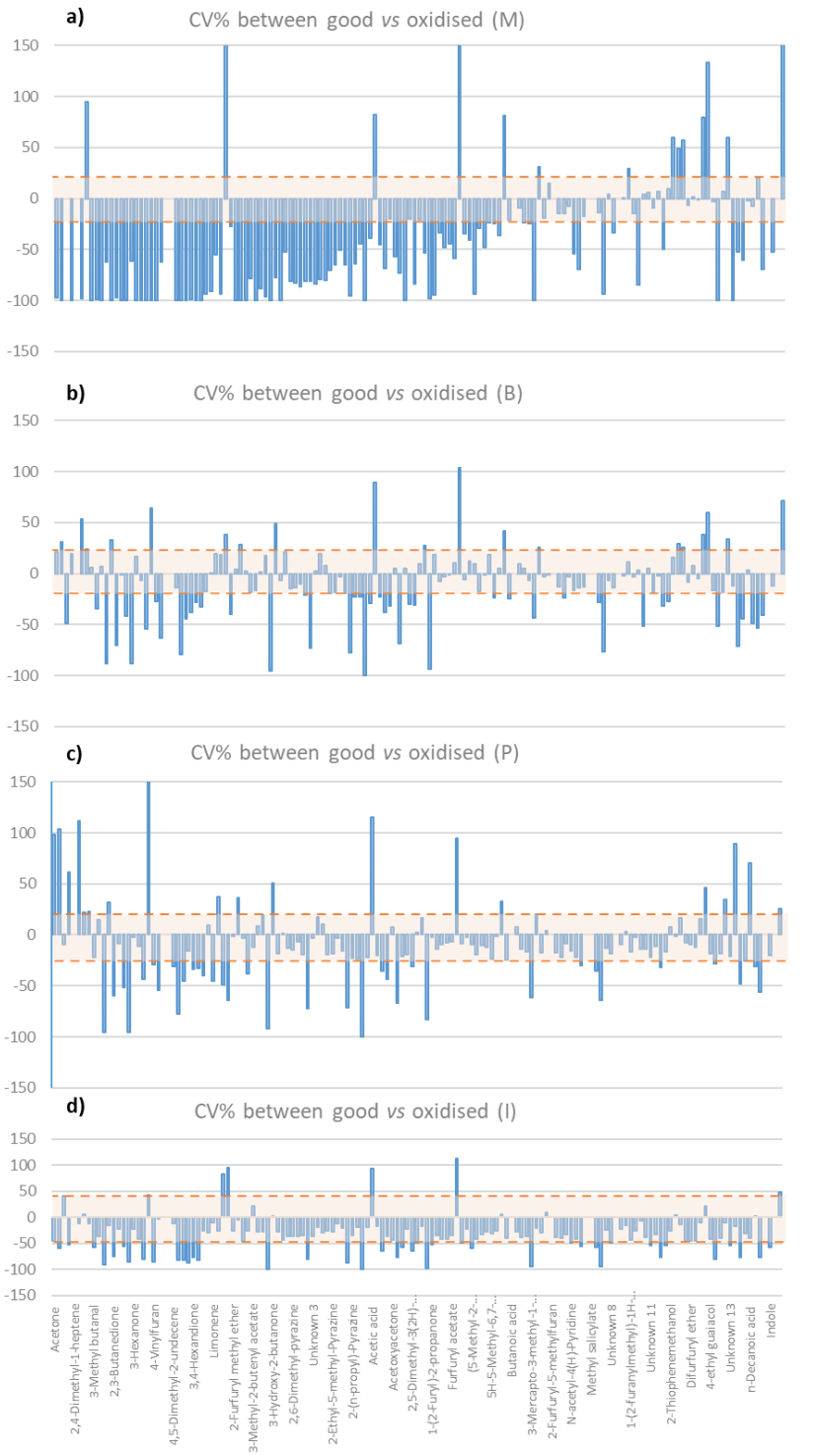

Table S1: lists the volatile substances that have so far been associated in the literature with the deterioration of the aroma of roasted coffee.

Compounds	Trend over time	Aging conditions	Notes
Indices			
2-Methylfuran and 2-butanone (M/B)	decrease	Linear decrease in air-permeable packages by about 0.1 unit a day [16]. Slower decrease in beans than in ground coffee samples; it remains constant for 7 months in beans and quickly decreases after grinding. Decrease during storage in dark roasted coffee[21].	Component related to freshness. Decrease related to staling. Decrease of M/B index due to oxidation and loss of 2-methylfuran through diffusion [3,16].
2-Butanone/2-methylfuran	increase	Increase with storage in coffee beans packed in paper and plastic composite films; slight decrease in packages with aluminium layer [20] .	Increase related to staling. Ratio mainly driven by the high volatility of 2-methylfuran; increase with packaging material allowing 2-methylfuran to evaporate. With packaging preventing its loss, the ratio is mainly governed by the small differences in intrinsic reactivity among the two compounds. This ratio increases in absence of an aluminium layer in the packaging material leads; very small variations over time are observed with packaging with aluminium layer [20].
2-Methylfuran/ methanol (M/M)	increase	Increase in air-permeable bags during 12 weeks' storage [16]	Indicator of ageing [21]. Related to freshness [3,5]
			Freshness index [5,8]
2-Methylfuran and 2,3-butanedione (M/BD)	decrease	Ratio decrease in coffee punctured packages [3,16]	Decrease related to staling [3]
2,3-Butanedione / 2-methylfuran	increase	Increase with storage in coffees packed in paper and plastic composite films. Slight decrease in packages with aluminium layer [16,20-21]	Index evolution depends on packaging [20]
2-Methylfuran and propanal (M/P)			Freshness index [3]
Methanethiol and 2,3-butanedione (MT/BD)			Freshness index [3]
Dimethyl disulphide/methanethiol	increase	Increase with storage in coffee capsules packed in aluminium layer package; stable with packages 100% aluminium [20]	Methanethiol is a highly volatile and reactive compound; it tends to oxidize and dimerize to dimethyl disulphide [20]
Methanethiol and hexanal (MT/HE)	decrease		Freshness index [3]
2-Butanone/methanethiol	increase	Increase with storage in coffee aluminium layered capsules, stable with 100% aluminium packages[20]	Methanethiol is a highly volatile and reactive compound; it tends to oxidize and dimerize to dimethyl disulphide [20]
Acetone / 2,3-butanedione	increase		Increase with storage [16,21]
Acetone /propanal	increase		Indicators of coffee ageing [16-21]
Thiophene/propanal	increase		Indicators of coffee ageing [21]. Increase with storage [16,21]
Thiophene/ 2,3-butanedione	increase		Indicator of coffee ageing; increase with storage [16,21]
2,3-Butanedione/2-methylfuran	decrease		Indicator of coffee ageing [21]
2-Methyl butanal/2,3-butandione	increase		Increase with storage [16]
2-Methyl butanal/propanal	increase		Increase with storage [16]
Aldheydes			
Hexanal	increase	Increase dependent on the time of exposure of roasted and ground coffee to air, in particular, with storage in air and in punctured packages [3]. More than double increase after 83 days of storage in valve bags under nitrogen atmosphere at 37°C (with less than 1% of oxygen) [14]	It is not a potent odorant, but its secondary oxidation volatile products develop oxidised off-notes in particular when the positive flavour compounds in roasted coffee start to decompose/evaporate [14]. Odour not characteristic of stale coffee. Not influenced by humidity [14]
Acetaldehyde		At room temperature, a small decrease for pads individually packed and hermetically sealed than in pads that were enclosed together in a secondary pack [6]. The loss is less drastic when pads were stored in plastic bottles [6].	
Propanal	decrease	Decrease by more than 80% in permeable bag [16,21]	
2-Methylpropanal	decrease	At room temperature, a small decrease for pads individually packed and hermetically sealed than in pads that were enclosed together in a secondary pack [6]. Decrease by more than 50% after 74 days storage in permeable bag [16].	Evaporation or degradation by oxidation [3]. Intense aromatic note in freshly roasted coffee [15].
2-Methylbutanal	decrease	At room temperature, a small decrease for pads individually packed and hermetically sealed than in pads that were enclosed together in a secondary pack [6]. Decrease by more than 50% after 74 days storage in permeable bag [16]. Degradation after 83 days of storage in valve bags at 37°C under nitrogen atmosphere (with less than 1% of oxygen with an oxygen concentration lower than 1%) [14]).	Quick degradation in coffees with high moisture content [14]. Evaporation or degradation by oxidation. Contribution to the freshness of roasted coffee aroma. Coffee ageing indicator [3,15]
3-Methylbutanal	decrease	At room temperature, a small decrease for pads individually packed and hermetically sealed than in pads that were enclosed	Fast degradation in coffee with the highest moisture content [14]. Evaporation or degradation by oxidation. It contributes to

Compounds	Trend over time	Aging conditions	Notes
		together in a secondary pack [6]. Decrease by more than 50% after 74 days storage in permeable bag [16] .	the freshness of roasted coffee aroma. Coffee ageing indicator [3,15].
ketones			
2,3-Butanedione	decrease	It significantly decreases in coffee packed under an air atmosphere than under a modified atmosphere [3]. At room temperature, a small decrease for pads individually packed and hermetically sealed than in pads that were enclosed together in an secondary pack [6]. Decrease by more than 50% after 74 days storage in permeable bag. Decrease of more than 80% in permeable bags [16]. Degradation after 83 days of storage at 37°C in valve bags under nitrogen atmosphere (with less than 1% of oxygen) [14].	Associate with buttery aroma in ground and brewed coffee [3,15]. Fast degradation in coffees with high moisture content [14].
2-Butanone	decrease		Indicator of roasted coffee staling and ageing. [21]
2,3-Pentanedione	decrease	At room temperature, a small decrease for pads individually packed and hermetically sealed than in pads that were enclosed together in a secondary pack [6]. Decrease by more than 50% after 74 days storage in permeable bag [16]. Degradation after 83 days of storage at 37°C in valve bags under nitrogen atmosphere (with less than 1% of oxygen) [14].	Associate with buttery flavour in ground coffee and coffee brew [3,15]. Degrades faster in the coffee with highest moisture content [14].
Sulphur compounds			
Dimethylsulphide	decrease	Degradation after 83 days of storage at 37°C in valve bags under nitrogen atmosphere (with less than 1% of oxygen) [14]	Fast degradation in coffees with high moisture content [14]. It contributes to the freshness of roasted coffee [14]
Dimethyldisulphide	decrease	Absence of significant variations throughout storage in the brews at 4 °C. Progressive and significant decrease in brews stored at 25 °C [28]. Losses during the storage of roasted coffee beans [15] and ground roasted coffee [16] in air.	
2-Furfurylthiol	decrease	Accelerated degradation in absence of protection from external conditions by proper packaging [3]. Significant reduction in coffee packed under air than in those under modified atmosphere [3]. Degradation after 83 days of storage at 37°C in valve bags under nitrogen atmosphere (with less than 1% of oxygen) [14]	Used as an indicator of coffee staling. Evaporation or degradation by oxidation [3]. Fast degradation with high moisture content [14].
Methanethiol	decrease	It showed a decrease over time [15].	Strong impact on aroma freshness [3]. Evaporation or degradation because of oxidation [3]. Its loss is correlated to the loss of freshness [5]
2-Furan methanethiol	decrease		It contributes to the pleasant aroma of fresh roasted coffee [15]
3-Methyl-2-buten-1-thiol	decrease		It contributes to the pleasant aroma of fresh roasted coffee [15]
Furfuryl mercaptan	increase	Increase during storage in permeable bags [16]	Responsible for stale flavor[5]
5-Methylfurfuryl mercaptan	increase	Increase during storage in permeable bags [16]	Responsible for stale flavor[5]
Dimethyl trisulphide	increase	Increase at atmospheric pressure and under vacuum [14]	It derives from oxidation of methanethiol. Large increase with increasing of moisture content, related to a faster oxidation of thiols [14].
Carbon disulphide	decrease	Decrease by more than 75% after 74 days storage in permeable bag [16]	
Thiophene	decrease	Decrease by more than 80% in permeable bag [16]	
Thiophene-3-aldehyde	decrease	Decrease by more than 80% in permeable bag [16]	
3-Methylthiophene	decrease	Decrease by more than 80% in permeable bag [16]	
Pyrazines			
2,6-Diethylpyrazine	increase [3] decrease [7]	It increases during storage in air and in punctured packages [3]. Decrease in Robusta coffee caps after 6 months of storage [7].	Odour not characteristic of stale coffee.
2,3-Diethylpyrazine	decrease	Decrease in Arabica coffee caps [7]	
2-Ethylpyrazine		No losses in two pads during storage due to its high boiling point (above 115°C) [6]	
2-Acetyl pyrazine	decrease	Decrease in Arabica coffee caps [7]. Decrease in Robusta after 6 months of storage [7].	
2-Ethyl-3,5-dimethylpyrazine	decrease	Decrease in Robusta after 6 months of storage [7].	
Esters			
Ethylacetate	decrease	Higher decrease in coffee packed under air than under modified atmosphere [3]	
Methyl acetate	decrease	Decrease by more than 80% in permeable bag [16]	
Methylthioacetate	decrease	Decrease by more than 80% in permeable bag [16]	
Furans			

Compounds	Trend over time	Aging conditions	Notes
2-Furfurylfuran	decrease	Decrease in Robusta after 6 months of storage [7].	
2-Acetylfuran	decrease	No losses in two pads during storage due to its high boiling point (above 115°C) [6]. Decrease in Robusta coffee caps after 6 months of storage [7].	
2-Methylfuran	decrease	Decrease by more than 50% after 74 days storage in permeable bag [20]	Indicator of staling roasted coffee and aging [21]. Formed by pyrolysis, decrease in presence of oxygen [21].
2,5-Dimethylfuran	decrease	Decrease by more than 80% in permeable bag [16]	Indicator of the change of ground coffee [21]
2-Ethylfuran	decrease	Decrease by more than 80% in permeable bag [16]	
Furfural	increase		Oxidation product of furfuryl alcohol [8]
Methyl formate	decrease	Decrease by more than 80% in permeable bag [16]	
3-Mercapto-3-methyl butyl formate	decrease	Degradation after 83 days of storage at 37°C in valve bags at nitrogen atmosphere (with an oxygen concentration lower than 1%) [14].	Decrease more in coffee with high moisture content [14]
Furanones			
Dihydro-2- methyl-3(2H)-furanone		No losses in two pads during storage due to the high boiling point (above 115°C) [6]	
2,5-Dimethyl-2,3-dihydrofuranone	decrease	Decrease in Arabica coffee caps [7]	
Furaneol	decrease	Decrease in Arabica coffee caps [7]	
Phenolic compounds			
4-Vinylguaiacol	increase [16] decrease [7]	Increase during storage in permeable bags [16]. Decrease in Robusta coffee caps after 6 months of storage [7].	Responsible for stale flavor [15]
4-Ethylguaiacol	decrease	Decrease in Robusta coffee caps after 6 months of storage [7].	
Guaiacol	decrease	Decrease in Robusta coffee caps after 6 months of storage [7].	
Phenol	decrease	Higher decrease in coffee packed under air than under modified atmosphere [3].	
Pyrroles			
1-Methylpyrrole	decrease	Higher decrease in coffee packed under air than under modified atmosphere [3]. Degradation after 83 days of storage at 37°C in valve bags under nitrogen atmosphere (with less than 1% of oxygen) [14].	Quick degradation in coffee with high moisture content [14].
2-Acetylpyrrole	decrease	Decrease in Robusta caps after 6 months of storage [7].	
1-Furfurylpyrrole	decrease	Decrease in Robusta coffee caps after 6 months of storage [7].	
1-Furfuryl-2-methylpyrrole	increase	Increase during storage in permeable bags [16].	Responsible for stale flavor
2-Formyl-1-methylpyrrole	decrease	Decrease in Robusta coffee caps after 6 months of storage [7].	
Pyridines			
N-acetyl-4H-pyridine	decrease	Decrease in Robusta coffee caps after 6 months of storage [7].	
Acids			
Acetic acid	increase	Volatile fraction of Arabica caps, stable during storage up to 12 months. Increase of free fatty acids (FFA), similar behaviour in Robusta coffee caps [7].	
Propionic acid	increase	Volatile fraction of Arabica caps, stable during storage up to 12 months. Increase of free fatty acids (FFA), similar behaviour in Robusta coffee caps [7].	
Tetradecanoic acid	increase	The volatile fraction of Arabica coffee caps, remains quite stable during shelf life up to 12 months of storage. After this period free fatty acids (FFAs) increase even in Robusta coffee caps	
Pentadecanoic acid	increase	Volatile fraction of Arabica caps, stable during storage up to 12 months. After this period FFAs increase even in Robusta coffee caps [7].	
Hexadecanoic acid	increase	Volatile fraction of Arabica caps, stable during storage up to 12 months. After this period, FFAs increase even in Robusta coffee caps [7].	
3-Methylbutanoic acid		No losses in two pads during storage due to its high boiling point (above 115°C) [6].	

*Table S2: Volatiles identified with their experimental and literature retention indices ITs, Target ion (Ti) and qualifier ions (Qis) and their mass spectral similarity index (SI). M, B, P and I are the different packaging/blends for which the CV% of the coffee profiling, presented in conditional format, is reported (in yellow CV%<-20 and in red CV%>20). *Volatiles confirmed by reference standard.*

N°	Compounds name	Rt	I^Tcalc	I^Tref	Ti	Qi	SI	M	B	P	I
1	Acetone*	2.22	821	820	43	58-42	98	-97.27	21.50	98.39	-44.69
2	Methyl acetate*	2.30	832	825	43	74-42	94	-100.00	31.45	103.40	-59.67
3	Tetrahydrofuran*	2.48	854	-	42	72-58	97	nd	-49.01	-9.19	40.89
4	2-Methyl-furan	2.62	871	868	82	53-81	98	-99.55	19.61	61.60	-52.88
5	2,4-Dimethyl-1-heptene	2.68	885	885	43	55-70	96	nd	nd	nd	nd
6	2-Butanone*	2.89	903	899	43	72-57	96	-98.10	53.26	111.98	-11.23
7	2,3-Dihydro-5-methyl-Furane	2.95	907	-	84	39-83	80	95.04	23.62	22.36	5.75
8	2-Methyl butanal	3.03	912	907	57	41-58	96	-99.57	6.25	22.49	-11.14
9	3-Methyl butanal	3.08	916	925	44	41-43	97	-99.37	-34.48	-21.75	-57.25
10	2,5-dimethyl-furane	3.58	958	958	96	81-53	97	-100.00	6.62	14.55	-36.44
11	1-Methyl piperidine	3.11	959	-	98	57-43-71	96	-62.27	-88.10	-95.87	-90.86
12	Unknown 1 (m/z 57; 43; 86; 41)	3.92	971	-	57	43-86-41	89	-100.00	32.65	31.86	-15.13
13	2,3-Butanedione*	3.99	977	998	43	86-42	99	-97.22	-70.20	-59.22	-74.93
14	Methyl 3-methylbutanoate	4.70	1043	1018	74	43-59-85	91	-100.00	-0.85	-8.82	-22.56
15	Thiophene*	4.79	1045	1022	84	58-45	93	-100.00	-41.94	-52.01	-56.38
16	N-Methyl-1,2,5,6-tetrahydropyridine	4.58	1039	-	96	54-82-68	95	-61.23	-87.97	-95.17	-85.11
17	3-Hexanone*	5.39	1064	1052	57	43-71-100	93	-100.00	16.55	-2.18	-22.28
18	Unknown 2 (m/z 110; 67; 95)	5.53	1068	-	110	67-95	91	-100.00	-6.66	-10.90	-42.11
19	2,3-Pentanedione*	5.68	1073	1085	43	57-100	98	-99.44	-54.21	-43.11	-79.92
20	Dimethyl disulphide*	5.94	1081	1071	94	79-61	91	-100.00	64.48	171.94	42.80
21	4-Vinylfuran	6.05	1084	-	94	65-66-39	96	-100.00	-27.21	-29.47	-86.08
22	Hexanal*	6.19	1088	1084	56	72-44	93	-62.17	-63.17	-54.61	-3.67
23	Unknown 2' (m/z 43; 57; 69)	6.77	1106	-	43	57-69	66	nd	nd	nd	nd
24	3,3,5-Trimethyl-1,5-heptadiene	6.63	1102	-	59	43-44-57	93	nd	nd	nd	nd
25	4,5-Dimethyl-2-undecene	6.97	1112	-	69	41-95-70	95	-100.00	-13.40	-30.64	-10.97
26	3-penten-2-one	7.37	1125	1110	69	41-43-39	87	-100.00	-79.31	-77.15	-82.32
27	2,3-Hexanedione*	7.67	1134	1136	43	71-41	93	-100.00	-44.12	-45.32	-81.16
28	1-Methyl-1H-pyrrole	7.90	1141	1137	81	80-53	96	-99.27	-37.86	-15.68	-86.77
29	3,4-Hexandione	7.94	1142	1151	57	114-58-56	98	-100.00	-28.39	-33.45	-75.84
30	2-Vinyl-5-methylfuran	8.39	1156	-	108	107-43	95	-99.62	-32.72	-33.07	-81.11
31	beta-Myrcene*	8.70	1165	1157	93	41-69-91	93	-93.49	-17.86	-39.94	-25.97
32	Pyridine*	9.04	1176	1188	79	52-51-50	98	-91.20	0.29	9.05	-29.83
33	Limonene*	9.72	1197	1205	68	111-93-136	95	-54.76	19.10	-45.30	-9.57
34	Pyrazine*	10.24	1211	1210	80	53-52	95	-93.84	18.49	37.49	-25.82
35	Butyl butanoate	10.14	1208	1212	71	89-56-43	84	178.85	38.13	-48.61	82.24
36	2-n-Pentylfuran*	11.16	1234	1230	81	138-94	91	-27.13	-39.79	-63.85	95.69
37	2-Furfuryl methyl ether*	11.46	1242	1243	81	53-112-82	91	-100.00	4.02	-1.64	-25.91
38	Thiazole*	11.69	1248	1246	85	58-45-57	95	-100.00	28.64	36.27	-4.72
39	3-Methyl-3-buten-1-ol*	11.80	1251	1245	56	86-41-68	92	-100.00	2.27	-2.83	-45.65
40	E-beta Ocimene	11.92	1254	1250	93	91-136-105	89	-78.07	-18.08	-38.52	-25.83
41	3-Methyl-2-butenyl acetate	11.85	1252	1251	43	86-68-53	90	-100.00	-16.60	-11.96	22.33
42	Methyl-pyrazine	12.22	1262	1264	94	67-40	99	-88.20	1.20	8.18	-26.97
43	Dihydro-2-methyl-3(2H)-furanone	12.33	1265	1269	43	72-100-44	99	-96.01	18.00	19.12	-27.59
44	2,5-Dimethyl-1H-pyrrole	12.56	1271	-	94	95-53	97	-100.00	-95.09	-91.86	-100.00
45	3-Hydroxy-2-butanone	13.12	1285	1277	45	43-88	94	-77.64	48.61	50.30	1.99
46	trans-2-Methyl-5-n-propenylfuran	13.39	1292	1267	122	79-43-121	94	-100.00	-6.93	-18.27	-27.64
47	1-Hydroxy-2-propanone*	13.12	1285	1274	43	74-42	99	-52.64	21.22	0.14	-43.67
48	2,5-Dimethyl-pyrazine	14.38	1317	1318	108	42-81	98	-81.37	-15.17	-12.90	-36.53
49	2,6-Dimethyl-pyrazine	14.66	1324	1330	108	42-40	98	-83.13	-14.27	-14.93	-35.93
50	2-Ethyl-pyrazine	14.94	1330	1334	107	108-80	99	-86.00	-10.17	-6.47	-36.85
51	2,3-Dimethyl-Pyrazine	15.32	1340	1335	108	67-59	96	-81.23	-21.05	-19.29	-35.15
52	2-Cyclopenten-1-one*	15.68	1349	1341	82	108-123	93	-80.82	-73.27	-72.19	-80.48
53	Unknown 3 (m/z 43 Ti; 71; 86)	15.87	1353	-	43	71-86	97	-84.10	2.44	-3.15	-35.78
54	2-Hydroxy-3-pentanone	16.08	1359	1361	45	57-58-84	98	-79.27	19.01	17.10	-19.21
55	2-Methyl-2-cyclopenten-1-one*	16.21	1362	1367	67	96-53	97	-80.09	7.49	10.57	-29.86

N°	Compounds name	Rt	I^Tcalc	I^Tref	Ti	Qi	SI	M	B	P	I
56	2-Ethyl-6-methyl-Pyrazine	17.00	1381	1375	121	122-94	99	-70.21	-19.38	-18.96	-26.23
57	2-Ethyl-5-methyl-Pyrazine	17.21	1386	1387	121	122-94	98	-65.21	-18.77	-18.27	-26.70
58	2,3,5-Trimethyl-Pyrazine	17.67	1397	1394	122	42-39-81	94	-50.68	-2.96	-3.57	-11.81
59	2-Ethyl-3-methylpyrazine*	17.76	1400	1397	121	122-94-67	79	-64.47	-19.53	-16.08	-20.59
60	2-Methyl-3(2H)-furanone	18.14	1409	1397	54	98-43-59	92	-95.56	-77.49	-71.73	-86.79
61	2-(n-propyl)-Pyrazine	18.33	1413	1404	94	107-122	95	-64.41	-22.99	-22.86	-34.60
62	2,6-diethyl-Pyrazine	19.01	1430	1415	135	136-175	96	-44.72	-23.19	-23.37	-19.24
63	2-Furfurylthiol*	19.38	1439	1434	81	53-114	64	-100.00	-100.00	-100.00	-100.00
64	2-ethyl-3,5-dimethyl-Pyrazine	19.43	1440	1435	135	136-42	95	-38.72	-28.82	-21.70	-18.98
65	2,3-Diethylpyrazine*	19.86	1451	1458	135	136-56-108	85	81.92	89.61	115.20	93.88
66	Acetic acid*	20.18	1461	1465	60	43-45	84	-44.82	-22.89	-20.52	-17.03
67	Furfural*	20.52	1467	1467	96	95-39	87	-68.79	-38.45	-35.34	-64.13
68	trans-Linalool oxide*	20.62	1469	1472	59	94-55-93	93	-20.50	-31.54	-43.72	-36.60
69	Acetoxyacetone	20.79	1474	1470	43	86-116-73	98	-56.87	4.86	7.71	-43.39
70	2-Methyl-6-vinyl pyrazine	21.25	1485	1488	120	52-121	98	-73.05	-68.67	-66.57	-76.99
71	Furfuryl methyl sulfide*	21.40	1488	1493	81	128-53	97	-100.00	4.89	-20.66	-58.08
72	3,5-Diethyl-2-methyl-pyrazine	21.46	1490	1496	149	150-120	96	-19.69	-30.11	-19.18	-21.63
73	2,5-Dimethyl-3(2H)-furanone*	21.72	1496	1492	40	68-112	97	-83.62	-31.17	-31.05	-64.88
74	2,5-Hexanedione*	21.93	1501	1505	43	99-114	96	-20.82	9.34	2.59	-48.78
75	2-Acetylfuran	22.05	1505	1510	95	110-126	98	-53.67	27.11	16.78	-17.42
76	4-Vinyltetrahydro-2H-pyran-2-one	22.73	1522	-	67	54-126	93	-98.02	-94.02	-82.92	-97.51
77	1-(2-Furyl)-2-propanone	22.80	1523	1524	81	124-116	95	-94.75	18.43	-2.16	-51.57
78	2,3-Dimethyl-2-cyclopenten-1-one*	23.00	1528	1530	67	110-95	94	-33.81	-7.82	-14.35	-35.06
79	2-oxopropyl propanoate	23.31	1536	-	57	43-100-87	98	-47.94	-3.10	-9.32	-41.40
80	1-Acetoxy-2-butanone	23.49	1540	1536	57	43-100-87	97	-43.87	-1.30	-7.75	-41.96
81	Furfuryl acetate*	23.66	1545	1541	81	98-140	99	-58.77	10.41	-6.46	-34.68
82	Propanoic acid*	23.11	1531	1555	74	45-57	92	164.69	103.41	94.90	112.32
83	5-Methyl-2-furancarboxaldehyde	24.77	1573	1570	110	53-81	98	-34.67	-5.68	-8.44	-47.87
84	2-Propionylfuran*	24.86	1575	1571	95	124-99	90	-40.96	12.05	-2.07	-22.46
85	(5-Methyl-2-furyl)methanethiol	25.07	1580	-	95	138-96	98	-93.81	9.07	-9.72	-58.88
86	(1-methylethenyl)-Pyrazine	25.41	1589	-	119	120-131	94	-28.97	-16.99	-19.66	-42.36
87	2-Acetylpyridine*	25.72	1597	1602	78	134-105	95	-47.45	-0.70	-10.46	-32.11
88	Furfuryl propanoate*	26.00	1604	1606	81	98-154	85	-23.44	18.52	-11.95	-27.68
89	5H-5-Methyl-6,7-dihydrocyclopentapyrazine*	26.21	1609	1616	119	134-137	92	-24.49	-23.80	-23.56	-31.82
90	1-methyl 1H-Pyrrole-2-carboxaldehyde*	26.46	1616	1620	109	108-53-80	92	-36.14	4.63	-1.09	-25.06
91	4-hydroxy-butanoic acid	26.66	1621	-	42	41-86	97	81.26	41.49	33.11	5.89
92	2-Isopropenylpyrazine	27.01	1630	-	120	119-93-65	86	-21.16	-24.96	-24.54	-39.47
93	Butanoic acid	26.59	1619	1628	60	87-112	84	nd	nd	nd	nd
94	2,5-dihydro-3,5-dimethyl-2-Furanone	27.48	1642	1640	69	112-97	85	-9.31	9.75	7.66	-27.89
95	1-(2-Furyl)-butan-3-one	27.58	1645	1651	81	138-67	95	-23.97	4.81	-13.48	-38.62
96	2-Acetyl-1-methylpyrrole*	27.77	1650	1657	108	123-53	95	-24.95	-6.54	-16.90	-37.12
97	3-Mercapto-3-methyl-1-butanol	28.17	1660	1658	41	69-71	97	-100.00	-43.63	-61.47	-94.05
98	Furfuryl alcohol*	28.49	1669	1660	98	81-69	99	30.78	25.40	20.50	-20.48
99	Unknown 3' (m/z 126; 84; 97)	28.74	1675	-	126	84-97	76	-19.18	-2.95	-17.23	-29.11
100	3-Methyl-butanoic acid	29.74	1701	1680	60	43-87	92	15.30	-1.38	3.60	8.94
101	2-Furfuryl-5-methylfuran	28.44	1667	1678	162	91-119	79	nd	nd	nd	nd
102	2-Acetyl-3-methylpyrazine*	29.08	1684	1686	136	94-67-108	77	-14.34	-12.87	-17.26	-37.22
103	Unknown 4 (m/z 134; 133; 81)	29.65	1699	-	134	133-81	95	-15.09	-23.75	-22.06	-39.76
104	Unknown 5 (m/z 97; 69; 126)	30.29	1716	-	97	69-126	83	-7.16	-3.12	-8.66	-32.18
105	N-acetyl-4(H)-Pyridine	30.45	1720	-	80	123-53	94	-53.74	-16.85	-15.76	-48.11
106	3-Methoxy-2-methyl-cyclohex-2-enone	30.77	1729	-	140	111-97	83	-69.30	-13.64	-22.16	-42.05
107	3-ethyl-4-methyl-2,5-Furandione	30.92	1733	-	67	53-140	94	-17.65	-13.30	-30.17	-55.12
108	2(5H)-Furanone*	30.64	1726	1712	55	84-150	90	nd	nd	nd	nd
109	Methyl salicylate*	31.45	1748	1753	98	55-137	65	nd	nd	nd	nd
110	Unknown 6 (m/z 95; 138; 150)	32.46	1775	-	95	138-150	95	-13.93	-28.16	-35.84	-56.57
111	Unknown 7 (m/z 94; 137; 78)	32.63	1780	-	94	137-78	94	-93.79	-76.21	-63.80	-93.55
112	3,5-Dimethyl cyclopentenolone	33.15	1794	-	126	69-111	91	4.00	-6.84	-13.06	-23.88

N°	Compounds name	Rt	I^Tcalc	I^Tref	Ti	Qi	SI	M	B	P	I
113	Unknown 8 (m/z 80; 137; 109)	33.18	1795	-	80	137-109	89	-33.53	-14.18	-18.38	-48.51
114	3-methyl-2-Butenoic acid	32.77	1784	1804	100	55-83	92	nd	nd	nd	nd
115	Unknown 9 (m/z 55; 83; 126)	33.84	1813	-	55	83-126	83	0.04	-2.28	-9.25	-22.08
116	2-hydroxy-3-methyl-2-Cyclopenten-1-one	34.27	1826	1807	112	69-83	94	28.86	11.02	3.04	-14.58
117	1-(2-furanylmethyl)-1H-Pyrrole	34.39	1829	1833	81	147-53	97	-14.57	-3.21	-16.43	-43.72
118	Unknown 10 (m/z 128; 112; 151)	35.23	1853	-	128	112-151	84	-84.92	3.19	-1.89	-26.20
119	n-butylbenzoate	35.37	1857		105	123-60	86	3.88	-51.13	-14.19	-6.97
120	2-methoxy-Phenol	35.44	1859	1859	109	124-81	98	6.00	5.19	-14.36	-37.37
121	Unknown 11 (m/z 109; 53; 152)	35.55	1862	-	109	53-152	97	-9.38	-19.46	-22.41	-54.18
122	3-ethyl-2-hydroxy- 2-Cyclopenten-1-one	36.55	1890	1891	126	83-97	87	6.79	-1.85	-11.39	-32.63
123	Unknown 12 (m/z 81; 161; 53)	36.80	1898	-	81	161-53	94	-49.90	-31.58	-32.12	-75.80
124	Phenylethyl Alcohol*	37.14	1908	1914	91	65-122	92	9.10	-27.43	-16.25	-53.71
125	2-Thiophenemethanol*	38.31	1942	1950	114	85-97	91	60.09	16.15	7.91	-24.96
126	Maltol*	38.83	1958	1952	126	71-97	98	49.28	28.83	-1.55	3.42
127	2-Acetylpyrrole*	39.18	1968	1966	94	109-66	99	56.64	25.79	16.67	-12.84
128	4(1H)-Quinazolinone	39.51	1978	-	146	93-118	92	-6.37	-8.40	-7.80	-44.99
129	Difurfuryl ether	39.75	1985	1980	81	82-178	97	1.12	8.04	-9.83	-44.57
130	4-Hydroxy-3-methylacetophenone*	40.10	1996	-	135	150-107	88	-1.37	-5.05	-12.10	-45.44
131	Phenol*	40.59	2010	1994	94	66-108	94	79.79	38.43	15.42	-10.38
132	1H-Pyrrole-2-carboxaldehyde*	40.90	2020	2012	95	66-39	99	133.27	59.47	46.09	20.86
133	4-ethyl guaiacol	41.15	2028	2034	137	152-122	95	-3.27	-16.10	-18.79	-42.21
134	2,5-Dimethyl-4-hydroxy-3(2H)-furanone (Furaneol)*	41.44	2037	2039	43	128-85	73	-100.00	-51.51	-27.84	-79.86
135	5-Acetyldihydro-2(3H)-furanone (Solerone)	42.00	2054	2096	85	57-128	96	6.71	-18.08	-18.14	-39.78
136	N-Methyl-2-formylpyrrol	43.47	2100	-	109	108-80	91	59.35	34.05	34.82	-10.28
137	Unknown 13 (m/z 57; 99; 149)	44.24	2125	-	57	99-149	75	-100.00	-12.07	-21.41	-54.20
138	4-Vinyl guaiacol*	46.35	2193	2185	150	135-107	99	-52.34	-71.37	89.29	-16.57
139	Nonanoic acid*	46.87	2210	2168	60	73-57-41	95	-60.06	-44.67	-47.97	-77.21
140	Unknown 14 (m/z 81; 175; 163)	47.64	2236	-	81	175-163	93	-2.30	3.29	-25.33	-31.64
141	n-Decanoic acid*	50.10	2319	2303	60	57-129	92	-7.90	-49.05	70.72	-38.97
142	2-Benzofuran-1(3H)-one	50.39	2329	2356	105	77-134	90	20.23	-53.08	-30.52	2.59
143	Unknown 15 (m/z 95; 39; 67)	51.95	2384	-	95	39-67	95	-69.61	-40.42	-55.80	-75.81
144	2,3-dihydro-Benzofuran	52.27	2395	-	120	91-119-65	84	nd	nd	nd	nd
145	Indole*	53.32	2434	2448	117	90-89-63	89	-52.19	-12.20	-20.45	-57.89
146	Benzoic acid*	53.27	2432	2433	105	122-77	84	nd	nd	nd	nd
147	5-(Hydroxymethyl)dihydro-2(3H)-furanone*	54.22	2467	-	85	57-43	92	227.90	71.38	25.43	47.26

2.2.3 Roasted coffee evolution: monitoring the changes of aroma across different types of packaging over time.

Roasted coffee evolution: monitoring the changes of aroma across different types of packaging over time.

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1. INTRODUCTION

Once the coffee package is opened, a very strong yet delicate aroma is released, a sign of the quality of the product (Buettner,2017). In order to make this aroma perceivable by our noses, aromatic volatile organic compounds (VOCs), first identified by Reichenstein and Staudinger in 1926, must be released and reach the olfactory epithelium, which contains the nerve endings that allow us to smell (Buettner, 2017). The chemical senses of both taste (gustatory) and smell (olfactory) and their interaction (flavor) are highly evolved and extremely sensitive. Humans use these senses to find acceptable food, recognize mating, detect disease, and determine the economic value (Buettner, 2017). It is therefore important to preserve and maintain coffee quality in terms of sensory properties since they also conditions its economic value, (Bhumiratana, 2011). Coffee quality is a multifaceted characteristic that is influenced by numerous chemical precursors, environmental factors and technological processes, including roasting and storage conditions, which involve sensory and chemical qualities. To maintain the sensory features, coffee must be protected from the external environment by appropriate packaging (Folmer, 2016; Sunarharum, 2014). Indeed, coffee goes through physical and chemical changes, while being stored, which can cause a quick drop down in quality. Different environmental factors have a significant impact on how coffee beans are stored, whether whole or ground. A number of deteriorative processes, including the volatilization of odorant molecules (VOCs), CO_2 release, oxidative reactions that result in the generation of off-notes and the development of rancidity, are influenced by the basic dynamic factors of humidity, temperature, and oxygen (Folmer, 2016; Subramaniam, 2016; Ross, 2006). The loss of VOCs and the emergence of oxidation products are typically responsible for these changes in sensory qualities. Coffee staling, caused by the aging-related loss of aroma freshness, has been intensively investigated since it affects consumer acceptability and defines the product's shelf-life (Flament, 2002). In fact, sensory tests are used often in industry as the gold standard to assess product quality, to evaluate the impact of the environment on storage conditions and to examine the impact of packaging on food products. However, these tests have significant drawbacks, including low repeatability and reproducibility, long time and high costs (Murray, 2001; Regueiro, 2017). Due to these factors, there is currently an active search for potential alignment with instrumental analytical measures that can collect, separate, identify, and extrapolate chemical data from a complex

food matrix such as coffee (Bressanello, 2018; Folmer, 2016). Numerous studies have been conducted with the aim of combining sensory and chemical analysis to define, through an objective analytical approach, the freshness of a product (Bröhan, 2009; Buffo, 2004; Calligaris, 2016; Cincotta, 2020; Glöss, 2014; Holscher, 1992; Kallio, 1990; Leino, 1992; Makri, 2011; Manzocco, 2016; Mardjan, 2019; Pérez-Martínez, 2008; Marin, 2008; Ross, 2006; Witik, 2019). The ultimate goal is to support or, when possible, to replace the more subjective sensory panel, with an objective chemical approach able to identify a collective pool of chemical markers, as potential indices of coffee quality degradation. This study a continuation of the previous one (Strocchi, 2022), in which the volatile fingerprinting delineated for oxidised samples, was investigated on different roasting and grounded samples monitored over time belonging the *Coffea Arabica* L. (Arabica) and *Coffea canephora* Pierre (Robusta) species and their blends in different packaging. The main problems in the search for chemical markers related to aging are due to the complexity and dynamic behaviour of coffee aroma and to the fact that single products have always been evaluated and never different blends/samples in the same investigation. The purpose, in fact, of this study was to search for one or more general chemical indices of aging that can be adopted for quality recognition in industry and, in particular, as unacceptability markers in products that were no longer viable, in different coffees (be they of different species, packages and blends). To achieve this goal, the same sample blends of roasted and ground coffees (R&G) suitable for Moka and espresso preparations, were monitored every 30-day under two different storage settings: ambient conditions (for a total of 24 months) and stressed conditions (for a total of 3 to 6 months depending on the packaging). Simultaneously, the samples were subjected to the sensory analysis to compare the results with the chemical information collected, in order to identify qualitative chemical markers that, in stressful situations, classified the product as unacceptable. To determine whether the molecular markers for the deterioration of sensory quality found under stress settings were the same for samples maintained under ambient conditions, the kinetics of evolution of these latter on samples was concurrently monitored. Then, acceptability/rejection threshold of aging-oxidation markers were established. A series of unknown representative samples in blinds were used to validate the identified chemical indicators.

2. MATERIALS AND METHODS

Coffee samples

Samples consist of R&G coffees suitable for espresso and Moka preparation, kindly supplied by Lavazza Group s.p.a. (Turin, Italy). Three type of commercial blends "P" and "B" from *Coffea arabica* biological cultivation (for ease expressed as "B" caps, suitable for espresso machine, and "M soft pack", available for Moka preparation) , and "I" consisting of 50-50 *Coffea robusta/Coffea arabica* were selected for their distinctive flavours. The three blends were available in two different types of modified atmosphere capsules: i) Eco Caps, consisting of a 100% ecological caps made of an innovative biopolymer capable of degrading in 180 days after disposal and becoming compost (PC, BC and IC); ii) the Standard caps (std) made of a copolymer based on polyethylene and aluminium (PS, BS and IS). The blend M soft pack (Msp), was available, even, in a controlled and modified atmosphere suitable for a Moka preparation. The samples, once produced, were subjected to both accelerated storage conditions at a temperature of 45°C and 65% relative humidity (RH), and to ambient storage conditions at a 20°C and 65% RH. The stressed samples were analysed at the following days (T0, T30, T60, T90) for the ecological caps and (T0, T30, T60, T90, T120, T150, T180) for the Standard caps. Three lots of each blend were investigated. Two lots of Moka samples, Msp, were analysed at the following day intervals (T0, T30, T60, T90, T120, T150). The ambient samples were analysed every 30-days from T0 to T690 days for both Eco and Standard caps and from T0 to T480 for Msp. The details on the samples analysed were reported in Table S1 of the supporting information.

Reference standards and solvents

Pure reference compounds were either purchased from Merck (Milan, Italy) or taken from the authors' laboratory collection for key-odorants identity validation. They were listed in Table S2 of the supporting information. The solvents cyclohexane, dibutyl phthalate and the homologous series of n-alkanes (from *n*-C9 to *n*-C25) for Linear Retention Index (I^T_S) determination were all purchased from Merck (Milan, Italy).

Headspace Solid Phase Microextraction (HS-SPME) devices and sampling conditions

A combi-PAL AOC 5000 Autoinjector equipped with SPME tool was coupled to a Shimadzu QP2010 GC-MS system provided with GC–MS Solution 2.51 software (Shimadzu - Milan, Italy).

Sampling conditions: Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) (df 65 μm, 1 cm length) SPME fibers were provided from Supelco (Bellefonte, PA, USA). Before use, fibers were conditioned in agreement with the manufacturer's recommendations. The internal standard had previously been uploaded onto the fiber (by sampling 5μL of a 1000 mg/L solution of n-C13 in Dibutyl phthalate (DBP) in a 20mL headspace vial for 20 min at 50°C, stirring speed 250rpm. 1,5 g coffee powder were placed in a 20mL vial and the headspace sampled for 40 min at 50°C at a stirring speed of 250 rpm.

Analytes were then recovered from the fibre by thermal desorption for 5 min at 250°C into the GC injector body, and then on-line transferred to the GC injector. All samples were analyzed in duplicate.

Chromatographic conditions: injector temperature: 250°C, injection mode: splitless; carrier gas: helium, flow rate: 1 mL/min; column: SGE SolGelwax (100% polyethylene glycol) 30 m x 0.25 mm dc x 0.25 μm df (Trajan- Melbourne, Australia). Temperature program, from 40°C (1min) to 200°C at 3°C/min, then to 250°C (5min) at 10°C/min. MSD conditions: ionization mode: EI (70 eV), temperatures: ion source: 200°C; transfer line: 250°C; scan range: 35-350 m/z.

Identification of volatiles was obtained through their linear retention indices (I^T_S) and EI-MS spectra, compared to those of authentic standards or, to those collected in in-house or in commercial libraries (Wiley 7N, FFSNC and Nist 14 Mass Spectral Data).

Sensory test

The Quantitative Descriptive Analysis (QDA) method was used for sensory evaluation, samples were tasted in monadic way. The evaluation of coffees following the SCAA Q cupping and grading protocol, assigning to each sensory attribute a score on a scale of 0 to 10. The attributes monitored over time are: direct odour, oxidation, aromatic

intensity, acidity, bitterness, astringency, body and aftertaste. From the average scores of the different attributes, the OK and KO ratings of samples were defined.

Statistical analysis

Data mining was carried out using Matlab R2021a (The MathWorks, Inc., Natick, MA, USA) with the following statistical packages: Principal component analysis by PCA toolbox (v1.5) (Ballabio, 2015) and Classification toolbox (v6.0) (Ballabio, 2013). XLSTAT software version 2021.2.1 was used for partial least square discriminant analysis (PLS-DA), (Addinsoft, New York, NY USA). PCA was used to explore information provided from samples along time, PLS-DA was used to extrapolate the variables important in the research of chemical indices.

3. RESULTS AND DISCUSSION

This study was divided into two parts and the first part was split into two sections: in the first section (3.1), the behaviour of the commercial capsules and Moka soft-pack coffees, placed under stressed conditions, was evaluated through unsupervised statistical analyses by PCA of samples from time T0 (reference) to Tn, depending on the packagings. In the second section (3.2), the samples stored in ambient condition were monitored over time by PCA. In the third part (3.3) volatiles describing the aged coffee were extracted through a step-wise supervised PLS-DA, to search unique chemical indices of ageing in stress conditions for all blends and packaging. In the 3.4 section, the indices extrapolated were validated with the samples under ambient condition and in the 3.5 section tested for the prediction capability on a blind samples set. Samples were at the same time submitted to sensory test to define their acceptability (OK) or unacceptability (KO).

Section 3.1: Monitoring of coffees stored in accelerated ageing conditions (ASLT) (45°C and RH 65%)

In this section, coffees in different packaging and blends stored in stressed conditions (45°C, 65% RH) were analysed to study the evolution of their volatiloma over time. In Figures 1 and 2, PCA on the first two PCs describe the patterns of the three blends. Figure 1a reports the

scores plot of the ecological samples spread in function of reference and aged samples along with the first PC (PC1), from right to left. Control (in blue) and aged (from red to pink) samples were discriminated from the first PC (PC1). More in depth, it was possible to notice that while in T0, the batches between the blends have a wider distribution, at the times T30, T60 and T90, the samples tend to cluster. On the hand, PC2 discriminates in function of the blend I from P and B; blends P and B were 100% Arabica while blend I was a 50/50 Arabica and Robusta mix (Figure 1b).

Figure 2 reports the distribution of the Standard caps in function of the time along with the first PC (PC1) and the discrimination of the blends along with the second PC (PC2). In Figure 2a, it was possible to observe that the tendency to clump was less strong than ecological capsules. This was probably due to the packaging's ability to be more resistant to accelerated conditions. On the other hand, Figure 2b reports the discrimination, along the PC2, of the blends. As Eco caps, in the Standards, it was possible to remark how the samples, from T90 clusters, compared to the greater spreading in the graph of the early days. Furthermore, the tendency for grouping according to the blend in Standard caps was much higher than in Eco caps. This reveals that the Standard samples, were less subject to the degradative activity over time, compared to the Eco caps; this was probably because, although the discrimination over time was on the first component (PC1), just as for the Eco caps, the Standard caps were found to be dependent on a higher variability (28.56%) to the sharper discrimination, along the second component (PC2), according to the blend.

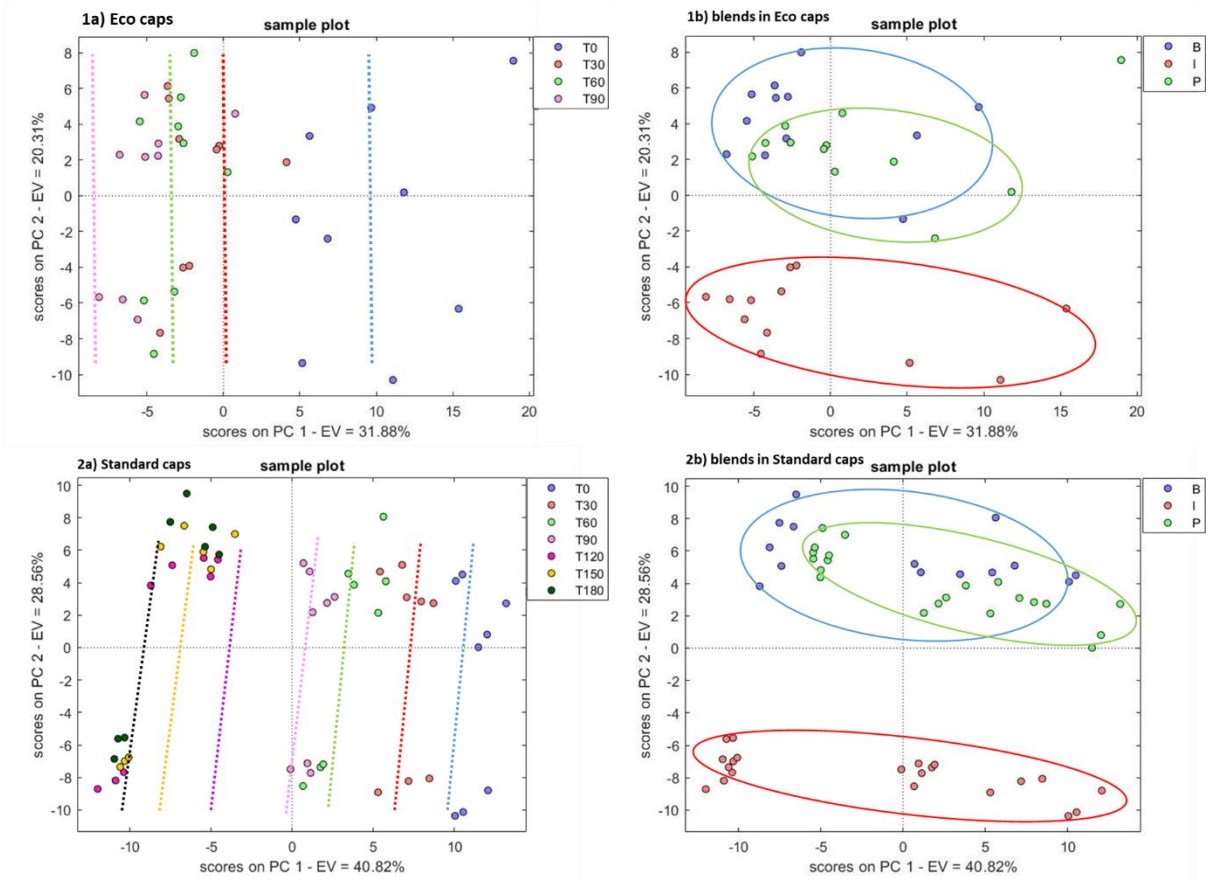

Figure 1 and 2: PCA of the cap samples investigated: 1a) score plot of Eco caps distribution over time, from T0 to T90, along PC1 vs PC2, 1b) score plot distribution of the blends I Eco caps (IC), B Eco caps (BC) and P Eco caps (PC) along PC1 vs PC2 (I circled by an ellipse in red, P and B by an ellipse in green and blue), 2a) score plot of Standard caps distribution over time, from T0 to T180, along PC1 vs PC2, 2b) score plot distribution of the blends I Standard caps (IS), B Standard caps (BS) and P Standard caps (PS) along PC1 vs PC2 (I circled by an ellipse in red, P and B by an ellipse in green and blue).

The behaviour of Msp is reported in Figures 3. The unsupervised analysis reports the scores plot of the soft pack spread in function of reference (T0) and aged samples along the first PC (PC1), from left to right with a curvilinear course which tends to cluster the samples in pairs, for the first 60 days (Figure 3). Indeed, with closer analysis, it can be remarked that these samples are subject to a batch effect, which is evident from Figure 4, in which it can be seen that individual batches move with different trends and abundances with the most characterizing compounds. The diagram shows the percent coefficients of variation (CV%) of the normalized responses of the reference sample compared to the KO samples of the different batches. Variances above and below 20% are shown, representing the relative

standard deviation of the analytical method (RSD% > 20 or < 20). CV% was calculated using equation (1):

CV% = -[(norm. response OK - norm. response KO)/norm. response OK] x 100

(Eq. 1)

This variation between the two batches could be explained by a possible micro leakage of one of the two packets, which undergoing greater air and moisture ingress, might experience chemical changes in aroma.

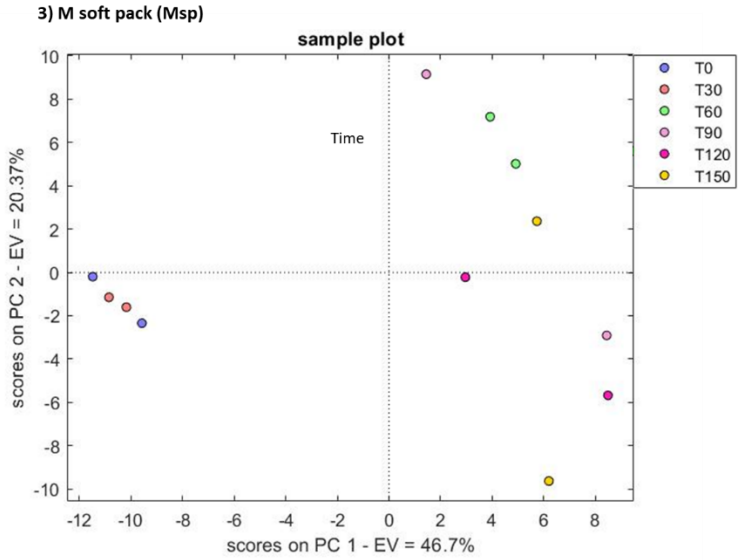

Figure 3: PCA of Moka soft pack (Msp) investigated: score plot of the Moka soft pack (Msp) distribution over time, from T0 to T150, along PC1 vs PC2.

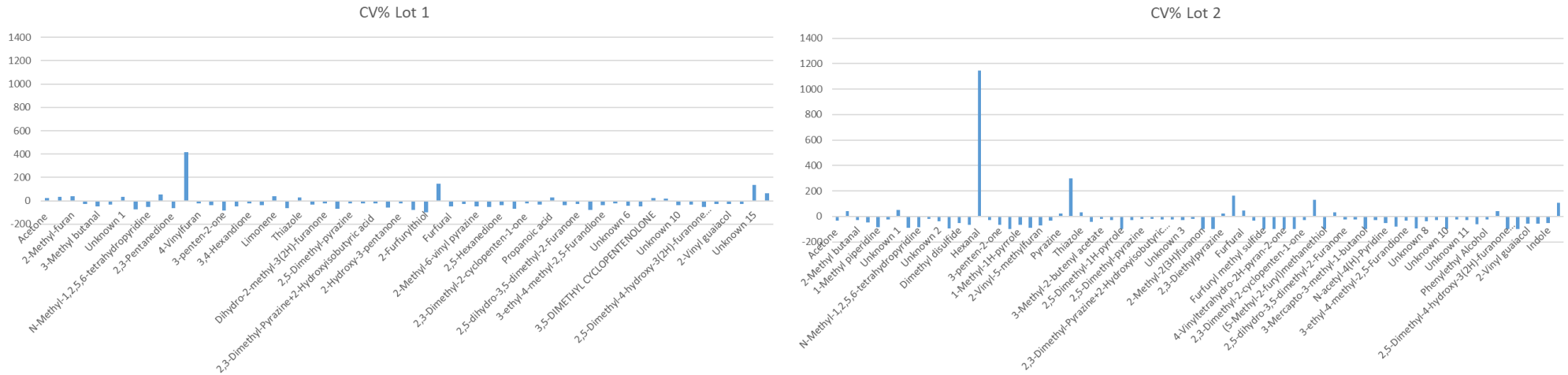

Figure 4: *Comparison of the % response variation (CV%) between* the two lots of Moka soft pack (Msp). Volatiles varying below 20% and above

Section 3.2: Monitoring of coffees stored in ambient conditions (20°C and RH 65%)

The tendency of coffee to age under ambient conditions proves to be less intense compared to stressed conditions showed in figure 1a. Figure 5a) and 6a) reports the PCA scores plot of the Eco and Standard caps stored in ambient conditions spread along the first PC (PC1), from right to left, for the Eco caps, and from left to right, for the Standard caps, with a curvilinear trend each. The third PC (PC3), for the Eco caps and the second PC (PC2), for the Standard caps, instead, explain much more the discrimination in function of the blends I from P and B; blends P and B were 100% Arabica while blend I was a 50/50 Arabica and Robusta mix (figure 5b) and 6b)).

As can be seen, the trend over time for the ambient samples, was slow and steady, with a tendency to distribute more compactly, compared to the stressed samples. In fact, the ambient caps appeared to have a more gradual change over time, regardless of the type of blend and with a curvilinear trend according to the PC1, in contrast to the stressed conditions (figures 1a) and 2a)).

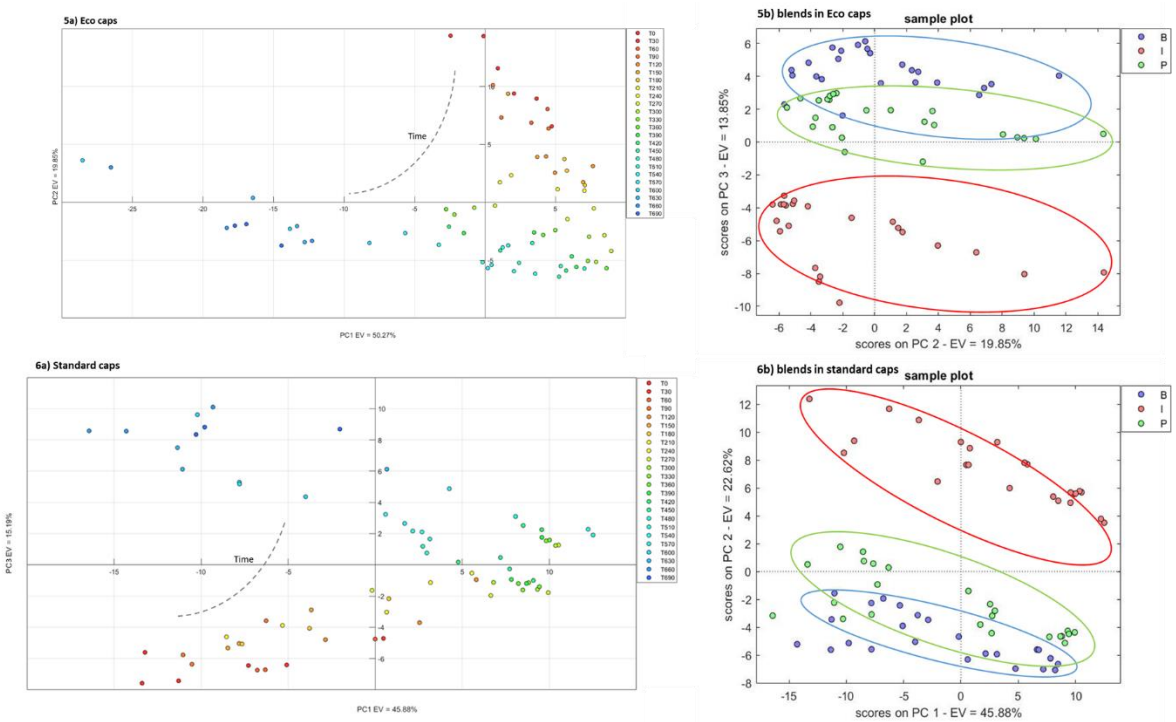

Figure 5 and 6: PCA of the cap samples investigated: 5a) score plot of Eco caps distribution in time, from T0 to T690, along PC1 vs PC2 (from right to left), 5b) score plot of the blends I Eco caps (IC), B Eco caps (BC) and P Eco caps (PC) distribution, along PC2 vs PC3 (I circled by an ellipse in red, P and B by an ellipse in blue), 6a) score plot of Standard caps distribution in time, from T0 to T690, along PC1 vs PC3 (from left to right), 6b) score plot of the blends I Standard caps (IC), B Standard caps (BC) and P Standard caps (PC) distribution, along PC1 vs PC2 (I circled by an ellipse in red, P and B by an ellipse in blue).

In the same way of the capsules, in Msp, there was not a linear trend in time. These samples present also a less stable behaviour over time with a clustering in the early T days and in the last times, as shown in figure 7.

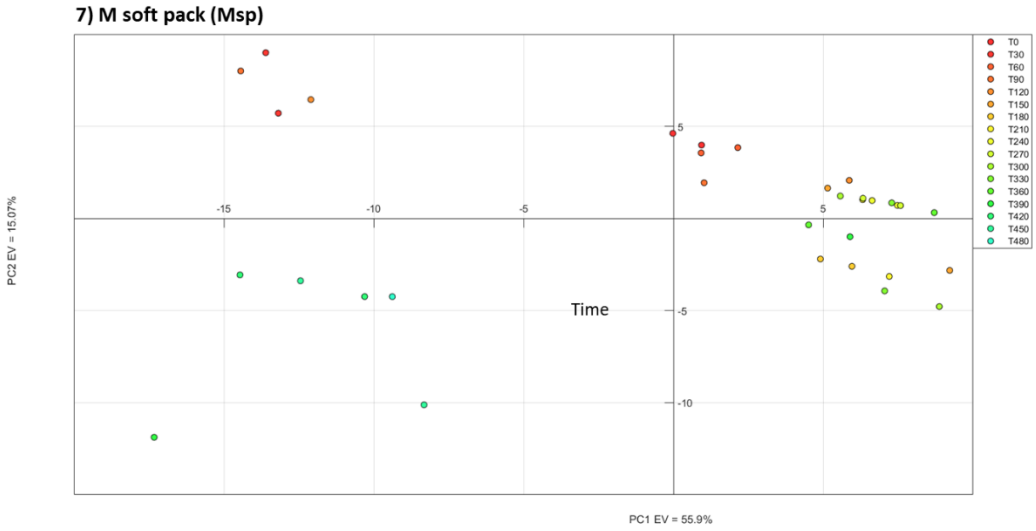

Figure 7: PCA score plot of the two lots of the Moka soft pack (Msp) distribution in time, from T0 to T150, along PC1 vs PC2 (from right to left).

Even in ambient conditions, it was possible to observe a different distribution of the samples in function of blend, (Figure 8a) and packaging (Figure 8b). The different distribution of the blends on the first and third PCs (PC2 and PC3), highlights that the samples were different for species (Figure 8a). I blend (100% of *Coffea Arabica*) discriminated along the third PC component (PC3), compared to the other capsules and Msp. Figure 8b shows the differentiation, along the second PC (PC2), between the Eco and Standard caps and the soft pack samples. The distribution highlighted that the Eco caps offer a different barrier effect during aging, compared to the Standard caps and the soft pack samples.

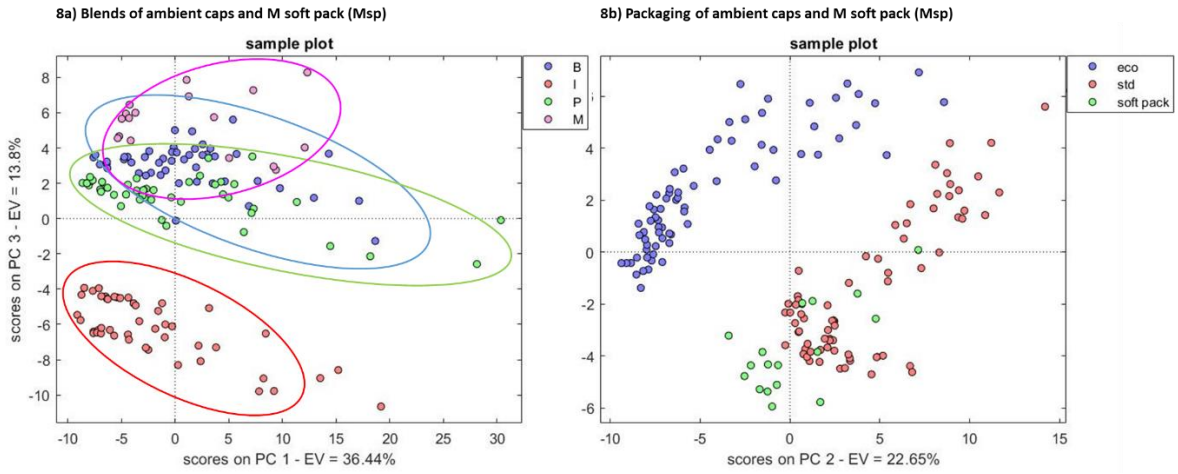

Figure 8a) score plot of the blends B, I, P (Eco and Standard) ambient caps and Moka soft pack (Msp) and along PC1 vs PC3 (I circled by an ellipse in red, P, B and Msp by an ellipse in blue), 8b) score plot of the packs Eco, Standard (std) and soft pack (sp) distribution along PC2 vs PC4.

Section 3.3: Searching for robust aging markers

To extract the informative volatiles from which subsequently obtain the descriptive indices of the aged coffee note, the entire set of samples stored under stress conditions was subjected to a step-wise supervised PLS-DA analysis.

Table 1 shows the list of 27 significant volatile components out 147 with VIP (variable important in the projection)> 1 (sorted in descending order) and that present a strong correlation (Pearson correlation coefficient (r<-0.7 or r>0.7)) with the aged samples. These compounds were listed in blue or yellow in the table below. It indicates that these substances

have a stronger impact on those samples that the sensory test considers to be no longer acceptable. The bounds at the 95% confidence level are additionally included together with the standard deviation for volatile components. The yellow compounds show a reduction over time, whereas the blue compounds show an increase over time. All of these changes were independent on the packaging or of blend.

Despite the fingerprint of compounds that describe ageing samples the industry needs a marker easiest to track compared to a footprint (Strocchi, 2022). A simple and robust method for assessing coffee ageing is therefore to look for ratios of headspace concentrations of selected VOCs (an index), which is useful for finding reliable relationships to coffee ageing and oxidation. The emphasis was therefore pointed to the ratios between volatiles and their behaviour over time when searching for indices that provide more reliable information on time-related markers since they represent associated trends of more than one variable.

A second PLS-DA elaboration was performed, starting with the results shown in Table 1, to identify the markers that have a more important weight in the discrimination between acceptable and undesirable samples. This operation has decreased the amount of volatiles that might affect the ratios and time markers. Table 2 reports the lowered aging-related markers with (r) values > 0.7 and <-0.7 together with the VIP coefficient. The table lists the volatiles whose percent coefficient of variation was higher than 20 in all of the blends and investigated packaging, although to different extents. The cut off of CV% > 20 is related to the analytical relative standard deviation, therefore only volatiles with a CV% higher than the RSD% of the analytical method were considered. The CV% was calculated using the equation 1.

Table 1: PLS-DA results by Pearson correlation coefficient (r) (in blue volatiles with a (r) value >0.7 and in yellow a (r) value <-0.7). In table were also reported VIP, standard deviation and lower and upper boundaries %.

Variables	p corr	VIP	Standard deviation	Lower bound(95%)	Upper bound(95%)
3-Mercapto-3-methyl-1-butanol	-0.93	1.7041	0.1607	1.3891	2.0191
Acetic acid	0.91	1.6723	0.0857	1.5043	1.8404
2,5-Dimethyl-3(2H)-furanone	-0.89	1.6376	0.1087	1.4246	1.8507
2-Methyl-6-vinyl pyrazine	-0.89	1.6319	0.0850	1.4653	1.7985
1H-Pyrrole-2-carboxaldehyde	0.88	1.6124	0.1199	1.3773	1.8474
2-Cyclopenten-1-one	-0.86	1.5776	0.1018	1.3781	1.7771
2-Methyl-2(3H)furanon	-0.86	1.5684	0.0858	1.4002	1.7367
2,5-Dimethyl-1H-pyrrole	-0.85	1.5520	0.0710	1.4129	1.6912
4-Vinyltetrahydro-2H-pyran-2-one	-0.84	1.5445	0.1134	1.3222	1.7668
3-penten-2-one	-0.84	1.5373	0.1349	1.2730	1.8016
N-Methyl-1,2,5,6-tetrahydropyridine	-0.83	1.5278	0.1085	1.3151	1.7405
Dimethyl disulfide	0.83	1.5202	0.1678	1.1914	1.8491
Furfuryl alcohol	0.83	1.5198	0.0862	1.3508	1.6888
Butanoic acid	0.81	1.4774	0.1747	1.1349	1.8199
3-ethyl-4-methyl-2,5-Furandione	-0.80	1.4703	0.1096	1.2554	1.6851
3,3,5-Trimethyl-1,5-heptadiene	-0.75	1.3833	0.1494	1.0905	1.6761
2(5H)-Furanone	0.74	1.3511	0.1655	1.0266	1.6756
1-Methyl piperidine	-0.73	1.3470	0.1856	0.9833	1.7106
trans-2-Methyl-5-n-propenylfuran	-0.73	1.3462	0.1459	1.0602	1.6321
Furfuryl methyl sulfide	-0.73	1.3420	0.2057	0.9388	1.7452
2,6-Dimethyl-pyrazine	-0.73	1.3406	0.1303	1.0851	1.5960
2,3-Butanedione	-0.72	1.3277	0.1755	0.9837	1.6717
3-Hydroxy-2-butanone	0.72	1.3262	0.1512	1.0298	1.6225
E-beta Ocimene	-0.72	1.3193	0.1814	0.9637	1.6749
2,3-Dimethyl-Pyrazine+2-Hydroxyisobutyric acid	-0.72	1.3117	0.1390	1.0392	1.5842
1-Hydroxy-2-propanone	0.71	1.3060	0.1679	0.9770	1.6351
2,3-dihydro-Benzofuran	-0.70	1.2808	0.1728	0.9422	1.6195

Table 2: PLS-DA results by Pearson correlation coefficient (r) (in red a (r) value >0.7 and in green a (r) value <-0.7). In table were also reported VIP correlations, standard deviation and lower and upper boundaries %. Furthermore were reported the % of the variation. CV%= eq.1.

Variable	p corr	VIP	dard devia	er bound(S	er bound(S	CV%BC	CV%IC	CV%PC	CV%BS	CV%IS	CV%PS	CV%Msp
Acetic acid	0.92	1.1574	0.0403	1.0785	1.2364	193.67	194.31	155.01	335.47	313.83	288.00	154.01
3-Mercapto-3-methyl-1-butanol	-0.90	1.1350	0.0457	1.0454	1.2246	-74.69	-100.00	-69.42	-79.45	-80.01	-75.50	-89.95
2-Methyl-6-vinyl pyrazine	-0.89	1.1282	0.0319	1.0657	1.1907	-94.14	-100.00	-71.14	-65.47	-68.11	-63.08	-42.16
2,5-Dimethyl-1H-pyrrole	-0.88	1.1064	0.0392	1.0295	1.1832	-100.00	-100.00	-78.02	-82.63	-79.72	-78.99	-83.01
4-Vinyltetrahydro-2H-pyran-2-one	-0.88	1.1139	0.0506	1.0147	1.2131	-100.00	-97.65	-71.85	-84.76	-74.58	-74.51	-83.38
2-Cyclopenten-1-one	-0.88	1.1110	0.0371	1.0384	1.1837	-79.97	-90.45	-70.08	-72.08	-74.46	-69.63	-43.58
1H-Pyrrole-2-carboxaldehyde	0.87	1.1020	0.0326	1.0381	1.1659	59.33	63.42	30.50	58.65	63.30	62.49	23.12
2,5-Dimethyl-3(2H)-furanone	-0.87	1.0966	0.0366	1.0250	1.1683	-41.68	-49.71	-42.83	-53.20	-52.98	-47.01	-74.47
3-penten-2-one	-0.86	1.0838	0.0519	0.9821	1.1855	-91.13	-73.66	-62.03	-81.29	-85.89	-79.64	-76.44
2-Methyl-2(3H)furanon	-0.83	1.0440	0.0586	0.9291	1.1589	-100.00	-100.00	-87.12	-100.00	-100.00	-100.00	-88.48

Figure 9 shows the process used to choose the aging indices (ratios between aging markers). Acetic acid/1H-pyrrole-2-carboxaldehyde and 2,5-dimethyl-3(2H)-furanone/1H-pyrrole-2-carboxaldehyde were the indices exhibiting the features mentioned above and coherency over time. These indices change throughout time, rising and falling correspondingly.

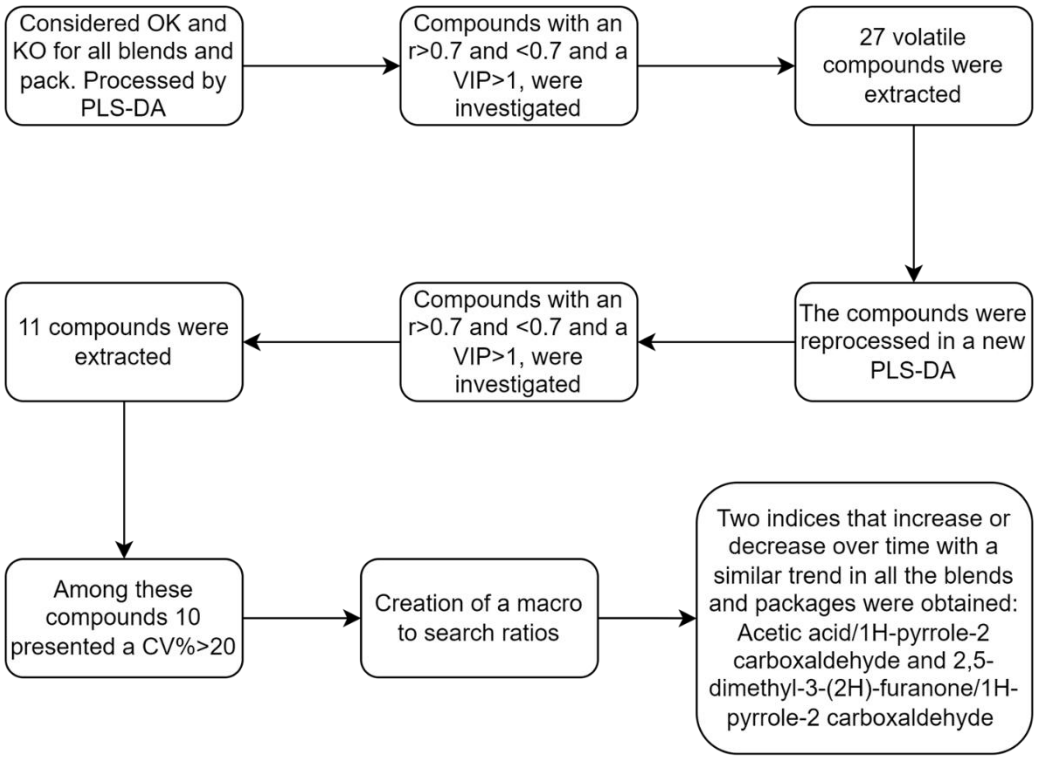

Figure 9: Flow of the steps for the identification of the 2 informative ratios.

The sensory tests carried out on samples were the driven guide to estimate the values of the chemical indices when the samples become KO. The sensory panel defined the following time limit for the no longer acceptability of coffees under stress conditions. At these times the chemical indices in stress conditions were calculated as average of the lots within each blends (table 3).

Table 3: Values of the two indices measured for each type of mixture stored under stress conditions (Acetic acid/1H-pyrrole-2-carboxaldehyde and 2,5-dimethyl-3(2H)-furanone/1H-pyrrole-2-carboxaldehyde) reported with the days each sample is KO.

Blend	**Days in which sensory acceptability is KO**	**Chemical Indices**	
		Acetic acid/1H-pyrrole-2-carboxaldehyde	2,5-dimethyl-3(2H)-furanone/1H-pyrrole-2-carboxaldehyde
BC	60	13131	241
IC	90	15694	197
PC	90	12860	287
BS	150	13400	263
IS	180	11376	220
PS	150	13705	286
Msp	120	12007	174

As determined by the sensory panel, coffee Eco caps begins to become unacceptable for the Acetic acid/1H-pyrrole-2-carboxaldehyde ratio in ASLT conditions at values of around 16000 for I and approximately 13000 for P and B. Approximately similar are the index values for samples stored in Standard capsules, thus 11000 for I and from around 14000 for P and B. On the other hand, the KO 2,5-dimethyl-3(2H)-furanone/1H-pyrrole-2-carboxaldehyde ratio Eco and for Standard caps was between 200 and 300.

The same values for both indices were revealed also for Msp, even if the standard deviation was very high at T90 and T120 for the 2,5-dimethyl-3(2H)-furanone/1H-pyrrole-2-carboxaldehyde ratio because of the different behaviour within the two lots observed (table 3).

These findings were interesting given that the trends of the two ratios pertain to different blends in different packaging.

Section 3.4: Validation of the chemical indices with sensory data on samples stored in ambient conditions

Of the two ratios identified only acetic acid/1H-pyrrole-2-carboxaldehyde resulted robust. This index varies over time independently of the type of sample examined (figure 10).

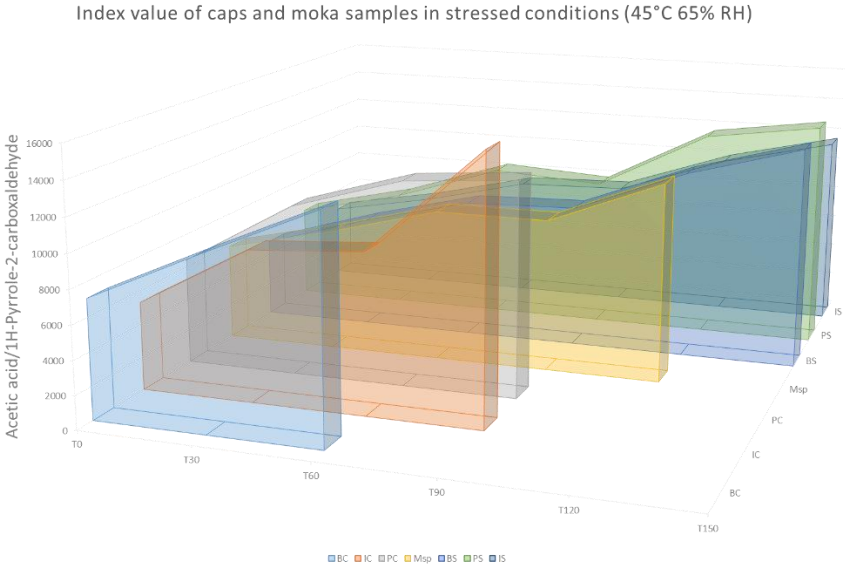

Figure 10: Acetic acid/1H-pyrrole-2-carboxaldehyde index trend over time for different Eco, Standard, and Moka soft pack (BC, IC, PC; BS, IS, PS and Msp) samples under stressed condition.

Using the mean value obtained from the index (the mean of the Eco caps and the mean of the Standard caps, table 3), we tested its ability to predict the non-acceptability of the sample under ambient conditions. Figure 11 reports the trend of the index in the samples under ambient condition.

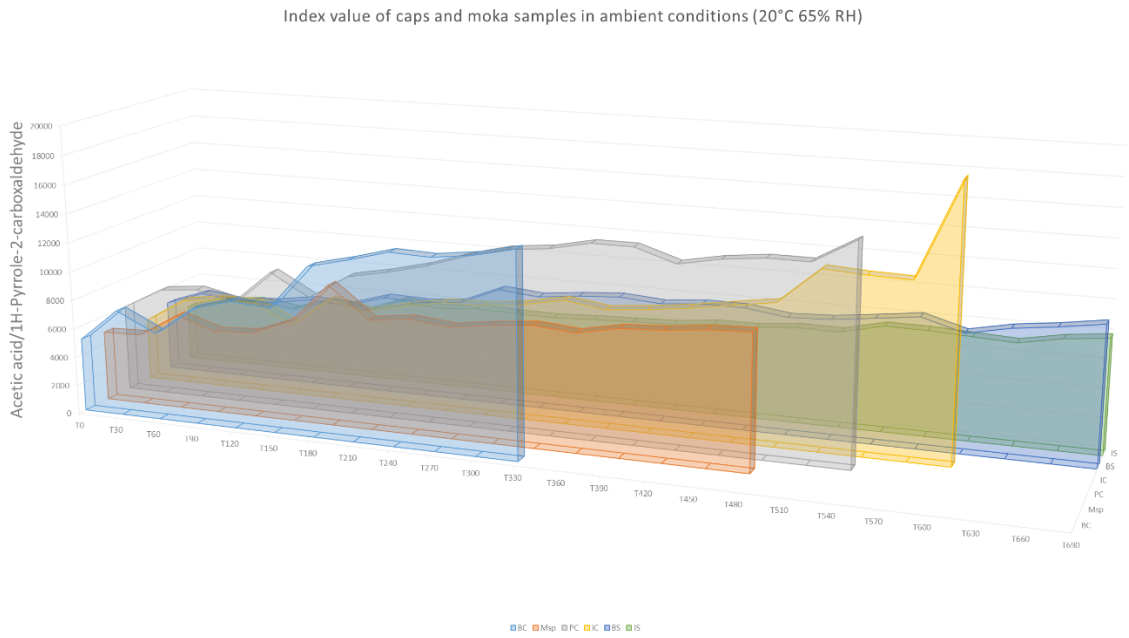

Figure 11: Acetic acid/1H-pyrrole-2-carboxaldehyde index trend over time for different Eco, Standard, and Moka soft pack samples (BC, IC, PC; BS, IS, PS and Msp) under ambient condition.

The chemical evaluation was then compared with the sensorial evaluation. Table 4 reports the results of the comparison between the sensory response (acceptable (OK)/not-acceptable (KO)) and the corresponding index value: these results highlights no differences between the two values. The only difference is given by the BS caps and the Moka samples for which anomalies in the blends from a chemical point of view have not yet been chemically clarified. However, these results underline an acceptable capability of predicting OK and KO samples with the chemical index.

Table 4: *Acceptable (OK) and not acceptable (KO) sensory results on coffees (Eco and Standard caps and moka samples) stored in ambient conditions, together with the* chemical evaluation using the index *Acetic acid /1H-pyrrole-2-carboxaldehyde. Green boxes OK products, red boxes KO products.*

	Validation	0	30	60	90	120	150	180	210	240	270	300	330	360	390	420	450	480	510	540	570	600	630	660	690
PC	Sensorial value																								
	Index value																								
BC	Sensorial value																								
	Index value																								
IC	Sensorial value																								
	Index value																								
BS	Sensorial value																								
	Index value																								
IS	Sensorial value																								
	Index value																								
Msp	Sensorial value																								
	Index value																								

Section 3.5: Testing the ability to define with an objective chemical index the sensory acceptability (Ok (Acceptable) and KO (not acceptable)) of coffees in a blind set of samples

An extra set of coffees was analysed in blind to evaluate the index's ability (Acetic acid /1H-pyrrole-2-carboxaldehyde) to discriminate between good and no longer acceptable samples. Given the fact that the blend or packaging was unknown, a single average value of the index obtained in stress conditions from all samples/packaging was calculated. Comparing the sensory evaluation to the chemical responses, only 7/28 samples resulted not matching, five of which are false negatives in the prediction of those samples that undergoes to oxidation (identified as good (OK) when they were not from the sensory panel) thus bringing the ability of the index in prediction to 75%. This result is at present under evaluation in a production factory to evaluate the acceptability of the risk to use a chemical index to define OK/KO samples (table 5).

Table 5: Acceptable (OK) and not acceptable (KO) sensory results on a blind samples set together with the chemical evaluation using the index Acetic acid /1H-pyrrole-2-carboxaldehyde. Green boxes OK products, red boxes KO products.

Prediction	Sensorial value	Index
1		
2		
3		
7		
8		
9		
10		
11		
15		
16		
18		
19		
20		
21		
22		
17		
23		
24		
25		
4		
5		
6		
8		
12		
13		
14		
26		
27		
28		

4. CONCLUSIONS

This study showed that some volatiles are related to the coffee ageing. Combining the simultaneous variation of these chemicals, a reductionist strategy was created to look for the most reliable aging markers by combining some of them and they exhibit identical patterns in all examined blends and packages. In particular, the ratios between some volatiles afforded the definition of two chemical indices that have been found to be correlated with coffee ageing, regardless of blends and packaging investigated.

From a sensory perspective, between the two indices identified the acetic acid/1H-pyrrole-2-carboxaldehyde index has proven to be much more robust when it comes to defining the acceptance or non-acceptance of the samples. It has been estimated that a ratio value of around 13000 indicates a KO coffee because the of aroma quality changes and in particular because aged and oxidised regardless blends and packaging.

The index was also evaluated on a set of blind samples, for which blend or packaging was unknown. Comparing the chemical and sensory evaluation responses 7 out 30 results do not match, five of which were however false negatives (i.e. they were identified as good (OK) from the sensory panel when they were not) thus indicating an ability of the index in prediction of 75%. This result is very good considering the high variability of coffees under investigation and it is now under evaluation in a production factory to evaluate the risk to use this objective tool for further studies on new packaging as a support to the panel test.

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SUPPLEMENTARY

Table S1: List of the investigated samples (B, M soft pack (Msp) and P (100% Coffea Arabica) and I (50% Coffea Robusta and 50% Coffea Arabica) in two different packaging (Eco and Standard) for two different preparations (caps available for espresso machine and Moka grind) their storage in stress and ambient conditions and time of aging.

Stress conditions (45°C - 65%RH) samples	T0	T30	T60	T90	T120	T150	T180
BC lot 1	BC1_T0	BC1_2T30	BC1_2T60	BC1_2T90			
BC lot 2	BC2_T0	BC2_2T30	BC2_2T60	BC2_2T90			
BC lot 3	BC3_T0	BC3_2T30	BC3_2T60	BC3_2T90			
BS lot 1	BS1_T0	BS1_2T30	BS1_2T60	BS1_2T90	BS1_2T120	BS1_2T150	BS1_2T180
BS lot 2	BS2_T0	BS2_2T30	BS2_2T60	BS2_2T90	BS2_2T120	BS2_2T150	BS2_2T180
BS lot 3	BS3_T0	BS3_2T30	BS3_2T60	BS3_2T90	BS3_2T120	BS3_2T150	BS3_2T180
IC lot 1	IC1_T0	IC1_2T30	IC1_2T60	IC1_2T90			
IC lot 2	IC2_T0	IC2_2T30	IC2_2T60	IC2_2T90			
IC lot 3	IC3_T0	IC3_2T30	IC3_2T60	IC3_2T90			
IS lot 1	IS1_T0	IS1_2T30	IS1_2T60	IS1_2T90	IS1_2T120	IS1_2T150	IS1_2T180
IS lot 2	IS2_T0	IS2_2T30	IS2_2T60	IS2_2T90	IS2_2T120	IS2_2T150	IS2_2T180
IS lot 3	IS3_T0	IS3_2T30	IS3_2T60	IS3_2T90	IS3_2T120	IS3_2T150	IS3_2T180
PC lot 1	PC1_T0	PC1_2T30	PC1_2T60	PC1_2T90			
PC lot 2	PC2_T0	PC2_2T30	PC2_2T60	PC2_2T90			
PC lot 3	PC3_T0	PC3_2T30	PC3_2T60	PC3_2T90			
PS lot 1	PS1_T0	PS1_2T30	PS1_2T60	PS1_2T90	PS1_2T120	PS1_2T150	PS1_2T180
PS lot 2	PS2_T0	PS2_2T30	PS2_2T60	PS2_2T90	PS2_2T120	PS2_2T150	PS2_2T180
PS lot 3	PS3_T0	PS3_2T30	PS3_2T60	PS3_2T90	PS3_2T120	PS3_2T150	PS3_2T180
Msp lot 1	SP1_T0	SP1_2T30	SP1_2T60	SP1_2T90	SP1_2T120	SP1_2T150	
Msp lot 2	SP2_T0	SP2_2T30	SP2_2T60	SP2_2T90	SP2_2T120	SP2_2T150	

Ambient conditions (20°C - 65%RH) samples	T0	T30	T60	T90	T120	T150	T180	T210	T240	T270	T300	T330	T360	T390	T420	T450	T480	T510	T540	T570	T600	T630	T660	T690
BC lot 1	BC1_T0	BC1_1T30	BC1_1T60	BC1_1T90	BC1_1T120	BC1_1T150	BC1_1T180	BC1_1T210	BC1_1T240	BC1_1T270	BC1_1T300	BC1_1T330	BC1_1T360	BC1_1T390	BC1_1T420	BC1_1T450	BC1_1T480	BC1_1T510	BC1_1T540					
BC lot 2	BC2_T0	BC2_1T30	BC2_1T60	BC2_1T90	BC2_1T120	BC2_1T150	BC2_1T180	BC2_1T210	BC2_1T240	BC2_1T270	BC2_1T300	BC2_1T330	BC2_1T360	BC2_1T390	BC2_1T420	BC2_1T450	BC2_1T480	BC2_1T510	BC2_1T540	BC2_1T570	BC2_1T600	BC2_1T630	BC2_1T660	
BC lot 3	BC3_T0	BC3_1T30	BC3_1T60	BC3_1T90	BC3_1T120	BC3_1T150	BC3_1T180	BC3_1T210	BC3_1T240	BC3_1T270	BC3_1T300	BC3_1T330	BC3_1T360	BC3_1T390	BC3_1T420	BC3_1T450	BC3_1T480	BC3_1T510	BC3_1T540	BC3_1T570	BC3_1T600	BC3_1T630	BC3_1T660	BC3_1T690
BS lot 1	BS1_T0	BS1_1T30	BS1_1T60	BS1_1T90	BS1_1T120	BS1_1T150	BS1_1T180	BS1_1T210	BS1_1T240	BS1_1T270	BS1_1T300	BS1_1T330	BS1_1T360	BS1_1T390	BS1_1T420	BS1_1T450	BS1_1T480	BS1_1T510	BS1_1T540	BS1_1T570				
BS lot 2	BS2_T0	BS2_1T30	BS2_1T60	BS2_1T90	BS2_1T120	BS2_1T150	BS2_1T180	BS2_1T210	BS2_1T240	BS2_1T270	BS2_1T300	BS2_1T330	BS2_1T360	BS2_1T390	BS2_1T420	BS2_1T450	BS2_1T480	BS2_1T510	BS2_1T540	BS2_1T570	BS2_1T600	BS2_1T630	BS2_1T660	
BS lot 3	BS3_T0	BS3_1T30	BS3_1T60	BS3_1T90	BS3_1T120	BS3_1T150	BS3_1T180	BS3_1T210	BS3_1T240	BS3_1T270	BS3_1T300	BS3_1T330	BS3_1T360	BS3_1T390	BS3_1T420	BS3_1T450	BS3_1T480	BS3_1T510	BS3_1T540	BS3_1T570	BS3_1T600	BS3_1T630	BS3_1T660	BS3_1T690
IC lot 1	IC1_T0	IC1_1T30	IC1_1T60	IC1_1T90	IC1_1T120	IC1_1T150	IC1_1T180	IC1_1T210	IC1_1T240	IC1_1T270	IC1_1T300	IC1_1T330	IC1_1T360	IC1_1T390	IC1_1T420	IC1_1T450	IC1_1T480							
IC lot 2	IC2_T0	IC2_1T30	IC2_1T60	IC2_1T90	IC2_1T120	IC2_1T150	IC2_1T180	IC2_1T210	IC2_1T240	IC2_1T270	IC2_1T300	IC2_1T330	IC2_1T360	IC2_1T390	IC2_1T420	IC2_1T450	IC2_1T480	IC2_1T510	IC2_1T540	IC2_1T570	IC2_1T600	IC2_1T630	IC2_1T660	
IC lot 3	IC3_T0	IC3_1T30	IC3_1T60	IC3_1T90	IC3_1T120	IC3_1T150	IC3_1T180	IC3_1T210	IC3_1T240	IC3_1T270	IC3_1T300	IC3_1T330	IC3_1T360	IC3_1T390	IC3_1T420	IC3_1T450	IC3_1T480	IC3_1T510	IC3_1T540	IC3_1T570	IC3_1T600	IC3_1T630	IC3_1T660	IC3_1T690
IS lot 1	IS1_T0	IS1_1T30	IS1_1T60	IS1_1T90	IS1_1T120	IS1_1T150	IS1_1T180	IS1_1T210	IS1_1T240	IS1_1T270	IS1_1T300	IS1_1T330	IS1_1T360	IS1_1T390	IS1_1T420	IS1_1T450	IS1_1T480	IS1_1T510	IS1_1T540	IS1_1T570	IS1_1T600	IS1_1T630		
IS lot 2	IS2_T0	IS2_1T30	IS2_1T60	IS2_1T90	IS2_1T120	IS2_1T150	IS2_1T180	IS2_1T210	IS2_1T240	IS2_1T270	IS2_1T300	IS2_1T330	IS2_1T360	IS2_1T390	IS2_1T420	IS2_1T450	IS2_1T480	IS2_1T510	IS2_1T540	IS2_1T570	IS2_1T600	IS2_1T630	IS2_1T660	IS2_1T690
IS lot 3	IS3_T0	IS3_1T30	IS3_1T60	IS3_1T90	IS3_1T120	IS3_1T150	IS3_1T180	IS3_1T210	IS3_1T240	IS3_1T270	IS3_1T300	IS3_1T330	IS3_1T360	IS3_1T390	IS3_1T420	IS3_1T450	IS3_1T480	IS3_1T510	IS3_1T540	IS3_1T570	IS3_1T600	IS3_1T630	IS3_1T660	IS3_1T690
PC lot 2	PC2_T0	PC2_1T30	PC2_1T60	PC2_1T90	PC2_1T120	PC2_1T150	PC2_1T180	PC2_1T210	PC2_1T240	PC2_1T270	PC2_1T300	PC2_1T330	PC2_1T360	PC2_1T390	PC2_1T420	PC2_1T450	PC2_1T480	PC2_1T510	PC2_1T540	PC2_1T570	PC2_1T600	PC2_1T630		
PC lot 3	PC3_T0	PC3_1T30	PC3_1T60	PC3_1T90	PC3_1T120	PC3_1T150	PC3_1T180	PC3_1T210	PC3_1T240	PC3_1T270	PC3_1T300	PC3_1T330	PC3_1T360	PC3_1T390	PC3_1T420	PC3_1T450	PC3_1T480	PC3_1T510	PC3_1T540	PC3_1T570	PC3_1T600	PC3_1T630	PC3_1T660	PC3_1T690
PS lot 1	PS1_T0	PS1_1T30	PS1_1T60	PS1_1T90	PS1_1T120	PS1_1T150	PS1_1T180	PS1_1T210	PS1_1T240	PS1_1T270	PS1_1T300	PS1_1T330	PS1_1T360	PS1_1T390	PS1_1T420	PS1_1T450	PS1_1T480	PS1_1T510	PS1_1T540	PS1_1T570	PS1_1T600	PS1_1T630	PS1_1T660	
PS lot 2	PS2_T0	PS2_1T30	PS2_1T60	PS2_1T90	PS2_1T120	PS2_1T150	PS2_1T180	PS2_1T210	PS2_1T240	PS2_1T270	PS2_1T300	PS2_1T330	PS2_1T360	PS2_1T390	PS2_1T420	PS2_1T450	PS2_1T480	PS2_1T510	PS2_1T540	PS2_1T570	PS2_1T600	PS2_1T630	PS2_1T660	PS2_1T690
PS lot 3	PS3_T0	PS3_1T30	PS3_1T60	PS3_1T90	PS3_1T120	PS3_1T150	PS3_1T180	PS3_1T210	PS3_1T240	PS3_1T270	PS3_1T300	PS3_1T330	PS3_1T360	PS3_1T390	PS3_1T420	PS3_1T450	PS3_1T480	PS3_1T510	PS3_1T540	PS3_1T570	PS3_1T600	PS3_1T630	PS3_1T660	PS3_1T690
Msp lot 1	SP1_T0	SP1_2T30	SP1_2T60	SP1_2T90	SP1_2T120	SP1_2T150	SP1_2T180	SP1_2T210	SP1_2T240	SP1_2T270	SP1_2T300	SP1_2T330	SP1_2T360	SP1_2T390	SP1_2T420	SP1_2T450	SP1_2T480							
Msp lot 2	SP2_T0	SP2_2T30	SP2_2T60	SP2_2T90	SP2_2T120	SP2_2T150	SP2_2T180	SP2_2T210	SP2_2T240	SP2_2T270	SP2_2T300	SP2_2T330	SP2_2T360	SP2_2T390	SP2_2T420	SP2_2T450	SP2_2T480							

Table S2: List of volatiles identified with their experimental and literature (retention indices) I^Ts, Target ion (Ti), and qualifier ions (Qi) and their mass spectral similarity index (SI).

Compounds name	**Rt**	I^T**calc**	I^T**ref**	**Ti**	**Qi**	**SI**
Acetone	2.22	821	820	43	58-42	98
Methyl acetate	2.30	832	825	43	74-42	94
Tetrahydrofuran	2.48	854	-	42	72-58	97
2-Methyl-furan	2.62	871	868	82	53-81	98
2,4-Dimethyl-1-heptene	2.68	885	885	43	55-70	96
2-Butanone	2.89	903	899	43	72-57	96
2,3-Dihydro-5-methyl-Furane	2.95	907	-	84	39-83	80
2-Methyl butanal	3.03	912	907	57	41-58	96
3-Methyl butanal	3.08	916	925	44	41-43	97
2,5-dimethyl-furane	3.58	958	958	96	81-53	97
1-Methyl piperidine	3.11	959	-	98	57-43-71	96
Unknown 1	3.92	971	-	57	43-86-41	89
2,3-Butanedione	3.99	977	998	43	86-42	99
Methyl 3-methylbutanoate	4.70	1043	1018	74	43-59-85	91
Thiophene	4.79	1045	1022	84	58-45	93
N-Methyl-1,2,5,6-tetrahydropyridine	4.58	1039	-	96	54-82-68	95
3-Hexanone	5.39	1064	1052	57	43-71-100	93
Unknown 2	5.53	1068	-	110	67-95	91
2,3-Pentanedione	5.68	1073	1085	43	57-100	98
Dimethyl disulfide	5.94	1081	1071	94	79-61	91
4-Vinylfuran	6.05	1084	-	94	65-66-39	96
Hexanal	6.19	1088	1084	56	72-44	93
Unknown 2'	6.77	1106	-	43	57-69	66
3,3,5-Trimethyl-1,5-heptadiene	6.63	1102	-	59	43-44-57	93
4,5-Dimethyl-2-undecene	6.97	1112	-	69	41-95-70	95
3-penten-2-one	7.37	1125	1110	69	41-43-39	87
2,3-Hexanedione	7.67	1134	1136	43	71-41	93
1-Methyl-1H-pyrrole	7.90	1141	1137	81	80-53	96
3,4-Hexandione	7.94	1142	1151	57	114-58-56	98
2-Vinyl-5-methylfuran	8.39	1156	-	108	107-43	95
beta-Myrcene	8.70	1165	1157	93	41-69-91	93
Pyridine	9.04	1176	1188	79	52-51-50	98
Limonene	9.72	1197	1205	68	111-93-136	95
Pyrazine	10.24	1211	1210	80	53-52	95
Butyl butanoate	10.14	1208	1212	71	89-56-43	84

Compounds name	Rt	I^Tcalc	I^Tref	Ti	Qi	SI
2-n-Pentylfuran	11.16	1234	1230	81	138-94	91
2-Furfuryl methyl ether	11.46	1242	1243	81	53-112-82	91
Thiazole	11.69	1248	1246	85	58-45-57	95
3-Methyl-3-buten-1-ol	11.80	1251	1245	56	86-41-68	92
E-beta Ocimene	11.92	1254	1250	93	91-136-105	89
3-Methyl-2-butenyl acetate	11.85	1252	1251	43	86-68-53	90
Methyl-pyrazine	12.22	1262	1264	94	67-40	99
Dihydro-2-methyl-3(2H)-furanone	12.33	1265	1269	43	72-100-44	99
2,5-Dimethyl-1H-pyrrole	12.56	1271	-	94	95-53	97
3-Hydroxy-2-butanone	13.12	1285	1277	45	43-88	94
trans-2-Methyl-5-n-propenylfuran	13.39	1292	1267	122	79-43-121	94
1-Hydroxy-2-propanone	13.12	1285	1274	43	74-42	99
Tridecane (ISTD)	13.85	1304	1300	57	71-99	98
2,5-Dimethyl-pyrazine	14.38	1317	1318	108	42-81	98
2,6-Dimethyl-pyrazine	14.66	1324	1330	108	42-40	98
2-Ethyl-pyrazine	14.94	1330	1334	107	108-80	99
2,3-Dimethyl-Pyrazine	15.32	1340	1335	108	67-59	96
2-Cyclopenten-1-one	15.68	1349	1341	82	108-123	93
Unknown 3	15.87	1353	-	43	71-86	97
2-Hydroxy-3-pentanone	16.08	1359	1361	45	57-58-84	98
2-Methyl-2-cyclopenten-1-one	16.21	1362	1367	67	96-53	97
2-Ethyl-6-methyl-Pyrazine	17.00	1381	1375	121	122-94	99
2-Ethyl-5-methyl-Pyrazine	17.21	1386	1387	121	122-94	98
2,3,5-Trimethyl-Pyrazine	17.67	1397	1394	122	42-39-81	94
2-Ethyl-3-methylpyrazine	17.76	1400	1397	121	122-94-67	79
2-Methyl-3(2H)furanone	18.14	1409	1397	54	98-43-59	92
2-(n-propyl)-Pyrazine	18.33	1413	1404	94	107-122	95
2,6-diethyl-Pyrazine	19.01	1430	1415	135	136-175	96
2-Furfurylthiol	19.38	1439	1434	81	53-114	64
2-ethyl-3,5-dimethyl-Pyrazine	19.43	1440	1435	135	136-42	95
2,3-Diethylpyrazine	19.86	1451	1458	135	136-56-108	85
Acetic acid	20.18	1461	1465	60	43-45	84
Furfural	20.52	1467	1467	96	95-39	87
trans-Linalool oxide	20.62	1469	1472	59	94-55-93	93
Acetoxyacetone	20.79	1474	1470	43	86-116-73	98
2-Methyl-6-vinyl pyrazine	21.25	1485	1488	120	52-121	98
Furfuryl methyl sulfide	21.40	1488	1493	81	128-53	97
3,5-Diethyl-2-methyl-pyrazine	21.46	1490	1496	149	150-120	96
2,5-Dimethyl-3(2H)-furanone	21.72	1496	1492	40	68-112	97

Compounds name	Rt	I^Tcalc	I^Tref	Ti	Qi	SI
2,5-Hexanedione	21.93	1501	1505	43	99-114	96
2-Acetylfuran	22.05	1505	1510	95	110-126	98
4-Vinyltetrahydro-2H-pyran-2-one	22.73	1522	-	67	54-126	93
1-(2-Furyl)-2-propanone	22.80	1523	1524	81	124-116	95
2,3-Dimethyl-2-cyclopenten-1-one	23.00	1528	1530	67	110-95	94
2-oxopropyl propanoate	23.31	1536	-	57	43-100-87	98
1-Acetoxy-2-butanone	23.49	1540	1536	57	43-100-87	97
Furfuryl acetate	23.66	1545	1541	81	98-140	99
Propanoic acid	23.11	1531	1555	74	45-57	92
5-Methyl-2-furancarboxaldehyde	24.77	1573	1570	110	53-81	98
2-Propionylfuran	24.86	1575	1571	95	124-99	90
(5-Methyl-2-furyl)methanethiol	25.07	1580	-	95	138-96	98
(1-methylethenyl)-Pyrazine	25.41	1589	-	119	120-131	94
2-Acetylpyridine	25.72	1597	1602	78	134-105	95
Furfuryl propanoate	26.00	1604	1606	81	98-154	85
5H-5-Methyl-6,7-dihydrocyclopentapyrazine	26.21	1609	1616	119	134-137	92
1-methyl 1H-Pyrrole-2-carboxaldehyde	26.46	1616	1620	109	108-53-80	92
4-hydroxy-butanoic acid	26.66	1621	-	42	41-86	97
2-Isopropenylpyrazine	27.01	1630	-	120	119-93-65	86
Butanoic acid	26.59	1619	1628	60	87-112	84
2,5-dihydro-3,5-dimethyl-2-Furanone	27.48	1642	1640	69	112-97	85
1-(2-Furyl)-butan-3-one	27.58	1645	1651	81	138-67	95
2-Acetyl-1-methylpyrrole	27.77	1650	1657	108	123-53	95
3-Mercapto-3-methyl-1-butanol	28.17	1660	1658	41	69-71	97
Furfuryl alcohol	28.49	1669	1660	98	81-69	99
Unknown 3'	28.74	1675	-	126	84-97	76
3-Methyl-butanoic acid	29.74	1701	1680	60	43-87	92
2-Furfuryl-5-methylfuran	28.44	1667	1678	162	91-119	79
2-Acetyl-3-methylpyrazine	29.08	1684	1686	136	94-67-108	77
Unknown 4	29.65	1699	-	134	133-81	95
Unknown 5	30.29	1716	-	97	69-126	83
N-acetyl-4(H)-Pyridine	30.45	1720	-	80	123-53	94
3-Methoxy-2-methyl-cyclohex-2-enone	30.77	1729	-	140	111-97	83
3-ethyl-4-methyl-2,5-Furandione	30.92	1733	-	67	53-140	94

Compounds name	Rt	I^Tcalc	I^Tref	Ti	Qi	SI
2(5H)-Furanone	30.64	1726	1712	55	84-150	90
Methyl salicylate	31.45	1748	1753	98	55-137	65
Unknown 6	32.46	1775	-	95	138-150	95
Unknown 7	32.63	1780	-	94	137-78	94
3,5-dimethyl cyclopentenolone	33.15	1794	-	126	69-111	91
Unknown 8	33.18	1795	-	80	137-109	89
3-methyl-2-Butenoic acid	32.77	1784	1804	100	55-83	92
Unknown 9	33.84	1813	-	55	83-126	83
2-hydroxy-3-methyl-2-Cyclopenten-1-one	34.27	1826	1807	112	69-83	94
1-(2-furanylmethyl)-1H-Pyrrole	34.39	1829	1833	81	147-53	97
Unknown 10	35.23	1853	-	128	112-151	84
n-butylbenzoate	35.37	1857		105	123-60	86
2-methoxy-Phenol	35.44	1859	1859	109	124-81	98
Unknown 11	35.55	1862	-	109	53-152	97
3-ethyl-2-hydroxy- 2-Cyclopenten-1-one	36.55	1890	1891	126	83-97	87
Unknown 12	36.80	1898	-	81	161-53	94
Phenylethyl Alcohol	37.14	1908	1914	91	65-122	92
2-Thiophenemethanol	38.31	1942	1950	114	85-97	91
Maltol	38.83	1958	1952	126	71-97	98
2-Acetylpyrrole	39.18	1968	1966	94	109-66	99
4(1H)-Quinazolinone	39.51	1978	-	146	93-118	92
Difurfuryl ether	39.75	1985	1980	81	82-178	97
4-Hydroxy-3-methylacetophenone	40.10	1996	-	135	150-107	88
Phenol	40.59	2010	1994	94	66-108	94
1H-Pyrrole-2-carboxaldehyde	40.90	2020	2012	95	66-39	99
4-ethyl guaiacol	41.15	2028	2034	137	152-122	95
2,5-Dimethyl-4-hydroxy-3(2H)-furanone (Furaneol)	41.44	2037	2039	43	128-85	73
5-Acetyldihydro-2(3H)-furanone (Solerone)	42.00	2054	2096	85	57-128	96
N-Methyl-2-formylpyrrol	43.47	2100	-	109	108-80	91
Unknown 13	44.24	2125	-	57	99-149	75
2-Vinyl guaiacol	46.35	2193	2185	150	135-107	99
Nonanoic acid	46.87	2210	2168	60	73-57-41	95
Unknown 14	47.64	2236	-	81	175-163	93
n-Decanoic acid	50.10	2319	2303	60	57-129	92

Compounds name	**Rt**	***I*^{T}calc**	***I*^{T}ref**	**Ti**	**Qi**	**SI**
2-Benzofuran-1(3H)-one	50.39	2329	2356	105	77-134	90
Unknown 15	51.95	2384	-	95	39-67	95
2,3-dihydro-Benzofuran	52.27	2395	-	120	91-119-65	84
Indole	53.32	2434	2448	117	90-89-63	89
Benzoic acid	53.27	2432	2433	105	122-77	84
5-(Hydroxymethyl)dihydro-2(3H)-furanone	54.22	2467	-	85	57-43	92

2.3 Taste quality

2.3.1 Evaluation of phenolic and alkaloids behaviour in roasted and ground coffee samples stored in different types of packaging: Implications for quality and shelf life.

Evaluation of phenolic and alkaloids behaviour in roasted and ground coffee samples stored in different types of packaging: Implications for quality and shelf life.

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1. INTRODUCTION

Coffee is one of the most popular drinks in the world, thanks to its psychoactive effect and its pleasant taste (Panusa, 2013). Taste and aroma are the most important properties to assess the quality of coffee andconsumer preference. Roasting is the main process that contributes to the development of flavour, essential for obtaining a high-quality coffee. Hundreds of chemical reactions occur simultaneously during roasting, favouring on one hand, the degradation of proteins, sugars, trigonelline and chlorogenic acids and, on the other, the formation of substances deriving from the Maillard reactions and Strecker degradation which will condition both the flavor and the aroma of the coffee drink. Many of these compounds, among which phenolics and alkaloids, contribute also to the basic taste sensation of coffee beverage, imparting bitterness, astringency, strength and body of coffee brew (Folmer 2016; Buffo, 2004; Clarke, 2014; Farah, 2006; dos Santos Scholz, 2018; Sunarharum, 2014).

Phenolic compounds are present in many parts of the coffee plant, but, in the seeds chlorogenic acids are predominant as a group of esters of one or more of the three analogues of *trans*-hydroxycinnamic acids (caffeic, ferulic, and p-coumaric, presented even in the free form) with quinic acid. Depending on the type and number of conjugated hydroxycinnamic acids, it is possible to have different groups of CQAs: 1) caffeoylquinic acids (CQA) (caffeic acid esterified with quinic acid), among which, 3-CQA, 5-CQA and 4-CQA stand in for chlorogenic acid, neo-chlorogenic acid and crypto-chlorogenic acid, respectively; 2) di-caffeoylquinic acids (di-CQA) (two caffeic acids esterified with quinic acid), 3) feruloylquinic acids (FQA) (ferulic acid esterified with quinic acid) and 4) coumaric acids (p-CoQA)(p-coumaric acid esterified with quinic acid). Furthermore, depending on the position of the ester bond(s), it is possible to have different isomers (Clifford, 2017; Farah, 2006; Panusa, 2013; Perrone, 2008). Figure 1 shows the different CQAs structures present in coffee, with their precursors (Farah, 2006; Perrone, 2008).

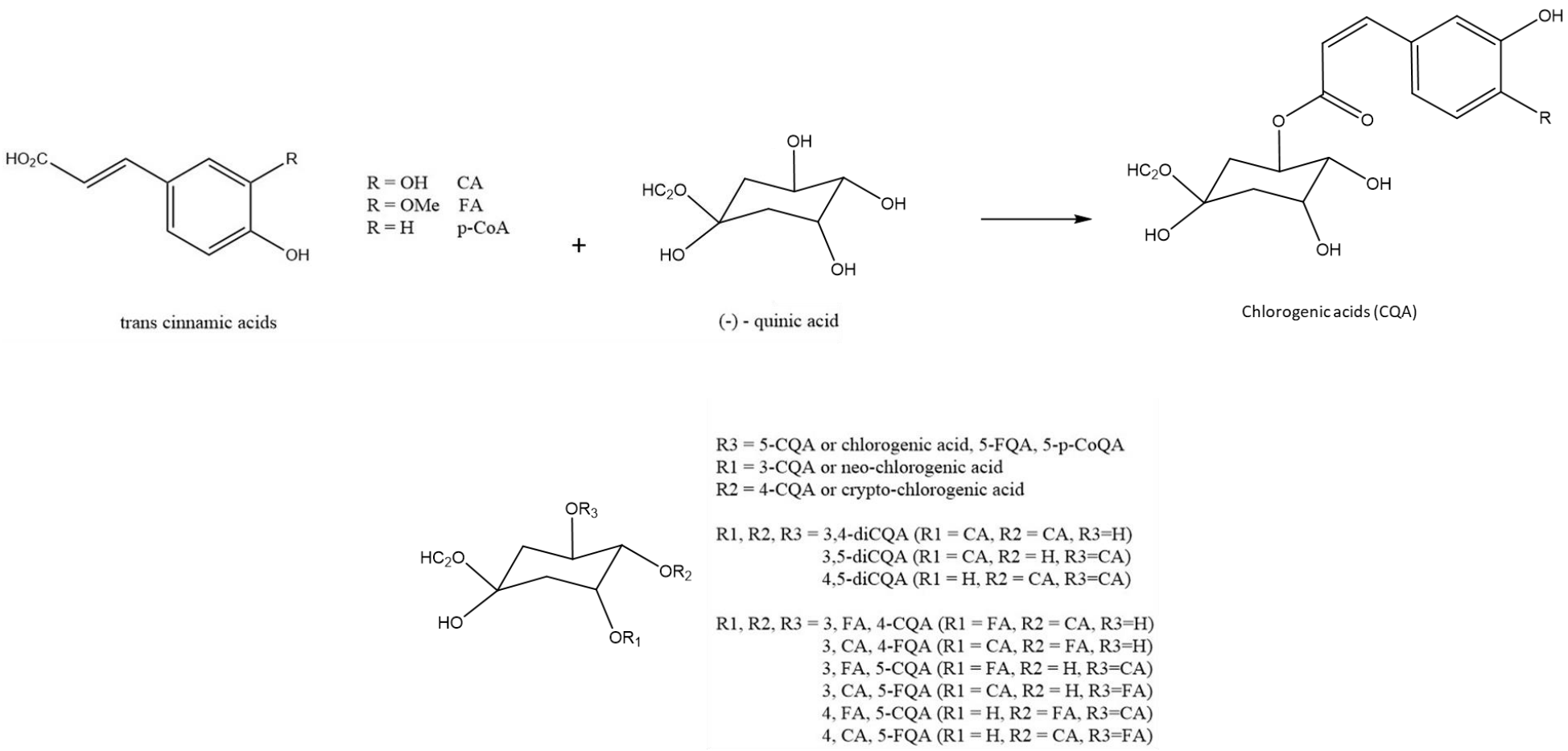

Figure 1: structure of chlorogenic acids precursors trans- cinnamic acids (quinic acid, caffeic acid (CA), ferulic acid (FA),p-coumaric acid (p-CoA)) and quinic acid. CGA main subclasses: caffeoylquinic acids (CQA), feruloylquinic acids (FQA), p-coumaroylquinic acids (p-CoQA),dicaffeoylquinic acids (diCQA).

Chlorogenic acids have a marked influence in determining coffee quality and play an important role in the formation of coffee flavour. In fact, during the roasting process, as a result of Maillard and Strecker's reactions, quinic acid remain relatively constant while the content of chlorogenic acids decrease since they decompose to quinic and caffeic acid responsible from one side for the increasing of acidity and to the other for the formation of phenolic volatiles and for the corresponding phenolic lactones (Correia, 2016; Perrone, 2008). The more drastic are the roasting conditions, the greater is the loss of chlorogenic acids components (up to 95% of the losses) (Farah, 2006). Coffee beans presented a different amount of chlorogenic acids depending not only on the species, (*Coffea canephora* Pierre (Robusta) contain the highest amount), but also on agricultural practices, on environmental conditions and on cultivar (Król, 2020). Trigonelline, a pyridine derivative, and caffeine, a xanthine derivative, (Figure 2) belonging to the alkaloids class, are responsible for the main stimulant effects and contribute to the bitter taste of the drink. While, trigonelline is rapidly degraded to nicotinic acid and volatile compounds such as pyridines and pyrroles, caffeine, on the opposite, is not affected by the roasting process and then its concentration strongly depends on the species and geographical area of origin and on the agricultural practices.

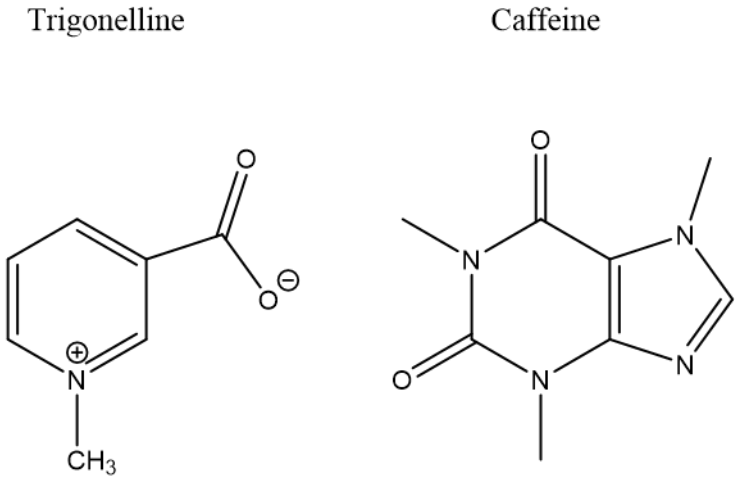

Figure 2: Chemical structure of the two alkaloids.

The two alkaloids and the phenolic acids have been studied mainly for their physiological effects as a source of psychoactive and antioxidant components and only few studies have focused on the effects that storage conditions over time have on these chemical compounds.

Krol *et al*. (Król, 2020) showed how, after 12 months of storage, the caffeine content did not change in the roasted coffee samples, while the content of phenolic components decreased, due to both the enzymatic and non-enzymatic oxidative effect, leading to an increase of caffeic, salicylic and gallic acids. The aim of this study is to evaluate the temporal evolution of coffee flavour in different and innovative packaging and try to investigate the chemistry involved with it. Compared to other research works, this project focuses on the performance of different packaging under the stressed conditions of temperature and humidity over time. The non-volatile coffee fraction and in particular phenolic compounds and alkaloids were here investigated as representative of astringency and bitter notes of the coffee flavour.

2. MATERIALS AND METHODS

Samples

Single served coffee capsules suitable for Espresso preparation, of three different roasting grounded coffee blends ("I", "P" and "B") were kindly supply by Lavazza group s.p.a (Turin, Italy). P and B were 100% *Coffea Arabica* , while I consisted of 50/50 *Coffea Arabica*/*Coffea Robusta*. The three blends were available in two different types of capsules: i) Eco capsules are 100% compostable caps made of an innovative biopolymer capable of degrading to compost in 180 days and becoming compost (IC, PC and BC), and ii) Standard capsules made of a copolymer based on polyethylene and aluminium (IS, PS and BS). The samples, were subjected to stress conditions at 45°C and a relative humidity corresponding to 65% (45°C; RH 65%). and then stored in freezer, before analysis. P blend in Eco capsules was also investigated at different relative humidity (RH 75%: PC75%) to evaluate how an increase in humidity affects coffee characteristics. The stressed samples were analysed at the following days (T0, T30, T60, T90) for both caps. Table S1 reports the details on the investigated samples of the supporting information.

Chemicals and standards

3-CQA, 5-CQA, 3,4- di-CQA, trigonelline, caffeine and caffeic, p-coumaric, ferulic, isoferulic and quinic acids were obtained from Sigma-Aldrich (Bellefonte), while 4-CQA, 3,5- di-CQA and 4,5- di-CQA were from Phytolab (Vestenbergsgreuth, Germany).

CQAs and di-CQA, hydroxycinnamic acids, quinic acid and alkaloids extraction

The beverage was obtained by percolation with 100 mL of distilled water at 90°C on 1g of coffee powder and then filtered through filter paper and then a 13 mm syringe filter (nylon 0.20 µm) to obtain 16mL of extract containing the compounds of interest. The extraction was carried out in two replicates for each sample.

LC-MS-UV/DAD

20 µL of each extract was analysed in duplicate with an Agilent 1200 system (Little Falls, DE equipped with an Agilent 1100 series spectra system UV diode array detector (Little Falls, DE). Samples were separated using a reversed phase C18 column (Eclipse XDB-C18) (250 x 4.6 mm, 80 Å, 5 µm) (Alltech, Deerfield), under controlled temperature condition at 25°C and at flow rate of 1.0 mL / min. The mobile phases were: solvent A: water with formic acid (999: 1, v/v), solvent B: acetonitrile with formic acid (999: 1, v/v). The gradient program was as follows: 15% B for 7 min, 15−55% B in 20 min, 55−100% B in 25 min and 100% B for 2 min, for a total running of 30 min. UV spectra were registered at 276 nm and 325 nm. The components were identified by comparing their retention times and UV spectra to those of authentic standards. The other components were tentatively identified. The stocks standard solutions were prepared at 0.1 mg/mL in ACN/water.

The chromatograms resulting from the analysis were processed through the Enhanced ChemStation software (MSD ChemStation F.01.03.2357-copyright 1989-2015 Agilent Technologies).

Statistical analysis

The data were processed with the statistical and data analysis package XLSTAT software version 2021.2.1 (Addinsoft, New York, NY USA) in particular principal component analysis (PCA), Spearman correlation and one-way ANOVA, to assess the statistical differences between the samples along time were applied.

3. RESULTS AND DISCUSSION

Analysis of phenols in coffee samples

In this study, cholorogenic acids (5-CQA, 4-CQA, 3-CQA), di-chlorogenic acids (3,4 diCQA, 3,5 diCQA, 4,5-diCQA), hydroxycinnamic acids (caffeic acid, p-coumaric acid, ferulic acid, isoferulic acid), quinic acid and alkaloids (caffeine, trigonelline) content of 24 samples of roasted coffee in various packaging was investigated.

The principal component analysis (PCA) shown below (Figure 3a and 3b), visualises how the different blends are mainly divided into two groups: 100% Arabica (P and B) and blend (50% Arabica and 50% Robusta - I). The LC-UV/DAD fingerprint was the base for this PCA, which grouped the various chemical classes to examine the differences between the blends:

- CQAs grouping all esterified chlorogenic acids (3-CQA; 4-CQA and 5-CQA) and di-chlorogenic acids (3,4-di-CQA; 3,5-di-CQA; 4,5-di-CQA);
- CINN grouping all the non-esterified hydroxycinnamic acids (ferulic, isoferulic, caffeic and p-coumaric acids) plus quinic acid;
- ALK with trigonelline and caffeine.

This PCA (Figure 3a, score plot) shows how the first component (F1), which accounts for 98.59% of the total variance, distinguishes the samples based on the blend. The biplot in figure 3b) further demonstrates that the I blend is richer in comparison to the other blends (P and B) of these components (CQAs, CINN and ALK) revealing significant discrimination regarding the composition of the blends (P and B respect to I and B). Two major groups are characterized: the blends in the capsules made entirely of Arabica powder (P and B) are on the left, and the I blend (50/50 Arabica/Robusta) is on the right (Figure 3b).

Observing how the samples are distributed along the second component (F2) can provide further relevant information mostly related to the time of ageing, even if with a trend not linear. This indicates that the phenolic component and the alkaloids are more resistant to degradation processes since they are less affected by the time of storage of the capsules . From these factors, it is clear that the composition of the blend, rather than the packaging, has the highest impact on the drink's flavour (F1; 95.28% vs F2; 3.31%).

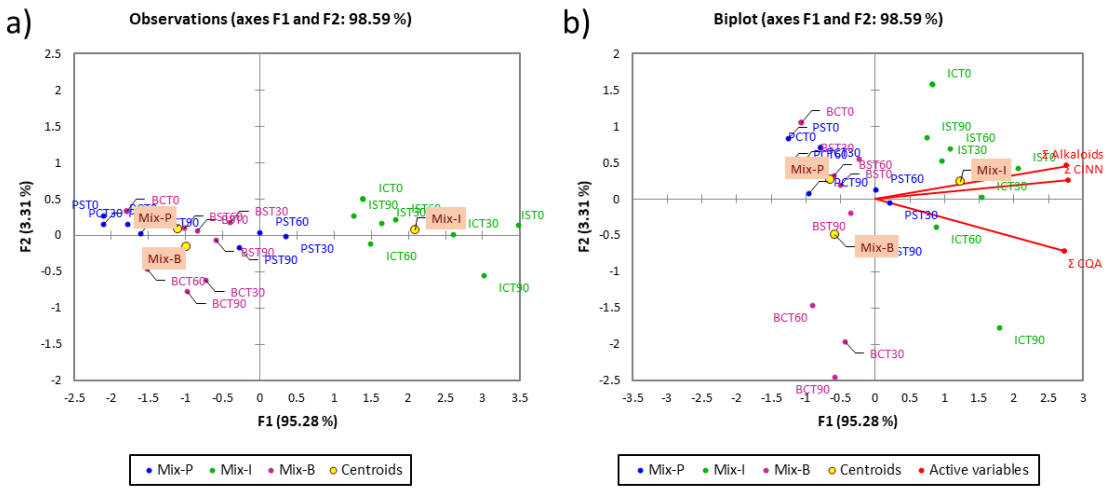

Figure 3: PCA on I, B and P of both Eco (IC, BC and PC) and Standard caps (IS, BS and PS) : a) Score plot and b) Biplot show the sample's distribution on the first and second PCs.

The variation in the abundance of the components over time was graphically represented For each coffee blend,, in function of the different packaging (Eco Caps or Standard Caps). Chlorogenic acids significantly contribute to the flavour of coffee, supporting the bitter, together with caffeine and trigonelline, and the astringent properties of the drink (Córdoba, 2021; del Campo, 2010; Heo, 2020; Ribeiro, 2011).

The variation over time of the CQAs, CINN and ALK, in the different samples, depending on the packaging is reported in figure 4 and 5 (Figure 4 for Eco caps and Figure 5 for Standard caps) as a sum of the chemical classes. First of all, Figure 4 and 5 show that the abundance of CQA and CINN in blend I is higher than that of other samples B and P, , confirming literature data indicating that *Coffea canephora* is distinguished by a higher proportion of phenolic compounds (Farah, 2006; Jeszka-Skowron, 2016; Narita, 2015). Therefore, the flavour of coffee blended with Robusta is known to be stronger than that of a 100% Arabica coffee. More in detail, as illustrated in Figure 4, the levels of CQAs, CINN, and ALK do not significantly vary over time in the Eco caps at 65% relative humidity. As validated by the ANOVA test, they were not significant at alpha=0.05, indicating a strong stability of their abundance during storage. However, only CQAs in IC show a small increase from T0 to T90 (figure 4a). Similarly CQAs grows in BC , but only from T0 to T30, after which they are stable over time (figure 4b). On the other hand, all the fractions in PC are stable over time, and under the same relative

humidity (RH 65%) (figure 4c), but when the storage humidity rises to 75%, the samples seems to be influenced by humidity-promoted degradative processes. Indeed, at T90 all fractions collapse (figure 4d).

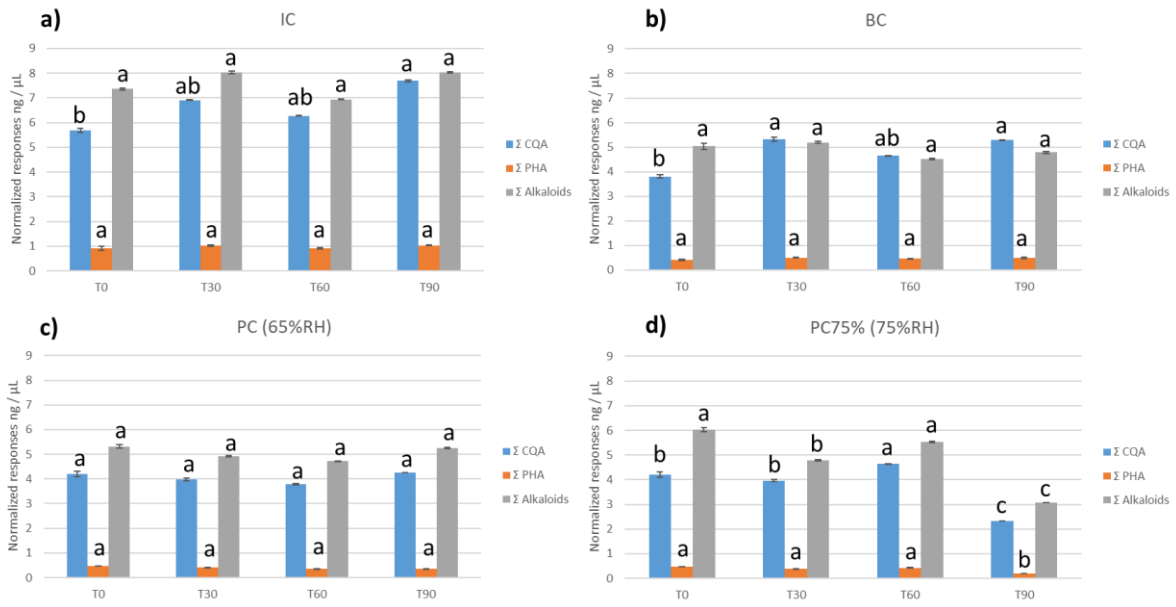

Figure 4: variation in time from T0 to T90 of the sum of CQAs that include the esterified chlorogenic acids (3-CQA; 4-CQA and 5-CQA) and di-chlorogenic acids (3,4-di-CQA; 3,5-di-CQA; 4,5-di-CQA); the sum of the CINN which group all the non-esterified hydroxycinnamic acids (ferulic, isoferulic, caffeic and p-coumaric acids) plus quinic acid and the sum of ALK that group trigonelline and caffeine in the Eco capsules. Mean of two extractions and two analytical replicates for each extract. 5a) IC; 5b) BC; 5c) PC (at 65% RH); 5d) PC75% (75% RH). Same letters correspond to no variation (p value >0.05).

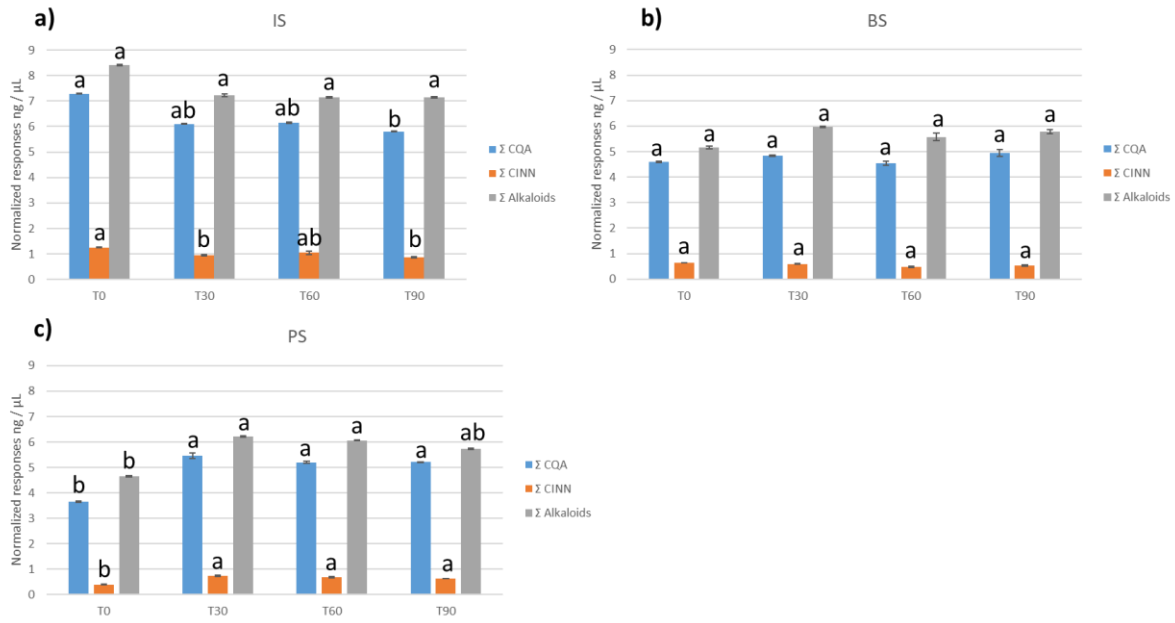

Figure 5: variation in time from T0 to T90 of the sum of CQAs that include the esterified chlorogenic acids (3-CQA; 4-CQA and 5-CQA) and di-chlorogenic acids (3,4-di-CQA; 3,5-di-CQA; 4,5-di-CQA); the sum of the CINN which group all the non-esterified hydroxycinnamic acids (ferulic, isoferulic, caffeic and p-coumaric acids) plus quinic acid and the sum of ALK that group trigonelline and caffeine in the Standard capsules. Mean of two extractions and two analytical replicates for each extract. 5a) IS; 5b) BS; 5c) PS. Same letters correspond to no variation (p value >0.05).

More specifically, in PC75% The Anova test (data not reported) shows that CQAs (as sum) vary the most compared to the dimers of CQAs (as a sum). The chlorogenic acids class is sensitive to moisture and it decreases more when coffee is stored at higher ambient humidity. This can be explained by the fact that an increase in water content leads to an increase in hydrolytic reactions that cleave the ester bond of chlorogenic acids forming caffeic acid and quinic acid (Dawidowicz, 2017).

An histogram chart showing the trends of individual compounds is reported below (Figure 6) to detectwhich chlorogenic acid isomers are more prone to degradation in both PC at 65% and at 75% RH . More in details, it is clear that the same sample placed in two different moisture conditions undergoes greater degradation in sample PC75% than in PC, especially with a rapid degradation of the 3 CQAs compounds at time T90 (Figure 6a and 6b). Among di-CQAs, all undergo to a degradation, which is much higher for the 3,4-diCQA isomer (Figure

6b). Contrary to what expected, direct hydrolytic degradation compounds of CQA acids, such as caffeic acid and quinic acid, do not appear to be related to mono- and di-chlorogenic acids. A possible explanation is that the degradation reaction of CQAs may not be related to a cleavage reaction that would lead to the formation of hydrolysis by-products, but to an oxidation-type reaction, given the strong antioxidant power these acids possess (Kamiyama, 2015; Vignoli, 2011). The other hydroxycinnamic compounds similarly behave between PC and PC75%, with caffeic acid being unstable at any humidity value (Figure 6c and d). Finally, among the alkaloids, the compound that is most affected by moisture over time is caffeine, while trigonelline seems to be nearly stable (Figure 6e and f). Although few studies related to caffeine reactions during storage of coffee samples are present in the literature, our data suggest a degradative effect influenced by time and humidity. These data appear to be in contrast to Król's results (Król, 2019) that show an increase in caffeine levels after 12 months, and in agreement with Vandeponseele (Vandeponseele, 2021) who reports a decrease in caffeine levels in a humid environment due to an enzymatic effect that would lead to xanthine formation. Telo reported an oxidative effect related to the degradation of this alkaloid to 1,3,7-trimethyluric acid (8-hydroxycaffeine) (Telo, 1997).

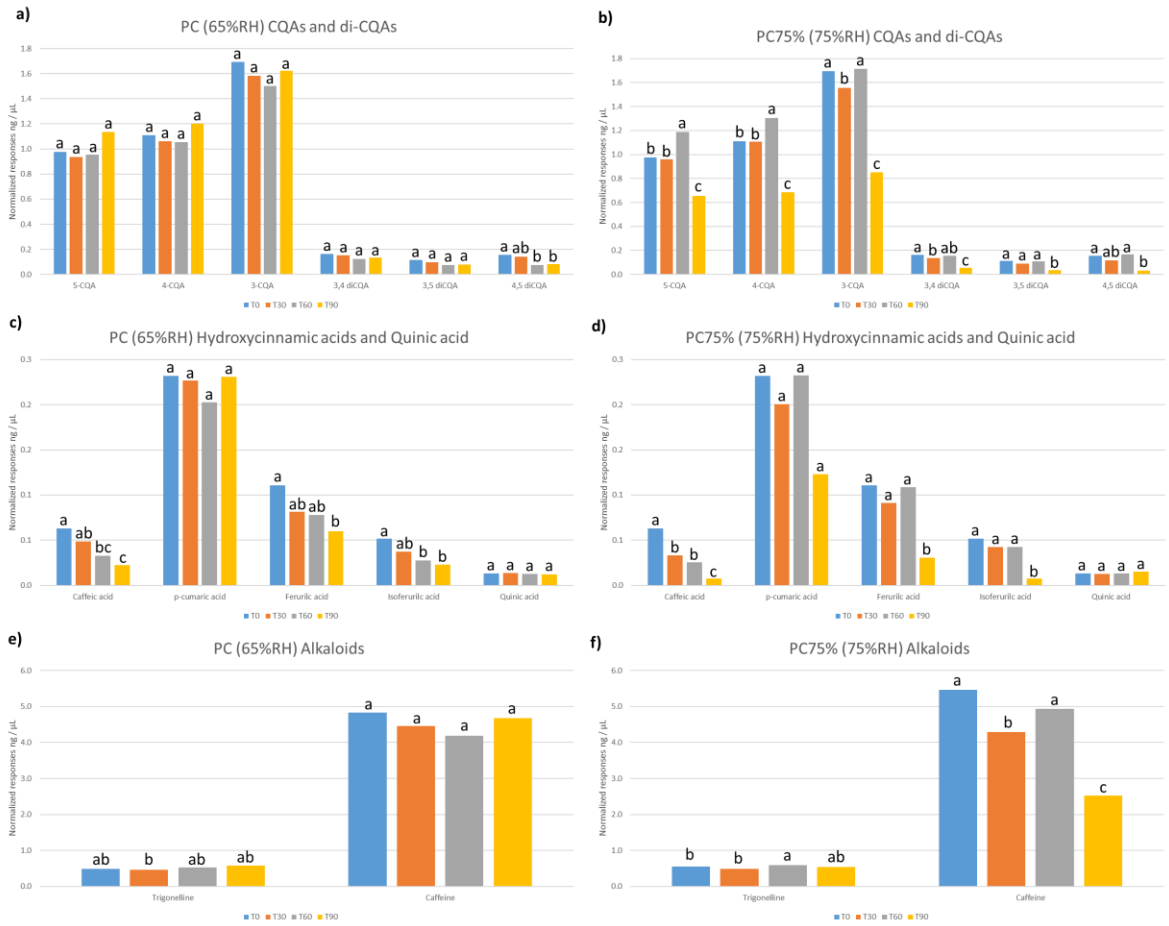

Figure 6: variation in time from T0 to T90 of the single phenolic components: a and b) CQAs that include the esterified chlorogenic acids (5-CQA; 4-CQA and 3-CQA) and the di-CQAs that include (3,4-di-CQA; 3,5-di-CQA; 4,5-di-CQA); c and d) that include all the non-esterified hydroxycinnamic acids (ferulic, isoferulic, caffeic and p-coumaric acids) plus quinic acid and e and f) that include the alkaloids: trigonelline and caffeine. 6a), 6c) and 6e) PC (65%RH 45°C); 6b), 6d) and 6f) PC75% (75%RH 45°C). Same letters correspond to no variation (p value >0.05).

The standard packed capsules maintained a greater stability over time than Eco, varying slightly over time in I and P (figure 5a) and 5c)) or not at all in B (figure 5b). This behaviour emphasizes the fact that this packaging, compared to Eco, has a better stability to the stress conditions and phenolic fraction is more stable over time.

4. CONCLUSIONS

From this study, it appears that the fractions that determine the taste of coffee strongly depend on the composition of the blend. In particular, PCAs showed a clear discrimination between 100% Arabica and 50% Arabica + 50% Robusta blends along the first main component. Another important factor influencing the variation in flavour quality is the storage conditions of the samples. The phenolic fraction in general remains nearly stable, although under different moisture conditions, CQAs degrade to a different extent depending on the isomers (i.e. mono CQAs more than dimers). In particular, caffeine and caffeic acid vary more compared to trigonelline and the other hydroxycinnamic acids. The above compounds were found to be more variable over time when stored in Eco caps compared to Standard caps, and an increase in the degradation of compounds related to bitter and astringency is observed with an increase in the relative humidity of the storage environment.

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SUPPLEMENTARY

Table S1: List of the investigated samples (B and P (100% Coffea Arabica) and I (50% Coffea Robusta and 50% Coffea Arabica) in two different packaging (Eco and standards) for espresso preparation (caps available for espresso machine) their storage in stress conditions and time of aging.

Stress conditions (45°C - 65%RH) caps	T0	T30	T60	T90
BC	BC1_T0	BC1_2T30	BC1_2T60	BC1_2T90
BS	BS1_T0	BS1_2T30	BS1_2T60	BS1_2T90
IC	IC1_T0	IC1_2T30	IC1_2T60	IC1_2T90
IS	IS1_T0	IS1_2T30	IS1_2T60	IS1_2T90
PC	PC1_T0	PC1_2T30	PC1_2T60	PC1_2T90
PS	PS1_T0	PS1_2T30	PS1_2T60	PS1_2T90

Stress conditions (45°C - 75%RH) caps	T0	T30	T60	T90
PC75%	PC1_T0	PC1_2T30	PC1_2T60	PC1_2T90

2.3.2 Study of lipid fraction in roasted and ground coffee packed in aluminium and environmentally friendly capsules for stability assessment as quality markers.

Study of lipid fraction in roasted and ground coffee packed in aluminium and environmentally friendly capsules for stability assessment as quality markers.

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1. INTRODUCTION

Consumption of coffee drinks produces hedonic pleasure as the result of a sophisticated multimodal experience involving all our senses. Peripheral processing of inputs received through the five senses is the starting point. Undoubtedly, the most significant factors in determining the flavour of coffee are aroma and taste. In particular, the balance between taste, which is detected through non-volatile molecules that bind at the level of the tongue and aroma, which is perceived through the sense of smell (orthonasal and retronasal), determines the quality of flavour perception (Folmer, 2016; Shepherd, 2006; Sunarharum, 2014). Lipids are of considerable importance to the sensory experience of coffee, especially espresso, where they influence the emulsion of the cream, the release of aroma components, the texture and the mouthfeel of the brew. (Moeenfard, 2020).

Coffee lipid fraction accounts for 11-17% of the chemical composition of beans depending on the species (i.e. *Arabica* or *Robusta*). The specific amounts of each depend on the type of coffee, the harvesting and post-harvesting procedures, the presence of damaged beans and the roasting technique (Yeager, 2021). Coffee lipids are dominated by a saponifiable fraction composed of 75% of triglycerides (TAGs), located for the major part in the endosperm of beans (Clarke, 2016). The most important unsaturated fatty acids in coffee are oleic (18:1n-9), linoleic (18:2n-6) and linolenic (18:3n-3) acids, with a different percentage between the two species (Speer, 2006; Toci, 2013). The remaining 25% of the lipid content consists of free fatty acids (FFA), esterified diterpenes, free and esterified sterols and substances such as tocopherols (α-tocopherol) and squalene (Clarke, 2016; De Oliveira, 2014; Moeenfard, 2020). The level of free fatty acids (FFA) is positively associated with the increase of temperature, oxygen content and moisture during the storage period, as the lipid fraction is a typical substrate for oxidation and/or degradation processes under enzymatic and non-enzymatic catalysis (Cincotta, 2020; Patui, 2014). Indeed, these elements significantly influence the stability of the chemical composition of roasted beans. High moisture content in food preservation systems can limit the amount of oxygen that comes into contact with the food, which tends to reduce oxidative reactions, but simultaneously can promote hydrolysis events. On the other hand, unsaturated FFAs, which are more prone to oxidative reactions than

esterified acids (EFAs), in presence of oxygen promote degradation processes (Toci, 2013). Oxidation of FFAs results in the formation of numerous volatile compounds, mainly carbonyl derivatives, a loss of freshness and also promote the formation of stale notes, thus affecting the sensory quality of the beverage (Cincotta, 2020; Goodman, 2015; Flament, 2002; Toci, 2013). The monitoring of the lipid fraction of coffee is therefore a crucial aspect in defining the shelf-life (Anese, 2006; Calligaris, 2016; Cincotta, 2020). Previous studies have often evaluated the behaviour of volatile composition over time, focusing on the stability of roasted coffee under various conditions of temperature, atmosphere, humidity and packaging, showing that these variables can influence the sensory quality of coffee (Manzocco, 2016; Strocchi, 2022). Few studies have been conducted on the temporal degradation of the lipid fraction in coffee, in particular those on the oxidative reaction that can occur during storage when the coffee is wrapped with different packaging materials. This study, therefore, aims to investigate the effects of storage on the lipid fraction of roasted and ground (R&D) coffee through a systematic approach. Commercial capsules for espresso preparation by three blends and packaged with different materials were monitored during storage in stress conditions. The behavior of lipid fraction together with the number of peroxides, p-Anisidine value, moisture %, pH and volatile compounds have been investigated to understand their relationship over time in blends and packaging.

2. MATERIALS AND METHODS

Samples

Single serve coffee capsules suitable for Espresso preparation, of three different roasting grounded coffee blends (“I”, “P” and “B”) were kindly supply by Lavazza group s.p.a (Turin, Italy). P and B, were from 100% *Coffea arabica* L. specie, while I, was from 50% of *Coffea arabica* L. and 50% of *Coffea canephora* Pierre ex A. Froehner *(robusta)*. The three blends were available in two different types of capsules: ecological capsules or Eco caps (IC, PC and BC), are composed by a 100% compostable material made of an innovative biopolymer capable of degrading in 180 days and becoming compost and Standard capsules or std caps (IS, PS and BS) made of a copolymer based on polypropylene and aluminium. The samples, were subjected to

stress conditions at a temperature of 45°C and relative humidity corresponding to 65% (RH 65%) and then preserved in freezer, before the analysis. P blend in Eco capsules was also investigated at different relative humidity (RH 75%: PC75%) to evaluate how an increase in humidity affects coffee. The stressed samples were monitored at intervals of 30 days from T0 to T90 for the ecological caps and from T0 to T180 for Standard capsules, except for BS capsules for which the time monitoring was from T0 to T120. The details on the samples analysed were reported in Table S1 of the supporting information.

Reagents and standards

Undecanoic acid methyl ester ≥98.0%, pure standards for normalization of individual fatty acids, n-alkanes (from n-C9 to n-C25) for linear retention indices (I^T_S) determination and the reference mixture for FAMEs identification (Supelco 37 components mix) were all from Supelco-Merck (Milan, Italy). Pure reference compounds were either purchased from Merck or taken from the authors' laboratory collection for key-odourants identity validation. n-Hexane 97% by VWR Chemicals (USA); Methanol (MeOH) for HPLC ≥ 99.9%, potassium hydroxide (KOH) and Sulfuric acid 98% (H_2SO_4) are from Sigma-Aldrich (Darmstadt, Germany).

Non-volatile fraction analysis: Analysis and identification of fatty acids methyl esters by gas Chromatography

Analyses were performed using an Agilent 6890 GC unit coupled to an Agilent 5973N MSD. A Supelco SLB™-IL 76 (Tri (tripropylphosphoniumhexanamido) triethylaminebis (trifluoromethanesulfonyl) imide) (30 m x 0.25 mm dc, 0.20µm df) column was used. The chromatographic conditions were: 1 µl of samples and FAME standards was injected in split 1:20 mode at a temperature of 250 °C; column temperature setting 60°C to 200 °C at 2°C/min and to 220° (5 min) at 5°C/min; detector MS 5973 Network, MS source temperature 230 °C; MS transfer line temperature 260 °C; Quadrupole temperature 150°C; carrier gas helium; Helium flow 1 mL/min, solvent delay 4 min. This method allowed to reach a separation of the FAMEs from the reference mixture (FAME37mix) with resolution at the baseline as shown by the chromatogram in figure S1 of the supporting information. The reliable identification and

the elution order of FAMEs in coffee lipid extracts occurred by comparing the peaks of the elution order of components in coffee, with retention times (Rt min) and I^T are listed in table S2 of the supporting information.

Volatile fraction analysis

A combi-PAL AOC 5000 Autoinjector equipped with SPME tool was coupled to a Shimadzu QP2010 GC-MS system provided with GC–MS Solution 2.51 software (Shimadzu - Milan, Italy).

Sampling conditions: SPME fibres Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) (df 65 µm, 1 cm length) was provided from Supelco (Bellefonte, PA, USA). Before use, fibers were conditioned as the manufacturer recommendations. The internal standard had previously been loaded onto the fiber (by sampling 5µL of a 1000 mg/L solution of n-C13 in Dibutyl phthalate (DBP) in a 20mL headspace vial for 20 min at 50°C, stirring speed 250rpm. 1,5 g coffee powder were introduced in a 20mL vial and the headspace sampled for 40 min at 50°C at a stirring speed of 250rpm. Analytes were then recovered by thermal desorption of the fibre for 5 min at 250°C into the GC injector, and then transferred on-line to the GC injector. All samples were analysed in duplicate.

Chromatographic conditions: injector temperature: 250°C, injection mode: splitless; carrier gas: helium, flow rate: 1 mL/min; column: SGE SolGelwax (100% polyethylene glycol) 30 m x 0.25 mm dc x 0.25 µm df (Trajan- Melbourne, Australia). Temperature program, from 40°C (1min) to 200°C at 3°C/min, then to 250°C (5min) at 10°C/min. MSD conditions: ionization mode: EI (70 eV), temperatures: ion source: 200°C; transfer line: 250°C; scan range: 35-350 m/z.

Identification of volatiles was made through their linear retention indices (I^T_S) and EI-MS spectra, compared to those of authentic standards or, to those collected in-house or in commercial libraries (Wiley 7N, FFSNC and Nist 14 Mass Spectral Data).

Peroxides, p-Anisidine and Oleic acids

The extracted coffee oil samples, were evaluated also by spectrophotometric measures using the CDR FoodLab® instrument, kindly supplied by the Department of Scienze agrarie, forestali

e alimentari of the University of Turin, determining the formation of secondary oxidative metabolites through p-Anisidine parameter, primary oxidation products through the number of peroxides and the acidity.

The Peroxides value evaluates the number of peroxides, expressed as milliequivalents of active oxygen per kg of extracted fat (mEq O2/kg). The value attests to the primary oxidation state. The peroxides oxidize the Fe^{2+} ions to Fe^{3+}, these reacting with the chromogen (i.e. N,N,-diethylparaphenylenediamine) form red colouring complexes whose intensity, measured at 505 nm, is directly proportional to the concentration of peroxides in the sample. The formation of peroxides occurs in the initial auto-oxidative phase. Once the threshold value is exceeded, propagation proceeds autocatalitically leading to the formation of secondary products.

The p-Anisidine analysis, whose value is expressed as AnV (p-Anisidine value), evaluates the presence of carbonyls, as secondary oxidation products. The carbonyls reacting with the p-Anisidine determine a measurable absorption at 366 nm.

The analysis of acidity measures the presence of free fatty acids in solution. The fatty acids in the sample, at pH <7.0, react with a chromogen developing a colour whose optical density, measured at 630nm, is proportional to the acidity concentration of the fat, expressed as % of oleic acid.

Moisture % and pH

Moisture content was determined according to the method of (Benković, 2018). Samples were dried at 100 °C for 2.5 h in an oven dryer and weighed on an analytical balance (Radwag Wagi Elektroniczne, Poland) prior to and after drying. The difference in weight before and after drying was recorded as the mass of the water contained in the sample.
The acidity was measured using the pH 70 portable pH-Mettler, Toledo© (Italy, Milan) on the espresso obtained from the samples. Measurements were done in triplicate.

Statistical analysis

Pearson correlation and heatmaps were performed using XLSTAT software version 2021.2.1 (Addinsoft, New York, NY USA).

3. RESULTS AND DISCUSSION

Since lipids are not thermostable substances, their extraction was carried out under moderate conditions to prevent autoxidative reactions. This was done by using and adjusting the extraction method for free (FFAs) and esterified fatty acids (EFAs), which allowed for a more detailed analysis of FFA development (Cialiè Rosso, 2021; Cincotta, 2020).

Extraction and separation of EFAs/FFAs

A protocol to extract and differentiate EFAs from FFAs qualitative profiling was adapted from Cialiè Rosso *et al.* (Cialiè Rosso, 2021) to enable a more in-depth investigation of the evolution of FFAs in the coffee matrix. The entire procedure is described in figure 1. Total lipids were extracted in duplicate from 1g of coffee powder with 30mL of organic solvents (n-hexane), by mixing them using an ultrasonic bath (Branson 3200 model) followed by centrifuge (R-8D Remi Motors LTD, Vasai, India).

Subsequently, the supernatant containing coffee lipids was collected, concentrated and stored at -20°C until the next analytical step of lipid class separation. The first reaction step aims at collecting EFAs and is by saponifying the lipid fraction with 2mL of MeOH in basic conditions, followed by a liquid-liquid extraction (LLE) of the organic phase with n-hexane. The residual methanolic phase containing FFAs is submitted to Fisher esterification with MeOH in acid ambient, followed by a liquid-liquid extraction (LLE) of the organic phase with n-hexane. Figure 2 reports the mechanism of saponification (Step 1), esterification (Step 2), and LLE.

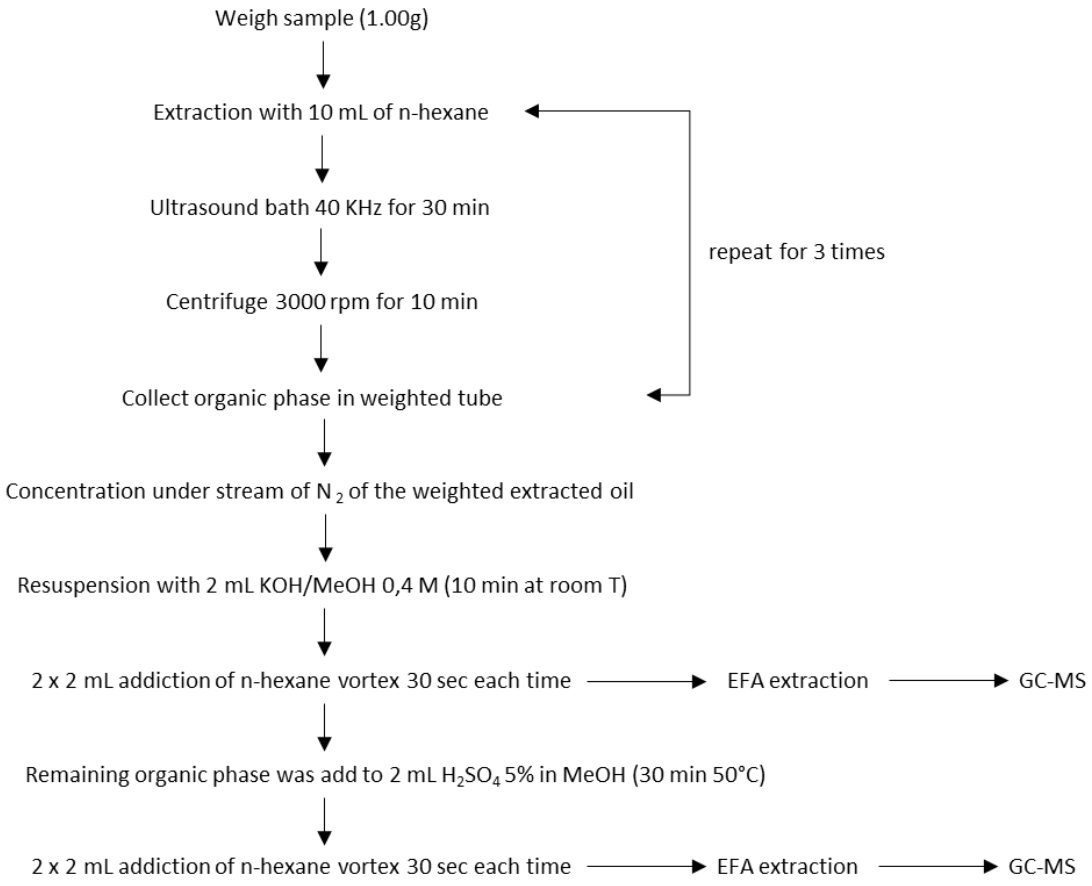
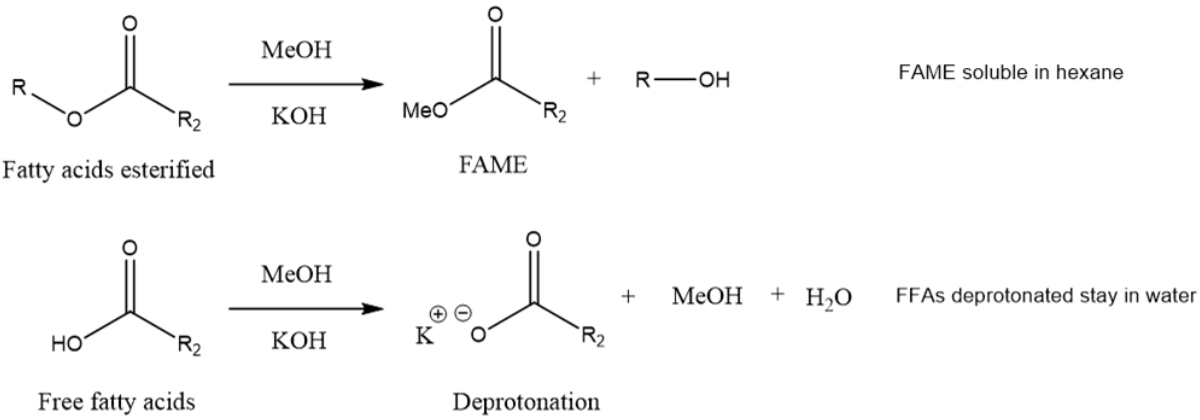


Figure 1: scheme of the completed reactions, from the extraction of the lipid phase, to the EFA and FFA reactions.

1^ Step saponification



2^ Step Fisher esterification

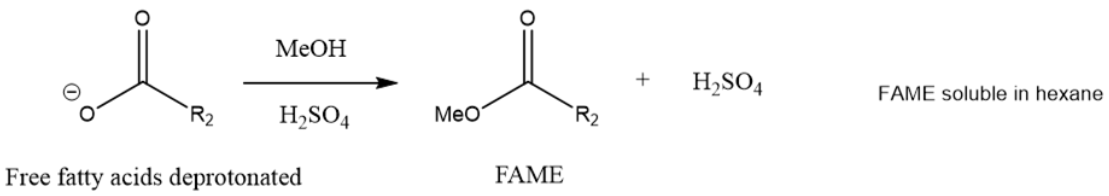


Figure 2: Scheme of the release of EFA and FFA in two steps.

The Soxhlet-based approach carried out according to the 920.39C method of Association of Official Analytical Chemists ("AOAC Official Methods of Analysis," 1990) was used as term of comparison to the proposed method of lipid extraction (n-hexane extraction). Table 1 compares the yields normalized to the weight of the extract with Soxhlet and n-hexane. The findings of the gravimetric analyses are shown in table 1:

Table 1: Results of the grams of roasting coffee fat extracted by the official AOAC method and by the adapted one.

Number test	**OAC method Soxhlet Extraction (g roasting coffee fat)**	**n-hexane Extraction (g roasting coffee fat)**
1	0.1290	0.1283
2	0.1378	0.1209
3	0.1388	0.1213
Average	0.1352	0.1235
RSD	0.005	0.004

The proposed alternative method resulted in a comparable extraction yield. The benefits of this LLE approach over the Soxhlet method relate to the time savings in preparation and extraction, the reduced amount of solvent, and the mild conditions that prevent FFAs from degrading before derivatization. Moreover, the semi-quantitative differences between the two methods for the methyl esters of EFA and FFA, as shown in table 2, are negligible.

Table 2: Semi-quantitative data for all FAMEs in the two fractions esterified fatty acids (EFAs) and free fatty acids (FFAs) with their relative standard deviation (RSD%).

	Soxhlet method				n-hexane extraction method			
	EFA		FFA		EFA		FFA	
Compounds	Mean A norm	RSD%	Mean A norm	RSD%	Mean A norm	RSD%	Mean A norm	RSD%
Tetradecanoic acid, methyl ester	109489	5.00	55908	19.14	229597	14.17	153279	23.77
Pentadecanoic acid, methyl ester	32188	0.92	16775	15.09	92381	14.38	57439	24.16
Hexadecanoic acid, methyl ester	47169376	10.44	24909900	15.73	83706016	10.47	56704598	16.70
Heptadecanoic acid, methyl ester	138573	14.00	58871	20.17	263329	13.70	151851	21.80
Octadecanoic acid, methyl ester	12999443	12.66	5997922	17.76	22597094	4.80	14683030	15.73
9-Octadecenoic acid (Z)-, methyl ester	5096300	11.75	2355541	17.25	6903894	11.67	4595686	17.27
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	20983821	10.38	10755376	15.65	36751735	8.26	24034648	15.82
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	1003396	14.19	418491	19.96	1875019	7.70	1080056	19.84
Eicosanoic acid, methyl ester	4810664	11.97	2178765	18.84	9057103	6.07	5272391	12.89
11-Eicosenoic acid, methyl ester	187864	19.16	82480	20.22	246201	24.19	159954	17.57
Heneicosanoic acid, methyl ester	98415	15.00	37145	21.78	179082	6.62	102722	23.04
Docosanoic acid, methyl ester	799711	13.52	311531	17.20	1242479	5.71	675211	19.58
Tricosanoic acid, methyl ester	98068	10.32	31629	17.46	178719	4.80	99717	17.34
Tetracosanoic acid, methyl ester	258059	6.66	96746	21.27	515031	6.50	282925	19.64

33 samples x 2 fractions were tested in duplicate for a total of 132 analyses. Ten saturated FAs, two monounsaturated and two polyunsaturated were investigated in both EFA and FFA fractions: seven are the most important fatty acids in coffee, in particular, palmitic (16:0; Hexadecanoic acid), stearic (18:0; Octadecanoic acid), oleic (18:1n-9; 9-Octadecenoic acid (Z)), linoleic (18:2n-6; 9,12-Octadecadienoic acid (Z,Z)), linolenic (18:3n-3; 9,12,15-Octadecatrienoic acid), arachidic (20:0; Eicosanoic acid) and behenic (22:0; Docosanoic acid). In agreement with the literature, palmitic and linoleic acids are the most abundant in both EFAs and FFAs fractions of the roasted samples (Toci, 2013). A reasonable amount of stearic

and oleic acids is also present. As reported by Speer & Kölling-Speer (2006) (Speer, 2006), stearic and oleic acids were found in almost equal quantities in Arabica coffee.

The oxidative kinetics over time of samples of different blends and packages, were evaluated by normalizing the increments in the amount in FFA at each time point, to the value recorded at T0. Samples of the two packages, Standard and Eco, were compared over time up to T90 for FFAs and EFAs (Figure 3 and 4 respectively). T90, for the Eco caps in stress condition, is the time in which the coffees is no longer acceptable for the sensory assessment of the industrial panel.

Figure 3a and 4a show an exponential increase in FFAs in the BS and PC samples, which is related to the hydrolysis of triglycerides, as confirmed by the behaviour of EFAs for the corresponding samples (Figure 3b and 4b). In all other samples, (BC, PS, IS and IC) the FFAs trend is decreasing over time, indicating a general degradation response that is also associated to a decay tendency in EFAs. These behaviours evidenced differences not only between the packages within the same blend (Eco and Standard), as expected, but also between the blends stored in the same type of packaging.

More in details, in B samples, for the same number of days (from T0 to T90), (BS and BC), depending from the packaging, an opposite FFAs trend is observed (Figure 3a and 4a). This potentially highlights that in the Eco packaging (BC), some chemical transformations might have affected the FFAs content during the storage period, due to the packaging, which promotes easier permeation to moisture or air. The rate of FFAs (Figure 3a and 4a) degradation might have exceeded the rate of their release by hydrolysis from EFAs in particular in environmental conditions that afford oxidation (Figure 3b and 4b). FFAs behaviour highlights therefore a rancidity evolution in which there is a continuing formation of FFAs by hydrolysis from EFAs as it is shown in Figure 5 from the increase acidity (resulting from oleic acid equivalents) and a contemporary formation of oxidation secondary products (volatiles acids, p-anisidine value) from FFAs degradation with a continuing increase of peroxides as the consequence of the oxidative process (Figure 5). The increase of the moisture content in the capsules and a decrease of the pH of the brew can be the responsible to produce an environment that trigger hydrolysis and speeding-up oxidative reactions; sensory assessment evaluated the coffee at T90 as more acidic with an oxidized note (Belitz, 2009,

Strocchi, 2022). Figure 6, for BC, reports the Pearson correlation between the different measures that show an inverse correlation (in red) between FFAs with p-Anisidine, Peroxides value, volatiles acids and acidity, meaning that degradation of FFAs is related to an increase of oxidative secondary products. In BS, the environment is less favourable to degradation as shown in the correlation map in figure 6, in which although with longer time (Figure 4a) FFAs decay, this is softer and the correlation with volatile acids, pH and moisture % is null or negligible.

For the same number of days (until T90), FFAs in PS (Figure 4a) tend to decrease over time, in contrast to the corresponding increase in PC (Figure 3a). Moreover, peroxides values, in PC, showed a direct correlation (in green) with FFAs, synonym of an initial autocatalytic formation of peroxides in which secondary oxidation products are not yet formed. In addition, there is also a strong direct correlation of FFAs with the pool of volatiles acids and a less or an inverse correlation with the other volatile compounds, meaning that, as the oxidation progresses, volatile amounts undergo to a decrease (Figure 6). The oscillating decrease of FFAs along longer storage time in PS (Figure 4a) is typical of the peroxides reactivity that present an induction phase in which peroxides start to form and a following decay due to their degradation and again an increase because of new formation in function of the availability of substrates (FFAs ready for the oxidative phase). At the same time, an increase in EFAs over time could be due to the presence of enzymes (i.e. lipase) that in the environment with high moisture, result active, despite the matrix being roasted, and thus capable not only of hydrolysing the ester bonds, but also of reforming them (Figure 4b) (Patui, 2014).
PC are also monitored under more stressful conditions, by increasing the RH% from 65% (Figure 7a and b) to 75% (Figure 7c and d). In these samples, in contrast to the same blend at 65% of RH%, a decrease of FFAs is observed, that is negatively correlated to Peroxides value, and p-Anisidine, thus showing that at higher RH%, during storage, the kinetic of degradation speeds up (Figure 8) (Belitz, 2009; Manzocco, 2016).

On the other hand, FFAs trend in I coffees (Figure 3a and 4a) in the two different packages (IC and IS), at the same time, decrease with two different progressions: IC soars until to T60 and then decrease at T90 (Figure 3a), in IS their decrease slowly (Figure 4a). EFA trend thereby stays more or less stable at the beginning of monitoring time then tend to decrease both for

IC and IS (Figure 3a and 4a). Figure 6 reports for both IC and IS a similar BC Pearson correlation trend between the different measures that show an inverse correlation between FFAs with p-Anisidine, Peroxide value, volatiles acids and acidity, meaning that degradation of FFAs is related to an increase of oxidation secondary products (Belitz, 2009).

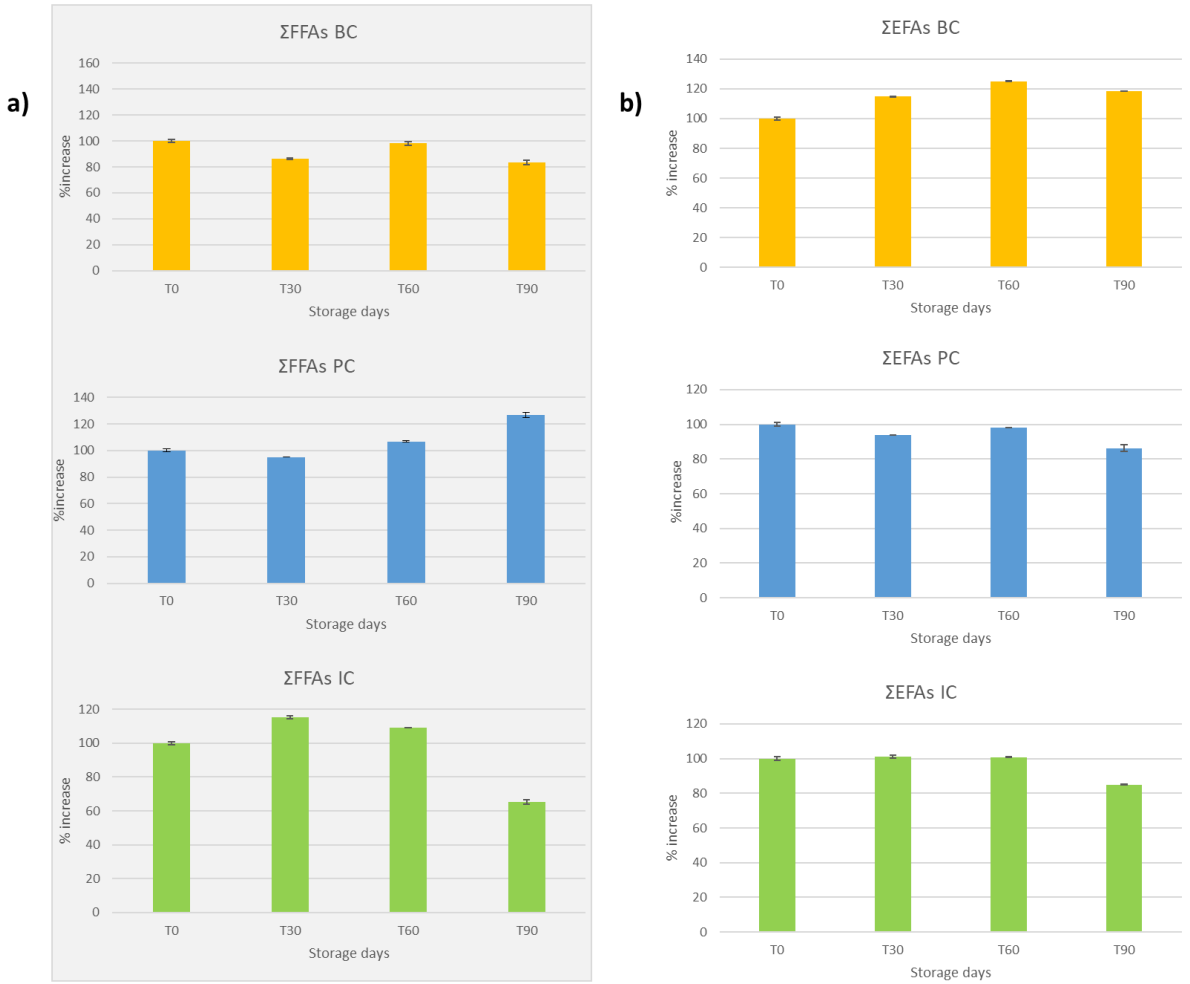


Figure 3: Evolution of total a) FFAs and b) EFAs, expressed as the percentage increase normalized over T0 for B Eco caps (65%RH 45°C) (PC) (T0-T90) in orange; P Eco caps PC (T0-T90) in blue I Eco caps (IC) (T0-T90) in green.

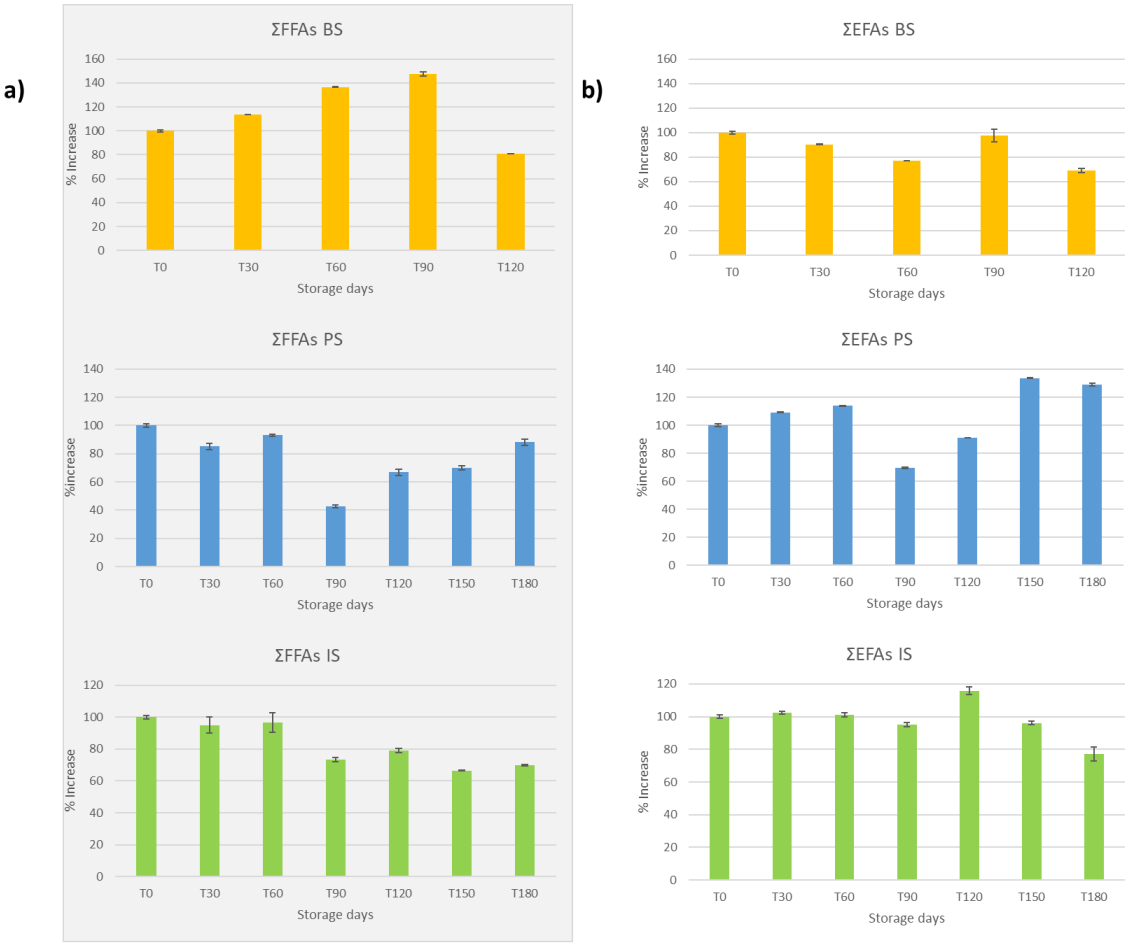


Figure 4: Evolution of total a) FFAs and b) EFAs, expressed as the percentage increase normalized over T0 for B Standard caps (BS) (T0-T120) in orange; P Standard caps (PS) (T0-T180) in blue and I Standard caps (IS) (T0-T180) in green.

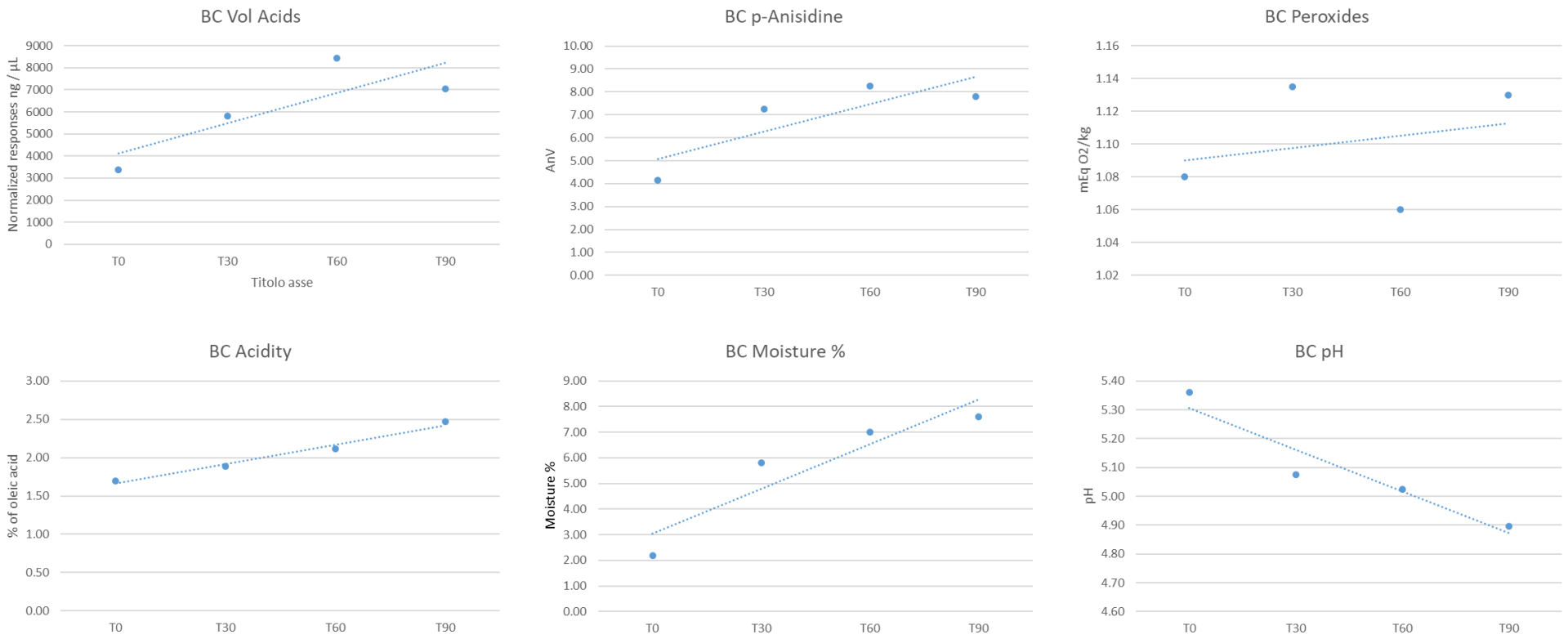


Figure 5: Trend from T0 to T90 of volatile Acids (Vol Acids), p-Anisidine, Peroxides value, acidity, moisture % and pH for B Eco caps (BC) (T0-T90).

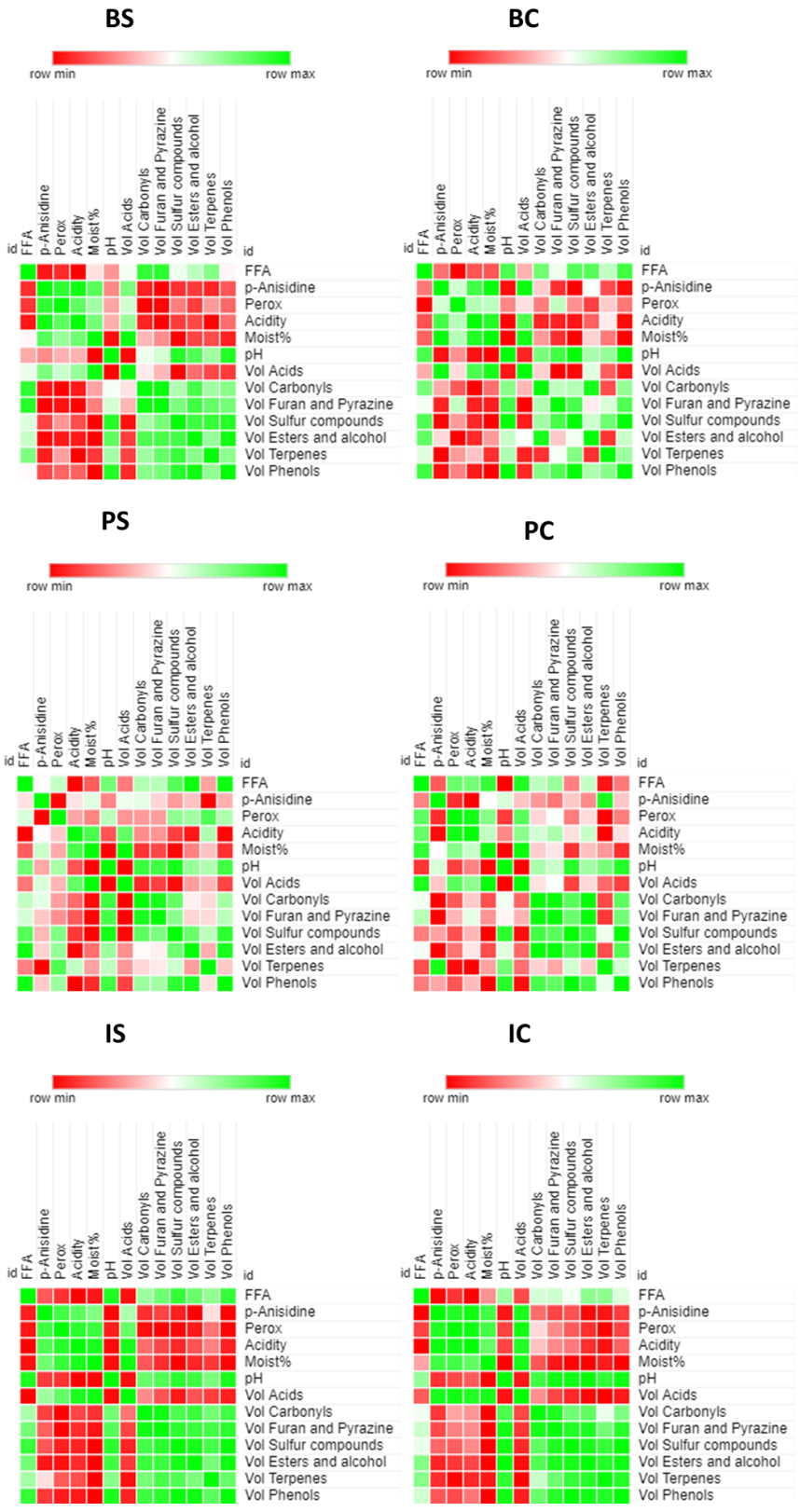


Figure 6: Pearson correlations between: Peroxides value, p-Anisidine, the sum of the free fatty acids (FFAs), acidity, moisture %, pH and the principal classes of volatiles compounds. B

Standard caps (BS), B Eco caps (BC), P Standard caps (PS), P Eco caps (65%RH 45°C) (PC), I standard caps (IS) and I Eco caps (IC). Data were pre-processed by autoscaling.

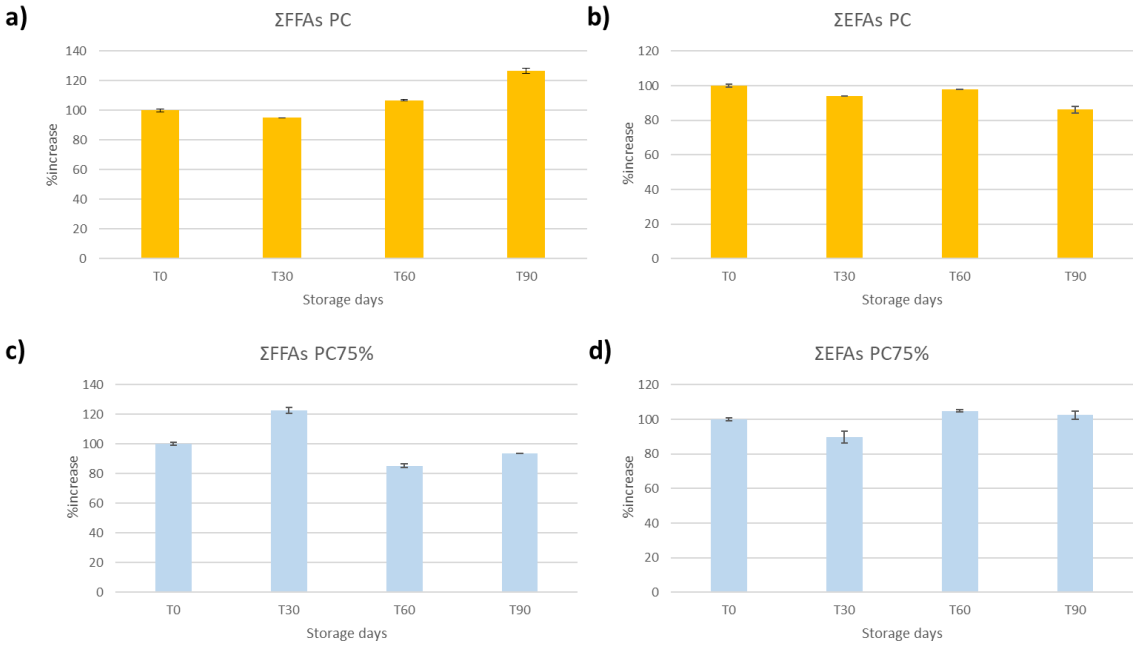


Figure 7: Evolution of a) total FFAs for P Eco caps (65%RH 45°C) (PC) reported in yellow; b) total EFAs for P Eco caps (65%RH 45°C) (PC) reported in yellow; c) total FFAs for P Eco caps (75%RH 45°C) (PC75%) reported in light blue and d) total EFAs for P Eco caps (75%RH 45°C) (PC75%) reported in light blue. The evolution of the total content of FFAs and EFAs for PC and PC75% (T0-T90) are expressed as the percentage increase normalized over T0.

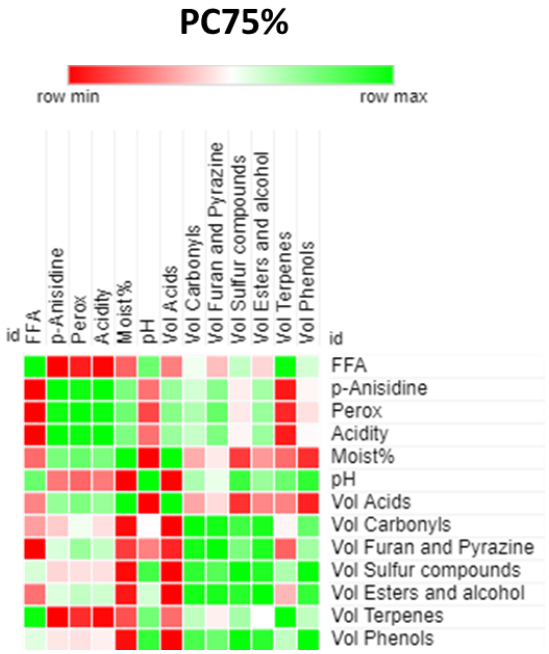


Figure 8: Pearson correlations between: Peroxides value, p-Anisidine, the sum of the free fatty acids (FFAs), acidity, moisture %, pH and the principal classes of volatiles compounds. P Eco caps (75%RH 45°C) (PC75%). Data were pre-processed by autoscaling.

4. CONCLUSIONS

This study assessed the free and esterified species of roasted coffee's fatty acids composition and the relationship over time with volatiles compounds, Peroxides and p-Anisidine values, as well as pH and moisture % measures.

For EFAs and FFAs, extraction and derivatization produced a variety of outcomes. The findings demonstrated that the shelf-life signatures of EFAs and FFAs varied and exhibit a different trend depending from blends, packaging and storing conditions.

In all samples, increased moisture and pH affect the release of fatty acids, their degradation, and the subsequent formation of peroxide compounds from which volatile secondary compounds oxidation products are formed. From the results so far obtained, it is moisture that most influences the oxidative effect. An example is the PC coffee sample, which under storage conditions at 65% RH, undergoes hydrolytic reactions of free-form release of fatty acids, with concomitant increase in peroxide compounds and initial autocatalytic phase of the same. The same sample placed under higher humidity conditions (75%RH), undergo to oxidative reactions with decrease in free fatty acid compounds. The increase in all secondary oxidative products also promotes the acceleration of degradation kinetics. Similarly, the BC, IC samples, because of their compostable packaging, exhibit similar behaviour to PC75%, while this is reached in a longer time for the same blends when packed up in standard packaging. The phenomena underlying this trend could be multifactorial and result from enzymatic and non-enzymatic activities. Thus, the change in the lipid fraction over time appears to be dependent on both blend and packaging, and moisture appears to be the main driving factor.

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SUPPLEMENTARY

Table S1: List of the investigated samples (B and P (100% Coffea Arabica) and I (50% Coffea Robusta and 50% Coffea Arabica) in two different packaging (Eco and Standards) for espresso preparation (caps available for espresso machine) their storage in stress conditions and time of aging.

Stress conditions (45°C - 65%RH) caps	T0	T30	T60	T90	T120	T150	T180
BC	BC1_T0	BC1_2T30	BC1_2T60	BC1_2T90			
BS	BS1_T0	BS1_2T30	BS1_2T60	BS1_2T90	BS1_2T120		
IC	IC1_T0	IC1_2T30	IC1_2T60	IC1_2T90			
IS	IS1_T0	IS1_2T30	IS1_2T60	IS1_2T90	IS1_2T120	IS1_2T150	IS1_2T180
PC	PC1_T0	PC1_2T30	PC1_2T60	PC1_2T90			
PS	PS1_T0	PS1_2T30	PS1_2T60	PS1_2T90	PS1_2T120	PS1_2T150	PS1_2T180

Stress conditions (45°C - 75%RH) caps	T0	T30	T60	T90	T120	T150	T180
PC75%	PC1_T0	PC1_2T30	PC1_2T60	PC1_2T90			

Figure S1: identification of 37 picks of FAME standard.

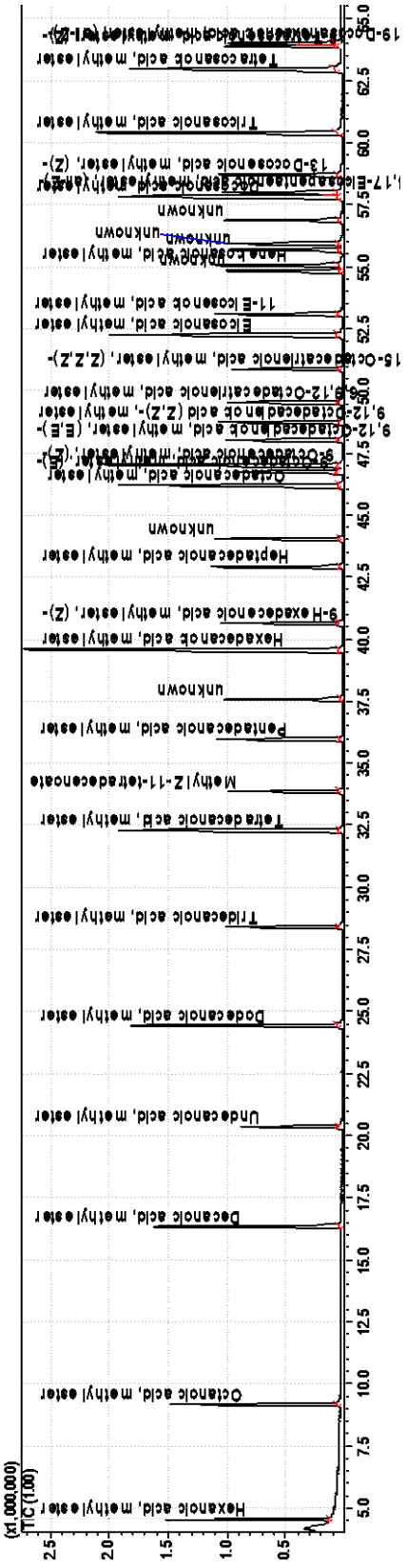


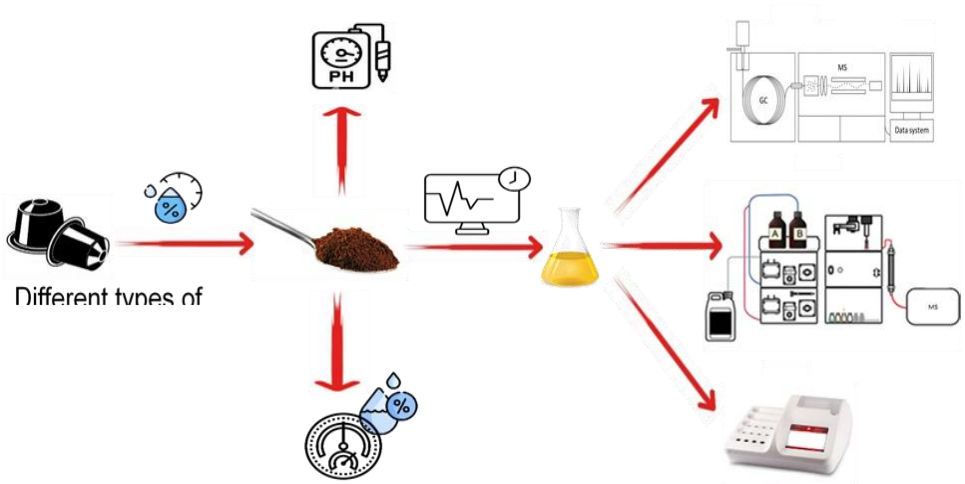
Table S2: The compounds find in a coffee oil extracts with their retention time.

Compounds name	**Rt min**
Tetradecanoic acid, methyl ester	32.255
Pentadecanoic acid, methyl ester	36.007
Hexadecanoic acid, methyl ester	40.117
Heptadecanoic acid, methyl ester	43.069
Octadecanoic acid, methyl ester	46.868
9-Octadecenoic acid (Z)-, methyl ester	47.422
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	49.364
9,12,15-Octadecatrienoic acid (Z,Z,Z)-, methyl ester,	50.994
Eicosanoic acid, methyl ester	52.628
11-Eicosenoic acid, methyl ester	53.303
Heneicosanoic acid, methyl ester	55.389
Docosanoic acid, methyl ester	58.176
Tricosanoic acid, methyl ester	60.847
Tetracosanoic acid, methyl ester	63.444

2.3.3 Diterpenes stability of commercial blends of roasted and ground coffees packed in copolymer coupled with aluminium and Eco-friendly capsules.

This study has been submitted to on “Food Research International”

GRAPHICAL ABSTRACT



Diterpenes stability of commercial blends of roasted and ground coffees packed in copolymer coupled with aluminium and Eco-friendly capsules.

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ABSTRACT

Diterpenes are compounds of the terpenic fraction of roasted coffee, present mainly in esterified form with fatty acids and accounting for about 7-20% (w/w) of the lipid fraction in the coffee beans. Several parameters can influence their occurrence in coffee beans and beverages including species and post-harvest processing. Diterpenes in coffee have been extensively studied, but to the best of the authors' knowledge, the literature does not report information on their stability over time. Coffee is a relatively stable product under optimal temperature, humidity, and oxygen conditions, but it can undergo a series of chemical and physical reactions during storage that changes its flavour and leads to rancidity, mainly due to the oxidative reaction occurring on the lipid fraction. In this study, the effect of long-term storage on the diterpene content of different commercial coffee blends and packaging is analysed and critically discussed. Results show relative stability for cafestol and kahweol over time and the evolution of their dehydro derivatives regardless of the blends and packaging. The concomitant evaluation of the free fatty acids normally esterified with these compounds and the peroxides values showed a different behaviour depending on blends and packaging.

Keywords: R&D coffee, quality, storage, diterpenes, packaging

1. INTRODUCTION

Coffee is a beverage consumed all around the world, becoming an asset of socialization. Coffee consumption, as part of our daily lives, is widespread not only because of the effects due to its bioactivity, but also and mainly for the pleasure associated with the symphony of tastes and aromas created during the roasting process and brewing (Choi, 2020). The flavour of coffee is important in defining the quality of beans and beverages and, thereby, it is determinant to define its economic value cost. In recent years, we have assisted in a packaging renewal now characterized by a visual identity associated with the description of the sensory profiles of the blend. It is therefore utmost essential to preserve and maintain its quality along the shelf-life (Bhumiratana, 2011).

Roasted coffee quality is correlated with a series of genetic precursors, environmental factors and post-harvest practices, such as de-pulping, drying fermentation, roasting and storage processes (Folmer 2017; Kitzberger, 2013; Novaes, 2015; Sunarharum, 2014). Storage conditions deeply affect the quality caused by the degradation of the product. Environmental conditions, such as humidity, temperature and compositions of the atmosphere around the coffee (air, inert gas or vacuum), and material used for packing can affect the rate at which the deterioration phenomena occur. The above factors play a fundamental role in oxidative kinetics. Although coffee is a relatively stable food, it could undergo staling events with volatilization of aroma compounds, CO_2 release, formation of off-notes and rancidity development (Anese, 2006; Nicoli, 2012). The staling effect, which determines the loss of aroma freshness, consequent to coffee ageing, has extensively been studied since it is decisive for the consumer's acceptance and delineates the shelf life of the product (Cheidig, 2007; Flament, 2002; Manzocco, 2016; Yeretzian, 2017). Some sensory features and compounds are positively correlated to a good cup quality and could therefore undergo deterioration over time. Among all the compounds that characterise coffee, lipids, have the function of precursors for many flavour components, conveying the active compounds and contributing to the perceived texture and mouthfeel of the brew (Moeenfard, 2020; Novaes, 2015).

Lipids are major components of green coffee ranging from 7-17% w/w, depending on the coffee species. Normally *Coffea robusta* contains lower amounts of this fraction, compared

to *Coffea arabica*. Oily fraction mainly consists of triglycerides (TAGs) (75% w/w), esterified sterols (2-5%) and a large number of esters of diterpenes (7-20%). The unsaponifiable fraction consists of free sterols, tocopherols, phosphatides, diterpenes, fatty acids (FAs), ceramides and other minor components (Moeenfard, 2020; Novaes, 2015; De Oliveira, 2014). After triglycerides, diterpenes are the main fraction of coffee oil. Diterpenes are mainly pentacyclic diterpene alcohols belonging to the kaurene family and the major representative components are cafestol ($C_{20}H_{28}O_3$) kahweol ($C_{20}H_{26}O_3$) and 16-O-methylcafestol or 16-OMC ($C_{21}H_{30}O_3$) (Figure 1) (Moeenfard, 2020; Novaes, 2015). Diterpenes are mostly found esterified with different FAs (18% w/w of the coffee lipids) and only a minor fraction is free (0.4% w/w of the coffee lipids) (Moeenfard, 2020; Moeenfard, 2020; Scharnhop, 2009; Speer, 2006). Up to 14 mono esterified derivatives of cafestol and 12 of kahweol had been found mostly with palmitic (C16) and linoleic (C18:2) acids, but even oleic (C18:1, cis-9) and stearic acids (C18:0) (Chartier, 2013; Dias, 2010; Moeenfard, 2020; Moeenfard, 2020; Novaes, 2015).

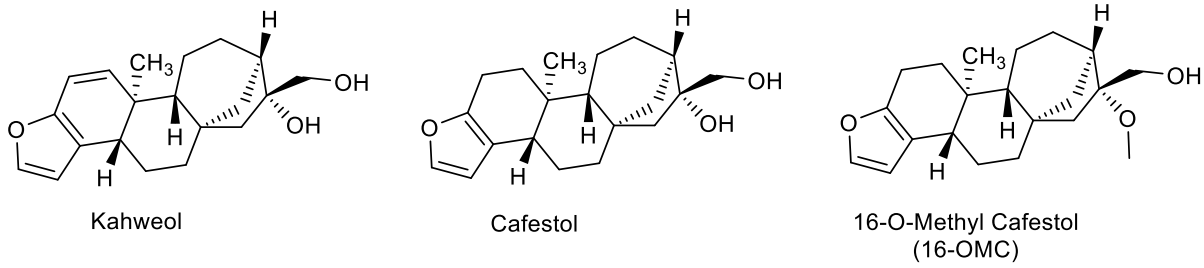

Figure 1: Chemical structure of cafestol, kahweol and 16-O-methylcafestol (16-OMC).

In roasted coffee, *Coffea arabica* contains higher amounts of cafestol (0.4% - 0.7% (w/w)), than *robusta* (0.2% up to 0.3% (w/w)). Kahweol seems to be more specific for *Coffea arabica*, compared to *C. robusta* (0.003% to 0.2% (w/w) (Dias, 2010; Kitzberger, 2013; Mori, 2016). Furthermore, *C. robusta* presents a specific derivative, 16-OMC (Mori, 2016), although, very recently, Gunning et al. (Gunning, 2018) detected this compound in both roasted species. The absence of 16-OMC in *C. arabica* has been used to control the authenticity of the products with appropriate methods to differentiate the two species (Scharnhop, 2009) along with sinapoyl quinic acid based as biomarkers (Badmos, 2019).

During roasting, diterpenes remain relatively stable, undergoing dehydration and dehydrogenation, with the formation of products such as kahweal, cafestal, isokahweol,

dehydroisokahweol and dehydrocafestol and dehydrokahweol (Dias, 2010; Moeenfard, 2020; Pacetti, 2012). This partial stability depends on the temperature and time of roasting that could influence the diterpenes profile, with a major reduction in *Coffea arabica*, due to the higher sensitivity of this specie to roasting (Dias, 2014; Speer, 1998; Sridevi, 2011; Williamson, 2019; Kitzberger, 2013) (Figure 2).

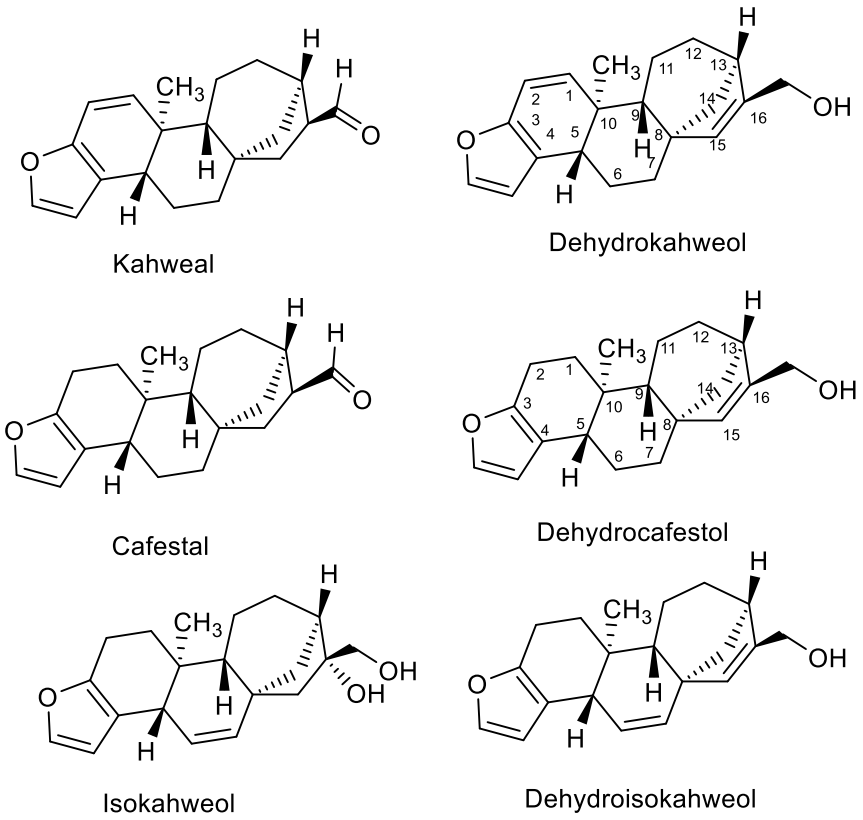

Figure 2: Chemical structure of decomposed and isomerized derivatives of cafestol and kahweol.

Diterpenes were widely studied since the 1930s, when kahweol and cafestol, were first detected, due to their relationship to human health (Kurzrock, 2001). The methodologies applied to extract this fraction, have mostly been used to study the difference between species or to measure the content of diterpenes in green and roasting beans, but how they vary over time during storage has not yet been investigated. Because of their molecular structure, diterpenes can easily react/degrade with moisture and oxygen under suitable environmental conditions. The lack of information about their variability during storage and the high number of parameters that may influence the coffee composition over time was the

base of this study that aims to evaluate the effect of long-term storage on diterpenes content of different commercial coffee blends and packaging (Eco and standard with aluminium barrier capsules), and trying to explain potential reactions which they go through.

1. MATERIALS AND METHODS

2.1 Coffee samples

Samples consist of roasted and ground coffee suitable for espresso machines, kindly supplied by Lavazza Group s.p.a. (Turin, Italy). Two commercial blends "P" based on *Coffea arabica* L. (Arabica) and "I" which is 50-50 of *C. canephora* Pierre ex Froehne (Robusta) and *C. arabica* were selected for their distinctive flavours, product marketing, and last but not least for the limited availability of blends sold in specific packaging (i.e. Eco-caps). The two blends were available in two different modified atmosphere capsules: Eco Caps (PC and IC), which consist of 100% compostable caps made of an innovative biopolymer suitable to be degraded in 180 days after disposal and becoming compost; the standard caps (PS and IS) are made of a copolymer based on polyethene and aluminium. The samples, once produced were frozen and then subjected to accelerated storage conditions at a temperature of 45°C and 65% relative humidity (RH%). The samples were analysed within 90 days and more precisely at the following times: T0, T15, T30, T45, T60, and T90.

2.2 Chemicals and Standards

Methanol (MeOH) and potassium hydroxide (KOH) were used for saponification and were from Carl Roth (Karlsruhe, Germany). *n*-Heptane and distilled water were used for extraction and clean up, acetonitrile (ACN) HPLC-grade (LC-MS grade; ≥ 99.9% purity) and formic acid > 95% purity for chromatographic analyses were from Merck (Darmstadt, Germany). Kahweol, Cafestol, 16-OMC and Dehydrocafestol standards were from PhytoLab GmbH & Co (Vestenbergsgreuth, Germany). Undecanoic acid methyl ester ≥98.0%, pure standards for normalization of individual fatty acids, n-alkanes (from n-C9 to n-C25) for linear retention indices (I^T_S) determination and the reference mixture for FAMEs identification (Supelco 37

components mix) and Sulfuric acid 98% (H_2SO_4) for the fatty acids transesterification were all from Merck (Milan, Italy).

2.3 Diterpenes extraction

The procedure for the diterpenes extraction was adapted from Dias (Dias, 2010). Since they are mainly present as esters, 1g of coffee powder was submitted to saponification at 80°C for 1 h with 2.0 mL of 2.5 mol/L KOH solution in MeOH to obtain the free form. The resulting solution was diluted with 2.0 mL of deionised water and submitted to liquid-liquid extraction with 2.0 mL of *n*-heptane (3 times) to separate the unsaponifiable fraction. After stirring and centrifugation (2 min at 3000 rpm), 1 mL of the organic phase was recovered, suspended in 2 mL of deionized water and then vortexed. The organic phase was recovered and evaporated to dryness under N_2. The dried extract was re-suspended in 2.0 mL of the mobile phase (acetonitrile/water 55:45, v/v), filtered through a 0.45 µm nylon membrane (Cromafil® AO-45/15 MS, Macherey-Nagel, Düren, Germany) and injected into the LC-MS-UV/DAD system. The extractions were performed in duplicate.

2.4 LC-MS-UV/DAD

Each extract (3 µL) was analyzed in duplicate with an Agilent 1100 system (Agilent Technologies, Santa Clara, USA) equipped with a G4225A 1260 online Degasser, G1312B 1260 Binary Pump, G1316A Column oven and a G1315C diode array detector in series to an ion trap mass analyser (HTC ultra, Bruker Daltonics®) provided with an atmospheric pressure chemical ionization (APCI) source (Bruker Daltonics®). Samples were analyzed on a Pursuit Xrs Diphenyl 150 x 3.0mm column (Agilent Technologies, Santa Clara, USA) under controlled temperature conditions at 25°C and flow rate of 0.5 mL/min. The analyses were carried out in gradient mode with mobile phases consisting of water/formic acid (0.01%) (solvent A) acetonitrile/formic acid (0.01%) (solvent B) in programmed as follows: 0 min, 45% B; 15 min, 65% B; 20 min, 85% B; 20.10 min, 45% B; 25 min, 45% B. The total analysis time was 25 min, with a retention time of 7.3 min for kahweol (m/z 299), 7.6 min for cafestol (m/z 297), 10.8

min for 16-OMC (m/z 331) and 13.6 min for dehydrocafestol (m/z 299) (Figure 3). UV spectra were registered at 210 nm wavelength.

MS operative parameters were as follows: positive ultra scan mode over the mass range 260-650 m/z; voltages: capillary, 106.8 V; skimmer, 40 V; trap drive 37 V; dry gas flow, 5L/min; dry temperature, 250°C; vaporizer temperature 400°C; nebulizer, 30 psi.

The four components were identified by comparing their retention times, UV and MS spectra to those of authentic standards (kahweol, cafestol, 16-OMC, dehydrocafestol). The other components were tentatively identified based on their UV spectra and mass spectral information, compared to those reported in the literature.

The stock standard solutions were prepared at 1000 mg/L in ACN/water (55:45%; v/v), for kahweol, cafestol and dehydrocafestol and in ethanol (EtOH) for 16-OMC.

Working solutions were obtained by dilution of these stocks at 10; 25; 50; 100 mg/L. Quantification was carried out in UV at 210 nm through an external calibration method using each diterpene standard for the unequivocally identification of compounds (kahweol, cafestol, 16-OMC and dehydrocafestol) at the four different concentrations. Kahweol was used for quantification of dehydrokahweol, since the standard was not available. Data were processed using the Bruker Compass DataAnalysis 4.2 software (Bruker, Daltonik GmbH).

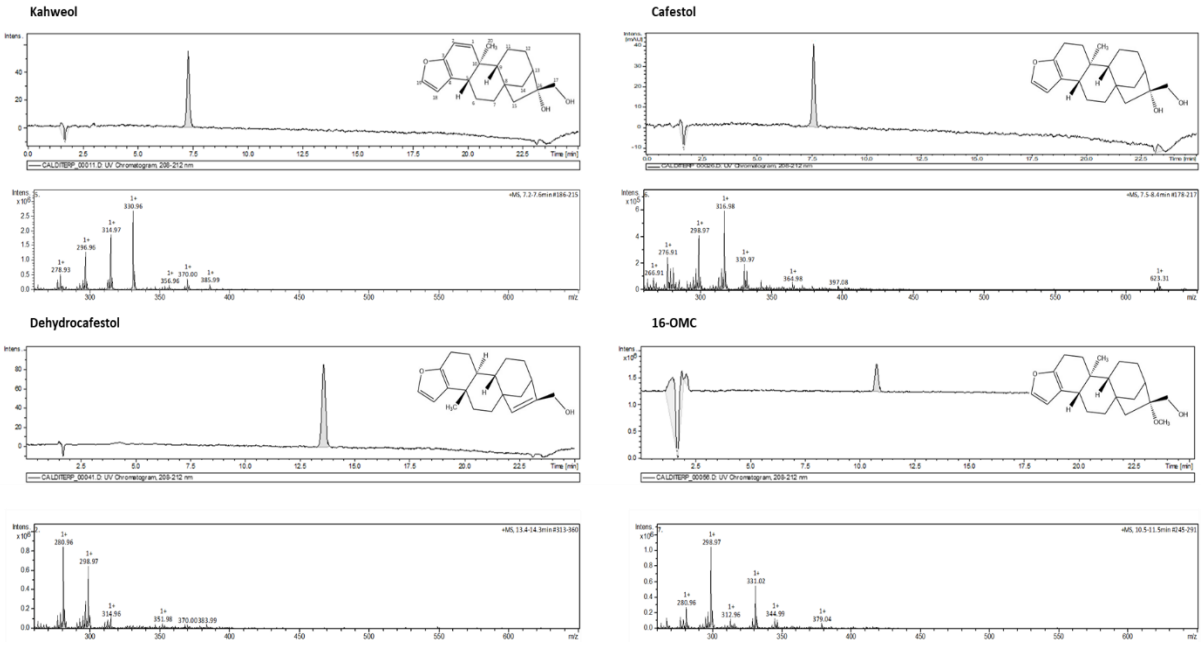

Figure 3: Standards at concentration of 0.1 mg/mL presented in UV at 210 nm with their APCI-MS spectrum in positive ion mode. In order at 7.3 min kahweol (m/z 299), 7.6 min cafestol (m/z 297), 10.8 min 16-OMC (m/z 331) and 13.6 min dehydrocafestol (m/z 299).

2.5 Extraction and separation of esterified fatty acids (EFAs)/free fatty acids (FFAs)

A protocol to extract and differentiate EFAs from FFAs qualitative profiling was adapted from Cialiè Rosso *et al.* (Cialiè Rosso, 2021) to enable a more in-depth investigation of the evolution of FFAs in the coffee matrix. Total lipids were extracted in duplicate from 1g of coffee powder with 30mL of organic solvents (n-heptane), by mixing them using an ultrasonic bath (Branson 3200 model) followed by centrifuge (R-8D Remi Motors LTD, Vasai, India).

Subsequently, the supernatant containing coffee lipids was collected, concentrated and stored at -20°C until the next analytical step of lipid class separation. The first reaction step aims at collecting EFAs and is by saponifying the lipid fraction with 2mL of MeOH in basic conditions, followed by liquid-liquid extraction (LLE) of the organic phase with n-hexane. The residual methanolic phase containing FFAs is submitted to Fisher esterification with MeOH in acid ambient, followed by liquid-liquid extraction (LLE) of the organic phase. FFAs were used for the statistical analysis.

2.6 Analysis and identification of fatty acids methyl esters by gas Chromatography

Analyses were performed using an Agilent 6890 GC unit coupled with an Agilent 5973N MSD. A Supelco SLB™-IL 76 (Tri (tripropylphosphoniumhexanamido) triethylaminebis (trifluoromethanesulfonyl) imide) (30 m x 0.25 mm dc, 0.20µm df) column was used. The chromatographic conditions were: 1 µl of samples and FAME standards were injected in split 1:20 mode at a temperature of 250 °C; column temperature setting 60°C to 200 °C at 2°C/min and to 220° (5 min) at 5°C/min; detector MS 5973 Network, MS source temperature 230 °C; MS transfer line temperature 260 °C; Quadrupole temperature 150°C; carrier gas helium; Helium flow 1 mL/min, solvent delay 4 min. This method allowed to reach a separation of the FAMEs from the reference mixture (FAME37mix) with resolution at the baseline as shown by the chromatogram in supporting information Figure S1. The reliable identification and the elution order of FAMEs in coffee lipid extracts occurred by comparing the peaks of the elution

order of components in coffee, with retention times (Rt min) and I^T are listed in Table S1 of the supporting information.

2.7 Determination of oxidative indices: Peroxides

The extracted coffee oil samples were evaluated by colourimetric measures using the CDR FoodLab® (CDR, Firenze, Italy).

The number of peroxides attests the primary oxidation state and it is expressed as milliequivalents of active oxygen per kg of extracted fat (mEq O2/kg). The formation of peroxides occurs in the initial auto-oxidative phase. Once the threshold value is exceeded, propagation proceeds autocatalytically leading to the formation of secondary products. The peroxides oxidize the Fe^{2+} ions to Fe^{3+} establishing a red-coloured complex solution whose intensity, measured at 505 nm, is directly proportional to the concentration of peroxides in the sample.

2.8 Moisture % and pH determination

Moisture content was determined according to the method of Benković et al.(Benković, 2018). Samples were dried at 100 °C for 2.5 h in an oven dryer and weighed on an analytical balance before and after drying. The difference in weight before and after drying was recorded as the mass of the water contained in the sample. Measurements were done in duplicate.

The pH was measured, in duplicate, using the pH 70 portable pH-Mettler, Toledo© (Columbus, Ohio, USA) on coffee beverages.

2.9 Statistical analysis

The analyses were performed using the quantitative results data as variables. Two replicates of extraction for each sample were injected in duplicate and the results were expressed as mean ± standard deviation. One-way ANOVA was used to assess the statistical differences between the samples over time. Principal component analysis (PCA) and correlation test were carried out using XLSTAT software version 2021.2.1 (Addinsoft, New York, NY USA). The correlation heat map was created by gene-e (https://software.broadinstitute.org/GENE-E/). Graphs were plotted using Excel.

2. RESULTS AND DISCUSSION

3.1 Quantification method

Table 1 reports the Validation parameters of the LC-MS-UV/DAD methods including *linearity, the limit of detection (LOD), the limit of quantification (LOQ)* and *precision*.

The linearity of the calibration curves was determined by the method of least squares and expressed by the determination coefficient (R^2). The regression lines in the investigated concentration range were linear with an R^2 higher than 0.999 for all analysed components.

The limit of detection (LOD) and the limit of quantification (LOQ) were estimated by serial dilution and detection of individual standard solutions. LOD and LOQ were calculated based on the standard deviation of the response and the slope $LOD = 3.3\sigma/S$ and $LOQ = 10\sigma/S$, where σ is the standard deviation of the response and S is the slope of the calibration curve. The validated method showed a LOD between 35 and 316 mg/L, and a LOQ between 145 and 960 mg/L.

The intraday precision of the considered compounds was assessed both for the individual standards and the sample IST0 which was selected as reference. The intraday repeatability was less than 0.8% for standards. The intraday repeatability of IS T0 capsules was between 1.67% and 4.35%.

3.2 Moisture % and pH evaluation

Preliminarily the relative humidity and the pH of both commercial blends stored in standard (PS and IS) or eco caps (PC and IC) are monitored from T0 to T90. As a general behaviour, the pH of all samples tends to decrease with an increase in the acidity of the product and the moisture increase due to the absorption of humidity. An increase in humidity could be caused by slow hydrolysis of chlorogenic acids releasing free caffeic acid during storage (Jaiswal, 2012). For the two Eco-capsules (IC and PC), the moisture % value increases faster than for the standard packaging (IS and PS) (i.e. polyethylene/aluminium copolymer), because of the higher permeability of the former (Figure 4). This behaviour can promote degradative processes over time on coffee components, such as diterpenes.

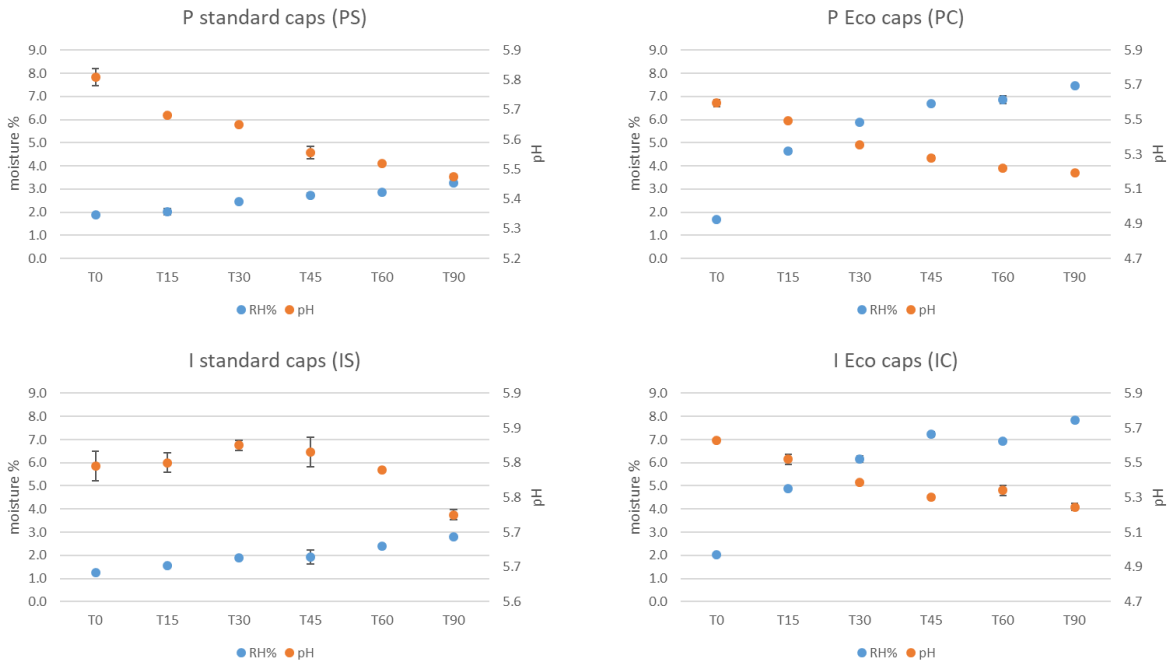

Figure 4: variation in time from T0 to T90 days of moisture % and acidity (pH) in analysed samples. Mean of two measures for each samples. P standard caps (PS), P Eco caps (PC), I standard caps (IS) and I Eco caps (IC).

3.3 Analysis of diterpenes in coffee samples

24 samples of roasted coffee in different packaging and blends were therefore analysed to determine a variation over time of their diterpenes content. The LC-MS-UV/DAD analysis revealed the presence of 5 diterpenes: kahweol, cafestol, 16-OMC and dehydrocafestol confirmed by standards and at 13.3±0.1 min the dehydrokahweol (Figure 5). The latter was tentatively identified by its mass spectrum in positive ion mode with diagnostic ions at m/z 297 $[M + H]^+$ and 279, in agreement with the data reported in the literature (Dias, 2014; Scharnhop, 2009).

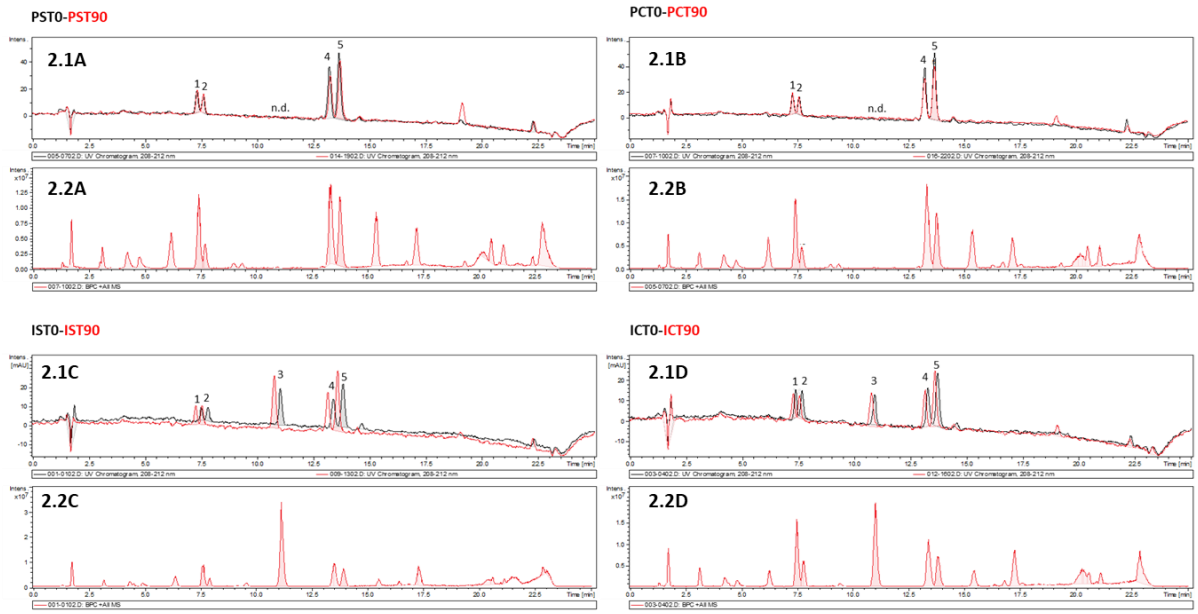

Figure 5: In 2.1A, 2.1B, 2.1C and 2.1D are reported the UV signals 208-212 nm spectrum of diterpenes in R&G coffees at of T0 (black) overlaid at T90 (red) (PS (Figure 2.1A); PC (Figure 2.1B), IS (Figure 2.1C); IC (Figure 2.1D)). The peaks corresponding to the diterpenes are indicated as (1) kahweol, (2) cafestol, (3) 16-OMe and (4) dehydrokahweol and (5) dehydrocafestol. 16-OMe are not detected in P samples (n.d.) (Figure 2.1A and 2.1B). Below the UV chromatogram, it is reported the MS signals (in red) in positive ionization mode for PS (2.2A), PC (2.2B), IS (2.2C) and IC (2.2D).

More in detail, principal component analysis (PCA) reveals the discrimination in terms of blends rather than on ageing over time. Figure 6 displays the PCA biplot of the first two PCs of I samples (IC and IS), in blue, and blend P, in green (PC and PS). On the first principal component (F1) IC and IS samples are mainly discriminated from PC and PS samples because of the higher amount of 16-OMC in their turn more characterized from cafestol and kahweol and their dehydro derivatives. The dehydro diterpenes are typical of roasting coffee, an formed during the high-temperature process (Dias, 2010; Moeenfard, 2020; Pacetti, 2012). On the other hand, 16-OMC, dehydrocafestol and dehydrokahweol differentiate samples on F2, mainly depending on packaging for IC and IS samples, although at different extent and not linearly with ageing over time.

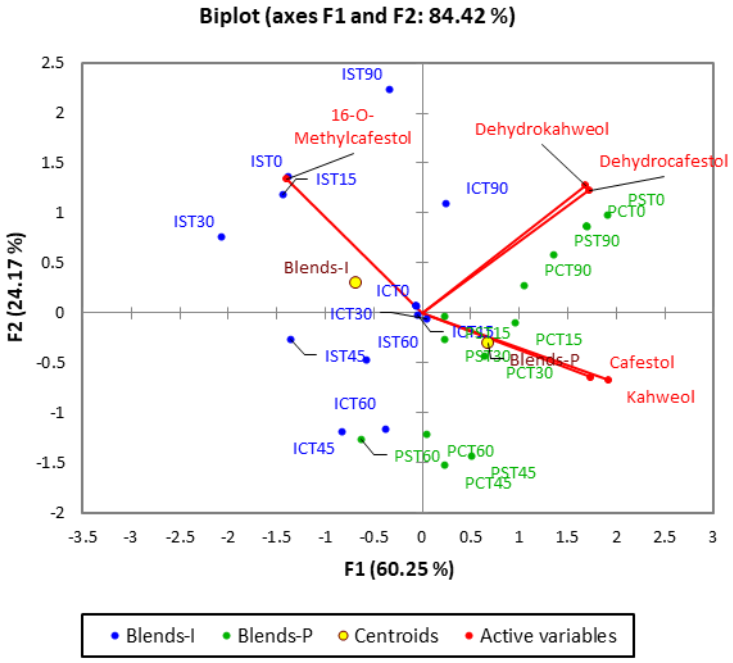

Figure 6: PCA Biplot on the investigated samples (P standard caps (PS), P Eco caps (PC), I standard caps (IS) and I Eco caps (IC) from T0 to T90 days) shows their distribution on the first and second PCs. Data were pre-processed by autoscaling.

In particular, as shown in Figure 7 16-OMC, dehydrocafestol, and dehydrokahweol show a trend that decreases up to T60 and then increases at T90 for all samples, regardless of packaging and blends. On the other hand, only small fluctuations are observed for kahweol and cafestol in all samples even if they are not significant at alpha=0.05 as confirmed by the ANOVA test.

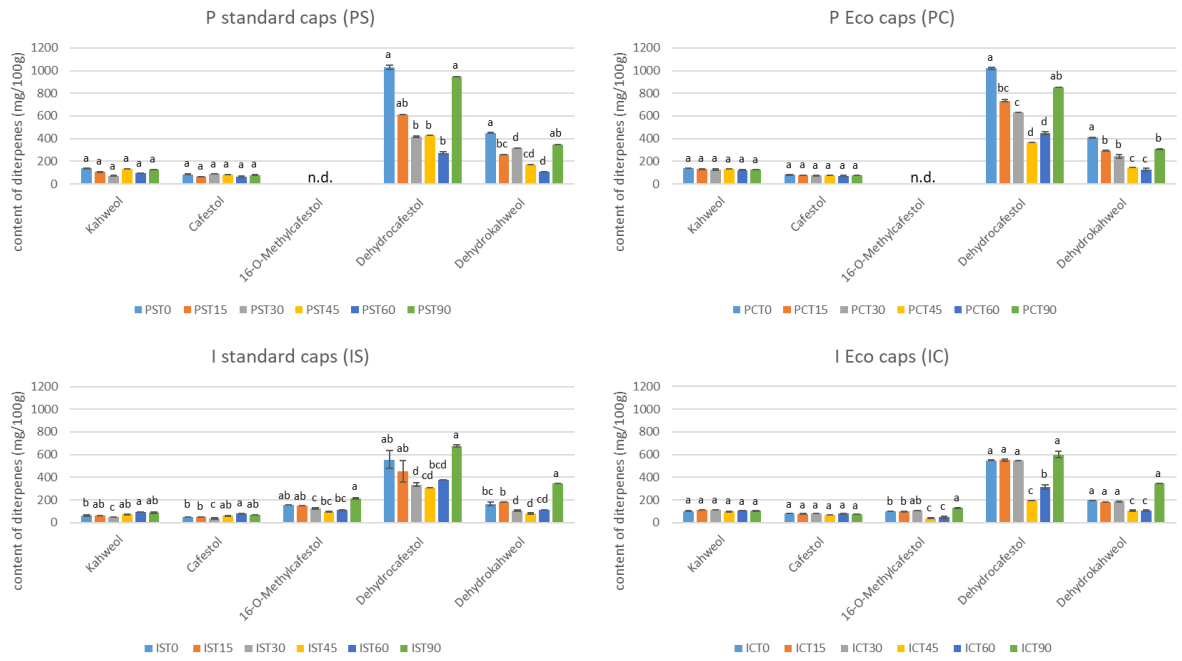

Figure 7: variation in time from T0 to T90 days of diterpenes in analysed samples. Mean of two extractions and two analytical replicates for each extract. P standard caps (PS), P Eco caps (PC), I standard caps (IS) and I Eco caps (IC). In P Eco and standard caps, 16-OMC was not detected. Same letters correspond to no variation (p value >0.05)

In both packages, Eco and standard, the trends of these three derivatives over time are quite similar, with a common decrease between 45-60 days, due to potential oxidative reactions occurring in the presence of oxygen, acidic environment (pH) and high moisture %. As depicted in figure 7, in IC caps a decreasing trend of the compounds begins at 45 days (in yellow), compared to the standards (IS), in which the decrease is more gradual, for all three compounds (i.e. 16-OMC, dehydrocafestol and dehydrokahweol). While similar decreasing trends over time starting from 15 days (in orange) are displayed for the P samples both in Eco and standard packaging (PC and PS). The staticity of cafestol and kahweol indicate that they haven't a direct correlation with the modification of dehydro derivatives over time (Figure 7). The variation in concentration for the diterpenes is reported in Table 1 between T0 and T90. Dehydrocafestol and dehydrokahweol result more affected by degradation than other diterpenes and it can be assumed that the increase of moisture % in acidic environments may promote oxidative reactions with the formation of primary and secondary oxidation products (i.e. peroxide derivatives and small volatile compounds). Indeed it is well known that the

double bond shows antioxidant activity and it may scavenge radicals as well as react with oxidative species such as oxygen to obtain epoxide intermediate and proceeds to the oxidative cleavage of the double bond (Cincotta, 2020; Flament, 2002; Toci, 2013). Based on the results reported in Figure 7 it can be hypothesised that the isolated double bond in position 15-16 (Figure 3) was responsible for this trend. Kahweol, despite having an unsaturation in position 1-2, showed higher stability and this could be correlated to the conjugated nature of its double bond with the furan aromatic heterocycle that has lower reactivity.

As already mentioned the analysis showed an unexpected increase of dehydro derivative contents after 90 days in all blends and packaging and surprisingly this behaviour cannot be attributed to the precursor compounds (i.e. cafestol and kahweol) that result stable over time. Since we observed that both standard and Eco samples (IS, PS and IC, PC) undergo an increase in moisture % and acidity, (Figure 4) we hypothesized that the environment can be responsible for a hydrolysis process (up to T90) within the matrix and that the increase of the amount of dehydrogenated compounds can be ascribed to a release of such compounds caused by acid catalysis. A related dehydration process from a tertiary alcohol forming presumably in a E_1-type elimination *via* a stable tertiary carbocation, has been reported for chlorogenic acids yielding at elevated temperatures shikimic acid derivatives (Jaiswal, 2010; 2011).

To try to explain the unusual behaviour not fully understood over time of the dehydro derivatives, the Pearson correlation of these compounds with moisture %, pH, peroxides and free fatty acids was searched and reported in Figure 8. The trends of these components up to T60 were correlated (positive in red and negative in blue) with FFAs, peroxides and acidity. The decrease of the two dehydro derivatives up to T60 indicates their susceptibility to temporal degradation when subjected to conditions that trigger oxidative processes for all samples at different extents. For PS samples (Figure 8a) is observed a low inverse correlation between dehydro derivatives with peroxides in blue (dehydro derivatives decrease and peroxides tend to slightly increase), while for PC (Figure 8b) there is a strong positive correlation in red (dehydro derivatives and peroxides decrease). The correlation of dehydro derivatives with moisture % and pH is responsible for their oxidative degradation probably justified by a continuous FFA deterioration and peroxides decay, meaning that PC favours a

strong rancidity event compared to other packaging (Belitz, 2009). In the same way, IS (Figure 8c) presented a similar behaviour of PS (Figure 8a), meaning that the standard packaging resulted more robust than the Eco caps. In IS, dehydro derivatives turn out to have an inverse correlation with peroxides, which justifies an initial formation of the primary metabolites derivate from oxidative reactions. On the other hand, IC (Figure 8d), presented a similar trend for peroxides and dehydro derivatives (strong inverse correlation), but similarly to PC (Figure 8b), there is a positive correlation between FFAs with peroxides. This trend could be linked to an effect in which the oxidative reactions promote a release of FFAs, and degradation of dehydro derivatives with a contemporary formation of peroxides. These results lead to the observation that the two blends have different susceptibilities to the reactions occurring when packaged in the same material, and within the same blend, the Eco-capsule showed a high permeation to humidity that result to be the driver of the oxidative reactions kinetic along time.

Table 1: Standard compounds with their Retention Time (Rt), mass/charge (m/z), LOD and LOQ, Intraday repeatability (RSD%) and the quantification of the samples at T0 and T90 (PS, PC, IS, IC).

								Quantification (mg/100g)							
Compounds	**Rt (min)**	**MS (m/z)**	**LOD (mg/L)[a]**	**LOQ (mg/L)[b]**	**RSD (%)[c]**	**R^2 (n=4)[d]**	**RSD (%)[e]**	**PST0**	**PST90**	**PCT0**	**PCT90**	**IST0**	**IST90**	**ICT0**	**ICT90**
Kahweol	7.3	299	73	246	4.35	0.9992	0.62	136 ± 2	128 ± 0.0	138 ± 0	123 ± 0.0	62 ± 7	85 ± 7	101 ± 5	104 ± 2
Cafestol	7.6	297	35	108	3.67	0.9994	0.81	84 ± 2	78 ± 4	79 ± 2	76 ± 0.0	48 ± 2	68 ± 2	79 ± 2	76 ± 0.0
16-OMC	10.8	331	48	145	2.48	0.9990	0.01	n.d.	n.d.	n.d.	n.d.	157± 0.0	214± 5	99 ± 0.1	129 ± 4
Dehydrocafestol	13.6	299	316	960	1.67	0.9999	0.61	1026 ± 19	949 ± 0.0	1021 ± 10	854 ± 0.0	556± 77	676± 10	549 ± 5	599 ± 29
Dehydrokahweol	13.3	297	81	246	0.00	-	-	450 ± 5	349 ± 0.0	407 ± 2	306 ± 2	165± 15	344± 0.0	193 ± 2	344 ± 0.0

[a]LOD (limit of detection) = 3.3*σ/slope of the calibration curve

[b]LOQ (limit of quantification) = 10*σ/slope of the calibration curve

[c]RSD% Intraday: Obtained by analysing 5 times the sample IS T0 in the same day.

[d]R^2 coefficient of determination, n number of calibration points

[e]RSD% Intraday: Obtained by analysing 3 times the standard solution at a concentration of 0.1 mg/L on the same day

PS	Decaf	Dekahw	Moist %	pH	Kahw	Caf	ΣFFA	Perox
Decaf	**1.0**	0.9	**-0.9**	**0.9**	0.6	0.3	-0.6	0.0
Dekahw	0.9	**1.0**	-0.9	**1.0**	0.2	0.6	-0.2	-0.2
Moist %	**-0.9**	-0.9	**1.0**	**-1.0**	-0.3	-0.1	0.4	0.3
pH	**0.9**	**1.0**	**-1.0**	**1.0**	0.3	0.4	-0.4	-0.2
Kahw	0.6	0.2	-0.3	0.3	**1.0**	0.2	**-0.9**	0.2
Caf	0.3	0.6	-0.1	0.4	0.2	**1.0**	0.1	-0.2
ΣFFA	-0.6	-0.2	0.4	-0.4	**-0.9**	0.1	**1.0**	-0.2
Perox	0.0	-0.2	0.3	-0.2	0.2	-0.2	-0.2	**1.0**

PC	Decaf	Dekahw	Moist %	pH	Kahw	Caf	ΣFFA	Perox
Decaf	**1.0**	**1.0**	**-1.0**	**0.9**	0.6	0.7	0.4	0.9
Dekahw	**1.0**	**1.0**	**-1.0**	**1.0**	0.7	0.8	0.3	0.8
Moist %	**-1.0**	**-1.0**	**1.0**	**-1.0**	-0.8	-0.8	-0.5	**-0.9**
pH	**0.9**	**1.0**	**-1.0**	**1.0**	0.8	0.8	0.2	0.8
Kahw	0.6	0.7	-0.8	0.8	**1.0**	**1.0**	0.1	0.6
Caf	0.7	0.8	-0.8	0.8	**1.0**	**1.0**	0.1	0.7
ΣFFA	0.4	0.3	-0.5	0.2	0.1	0.1	**1.0**	0.7
Perox	0.9	0.8	**-0.9**	0.8	0.6	0.7	0.7	**1.0**

IS	Decaf	Dekahw	Moist %	pH	Kahw	Caf	ΣFFA	Perox
Decaf	**1.0**	0.9	-0.8	-0.6	-0.1	-0.2	0.7	-0.6
Dekahw	0.9	**1.0**	-0.7	-0.6	-0.2	-0.3	0.3	-0.7
Moist %	-0.8	-0.7	**1.0**	0.1	0.6	0.7	-0.6	0.1
pH	-0.6	-0.6	0.1	**1.0**	-0.7	-0.6	-0.1	**0.9**
Kahw	-0.1	-0.2	0.6	-0.7	**1.0**	**1.0**	-0.4	-0.5
Caf	-0.2	-0.3	0.7	-0.6	**1.0**	**1.0**	-0.4	-0.5
ΣFFA	0.7	0.3	-0.6	-0.1	-0.4	-0.4	**1.0**	0.0
Perox	-0.6	-0.7	0.1	**0.9**	-0.5	-0.5	0.0	**1.0**

IC	Decaf	Dekahw	Moist %	pH	Kahw	Caf	ΣFFA	Perox
Decaf	**1.0**	**1.0**	-0.7	0.8	0.7	0.8	-0.3	-0.3
Dekahw	**1.0**	**1.0**	-0.8	0.8	0.6	0.7	-0.5	-0.5
Moist %	-0.7	-0.8	**1.0**	**-1.0**	0.0	-0.5	0.5	0.1
pH	0.8	0.8	**-1.0**	**1.0**	0.2	0.5	-0.5	-0.1
Kahw	0.7	0.6	0.0	0.2	**1.0**	0.7	0.0	-0.3
Caf	0.8	0.7	-0.5	0.5	0.7	**1.0**	0.3	0.1
ΣFFA	-0.3	-0.5	0.5	-0.5	0.0	0.3	**1.0**	0.5
Perox	-0.3	-0.5	0.1	-0.1	-0.3	0.1	0.5	**1.0**

Figure 8: Pearson correlations between: Peroxides value, the principal free fatty acids normally esterified with the diterpenes (two saturated fatty acids: hexadecanoic acid, octadecanoic acid; a monounsaturated fatty acid: 9-octadecenoic acid (Z)-; a polyunsaturated fatty acid: 9,12-octadecadienoic acid (Z,Z)-), dehydrocafestol and dehydrokahweol, moisture %, pH, kahweol and cafestol. a) P standard caps (PS), b) P Eco caps (PC), c) I standard caps (IS) and d) I Eco caps (IC). Data were pre-processed by autoscaling.

4. CONCLUSIONS

This study is a development in the investigation of the temporal behaviour of diterpene compounds when roasted coffee is stored under stress conditions. This unsaponifiable fraction would have been expected to be more reactive, with an increased degradation activity, especially under suboptimal temperature and humidity conditions.

The storage conditions heavily affect the chemical and physical parameters of the roasted coffee stored in the capsules with changes in moisture % and pH. In particular, all samples investigated showed an increase in humidity and a decrease in pH that mainly vary in samples packed in Eco-capsules (PC and IC) favouring a more reactive environment.

At a molecular level, the investigation of the diterpene fraction shows different behaviour for hydro and dehydro derivatives.

Cafestol and kahweol appear to be stable over time. On the contrary, dehydrogenated compounds show a degradation over time up to T60 that unexpectedly is not related to their

precursors. In a condition of high humidity and acidity, the degradation reactions are observed with reaction rates that depend principally on the type of packaging. While in PS and IS there is a trend in which peroxides tend to increase due to an autoxidation phase in which their threshold value is exceeded resulting in autocatalytic propagation of peroxides, in PC, on the other hand, peroxides have a tendency to degrade due to lysis reactions resulting from very driven oxidative processes. A different argument can be made for IC, which behaves more similarly to standard samples (PS and IS), with initial formation peroxides attesting the primary oxidation state. However, it has still to be clarified what happened to the latter components after 60 days under stress conditions, that deserves further investigation.

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SUPPLEMENTARY

Figure S1: identification of 37 picks of FAME standard.

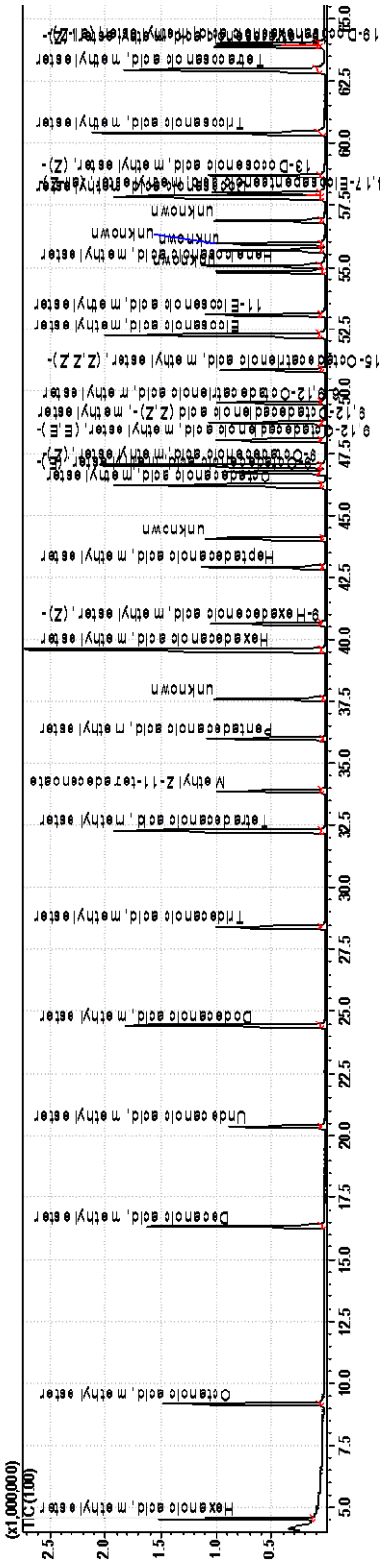

Table S1: Fatty acids detected in the coffee oil extracts with their retention time.

Compounds name	**Rt min**
Tetradecanoic acid, methyl ester	32.25
Pentadecanoic acid, methyl ester	36.00
Hexadecanoic acid, methyl ester	40.11
Heptadecanoic acid, methyl ester	43.06
Octadecanoic acid, methyl ester	46.86
9-Octadecenoic acid (Z)-, methyl ester	47.42
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	49.36
9,12,15-Octadecatrienoic acid (Z,Z,Z)-, methyl ester,	50.99
Eicosanoic acid, methyl ester	52.62
11-Eicosenoic acid, methyl ester	53.30
Heneicosanoic acid, methyl ester	55.38
Docosanoic acid, methyl ester	58.17
Tricosanoic acid, methyl ester	60.84
Tetracosanoic acid, methyl ester	63.44

3. Conclusions

Food-*omics* is a cutting-edge methodology that uses sophisticated analytical platforms and multivariate data management techniques to analyse foods holistically and link their chemical composition to biological effects in the human body. As consumers become increasingly aware of the importance of food composition, the need for information and control over the health effects of what we eat and drink is growing. Sensory impact is a primary characteristic when choosing products, especially for those classified as "comfort foods," which are devoid of nutritional content but are often consumed for their pleasurable effects. The sensory qualities of a product such as coffee mainly depend on the supply chain as a whole, which includes different production steps, from the arrival of raw beans, to the selection of single-origin beans that may or may not be blended together, to storage in increasingly innovative packaging, before consumption. This research set out to establish a relationship between aroma and flavour profiles to characterize the chemical evolution of coffee flavour over time in the presence of new packaging and reduce food waste by extending the product's expiration date,. In this context, food quality is a dynamic and complex term, influenced by both intrinsic and extrinsic elements that can change the final product and have an effect on quality at various levels, including safety, sustainability and sensory.

Being linked by a relationship of trust, safety is the most crucial aspect of quality. Indeed, safety is a prerequisite of quality, and its absence can impact both customers' quality expectations and human health. Safety, however, is also an extremely unpredictable feature. Supply chains are implementing more monitoring systems due to the emergence of new hazards, the complexity of food and the low concentrations of contaminants in the product, yet it is consumed in large quantities. Despite these possible outcomes, both green coffee beans and roasted coffee can present a number of risks related to pathogen attack, environmental contaminants, and, in the case of roasted coffee, the formation of toxic compounds such as acrylamide, which IARC has classified as a probable human carcinogen. According to exposure statistics, coffee is a source of this toxic substance. Several mitigation measures are needed to reduce acrylamide along the supply chain, from the selection of green beans to the roasting stage. Unlike other heat-processed foods, reducing acrylamide in coffee while maintaining the sensory quality of the product is difficult.

Sensory analysis can regulate and control the sensory component(s). Panel tests are currently the evaluation method in industry, but the cupping protocol has revealed a number of

problems: it is time-consuming, needs experienced panellists and, sometimes, subjective. Because of these factors, analytical methodologies that support sensory panel testing in routine quality control are needed in Industry 4.0. The shift from the conventional analytical technique, in which each food characteristic is addressed independently, to a -omics approach, in which all attributes are considered as part of a network, a multidimensional experience of coffee flavour is particularly useful. To enable comprehensive food characterization, it is essential to incorporate several fields, including food chemistry, advanced analytics, and machine learning.

The flavour of packaged coffee has been studied using omics approaches to explore the evolution of the chemical composition. This association would be exploited to create a useful model for predicting when coffee is no longer acceptable, with the aim of increasing its end-of-life and thus promoting greater sustainability, especially in light of the increased consumption of coffee capsules, which have a significant environmental impact. For this reason, the coffee industry is working to develop environmentally friendly packaging that does not affect the final quality of the coffee. Industry want to adopt a circular economy, passing from the assumption "take, use and throw away" to "take, use and recycle." The goal of circular economy is to maximize material value by understanding the finite nature of natural resources and reducing waste. Roasted coffee is a commodity that can be stored on a shelf without losing its quality, it can undergo a number of physical and chemical changes during storage. Extraneous variables, including oxygen, humidity, and temperature, impact commercial coffee and can promote staling through the loss of volatile components, production of off-notes, and oxidative reactions. For these reasons, companies are working to develop new materials and packaging technologies to reduce the occurrence of staling. To better understand some phenomena that occur in samples packaged in various types of materials, it is therefore even more important to explore the chemistry behind these aspects.

Because of the wide range of variations , coffee is a very fascinating and complex matrix; comprehensive chemical characterization of its profiles is still difficult. Some broad considerations can be made taking into account these variables and the experience gained over the past three years. In particular, especially acrylamide appears not to vary with storage, as inferred in the literature, so its formation should be prevented upstream. The aroma constitutes the dominant fraction to explain what happens in aged coffee, despite the

various types of volatile chemical ingredients and their modest amount. In fact, an increase in volatile compounds that produce undesired aroma notes was found during the storage of the samples, such as acid and oxidized notes, was found during the storage of the samples, both at the sensory and at the chemical levels. From these molecules, it was possible to extrapolate an index easier to use in the industry and with good predictive ability, comparable to sensory panel tests. On the other hand, the non-volatile part is ultimately the most stable compared to the other fractions investigated. Chlorogenic acids and alkaloids are the non-volatile groups that do not change substantially with storage over time, while lipids are have the greatest effects on aged coffee, and the fatty acid fraction undergoes the most substantial age-related oxidative reactions. To confirming some unexpected data on the lipidic fraction, further analysis was performed on a second set of samples, which led to the potential presence of an active lipase, but further confirmation are necessary to explain some abnormal behavior. From these studies emerges, also, that moisture is the main responsible for the compositional change of packed coffee quality during storage. Its increase associated with a pH decrease creates a favourable environment to speeding up of degradation reactions. In terms of packaging, standard capsules offer the longer shelf life of coffee, but at a significant environmental cost. On the other hand, Eco-capsules are a good alternative to reduce the environmental impact of single-serve coffee consumption while keeping the sensory quality of the powder, albeit with a shorter shelf life. A preliminary study carried out on the packaging structure showed that some particular behaviours observed in the samples are indeed justified and related by the molecular structure of the packaging itself. Further studies has to be carried out as this work is in progress. The adopted approach showed that the aroma is the driving fraction to monitoring the perception of the evolution of the coffee quality over time also as an endpoint of the changes occurring in the other fractions (i.e. lipids, alkaloid and phenolic compounds). Therefore, we can conclude that the science of coffee quality is a never-ending research process in continuous progress.

4. Publications

SCIENTIFIC PUBLICATIONS

- Diterpenes stability of commercial blends of roasted and ground coffees packed in copolymer coupled with aluminium and Eco-friendly capsules. **Strocchi G.**, Müller A., Kuhnert N., Martina K., Bicchi C., Liberto E. (2023) submitted to **Food Research International**

- Acrylamide in coffee: what is known and what still needs to be explored. A review.. **Strocchi G**.; Rubiolo P; Cordero C; Bicchi C.; Liberto E. (2022)**Food Chemistry, 393, 1333406.**

- Potential Aroma Chemical Fingerprint of Oxidised Coffee Note by HS-SPME-GC-MS and Machine Learning. **Strocchi G**., Bagnulo E., Ruosi M.R., Ravaioli G., Trapani F., Bicchi C., Pellegrino G. and Liberto E. (2022). **Foods 11(24), 4083.**

- Chromatographic Fingerprinting Strategy to Delineate Chemical Patterns Correlated to Coffee Odor and Taste Attributes.. Bressanello D., Marengo A., Cordero C., **Strocchi G**., Rubiolo P., Pellegrino G., Ruosi M.R., Bicchi C., Liberto E. (2021) **Journal of Agricultural and Food Chemistry 69(15), 4550.**

- Cocoa smoky off-flavour: A MS-based analytical decision maker for routine controls. Scavarda C., Cordero C., **Strocchi G**., Bortolini C., Bicchi C., Liberto E. (2021). **Food Chemistry 336, 127691.**

- Large-scale evaluation of shotgun triacylglycerol profiling for the fast detection of olive oil adulteration. Quintanilla-Casas B., **Strocchi G**., Bustamante J., Torres-Cobos B., Guardiola F., Moreda W., Martinez-Rivas J.M., Valli E., Bendini A., Gallina Toschi T., Tres A., Vichi S. (2021) **Food control, 123, 107851**

- HS-SPME-MS-Enose Coupled with Chemometrics as an Analytical Decision Maker to Predict In-Cup Coffee Sensory Quality in Routine Controls: Possibilities and Limits. Liberto E., Bressanello D., **Strocchi G**., Cordero C., Ruosi M.R., Pellegrino G., Bicchi C., Sgorbini B. (2019). **Molecules, 24(24), 4515.**

CONGRESS CONTRIBUTIONS

- Coffee sensory HS-SPME-GC-MS fingerprints for the "identitation" of the coffee oxidized note. **Strocchi G.**, Liberto E., Pellegrino G., Ruosi M.R., Bicchi C. **28th ASIC Conference** (Poster), Montpellier-Francia, 28 giugno-1 luglio 2021

- The role of chemometrics in the characterization of coffee quality. Liberto E., **Strocchi G.**, Ruosi M.R., Pellegrino G., Bicchi C. **28th ASIC Conference** (Oral presentation), Montpellier-Francia, 28 giugno-1 luglio 2021

- Coffee sensory properties: a complementary data fusion to simulate odor & taste integration by instrumental approach. Possibilities and limits. **Strocchi G.**, Liberto E., Pellegrino G., Ruosi M.R., Bicchi C. **16th Weurman Flavour Research Symposium** (Poster) Montpellier-Francia, 4-6 Maggio 2021

- The chemistry of the temporal evolution of quality coffee flavour. **Strocchi G.**, Ruosi M.R., Ravaioli G., Trapani F., Pellegrino G., Liberto E. 7th **MSFood Day 2022** (Poster), Firenze 5-7 Ottobre 2022

- Identification of potential aroma markers of coffee oxidized note. Strocchi G., Bagnulo E, Ruosi M.R., Ravaioli G., Trapani F., Bicchi C., Pellegrino G., Liberto E. 7th **MSFood Day 2022** (Poster), Firenze 5-7 Ottobre 2022

Ho tante famiglie, una più bella dell'altra. Sono stata una figlia, una sorella, una nipote, una compagna di giochi, una figlioccia, una principessa, una compagna di banco, una studentessa (ormai da troppi anni), un'amica e un'amante. Ho avuto accanto a me tante persone e tante personalità diverse che mi hanno messo al mondo, accolta, cresciuta, viziata e insegnato. Ho appreso tanto da ognuna di loro. Vorrei ringrazire prima fra tutti, la Professoressa Liberto, che con alti e bassi, mi ha portata fino a questo traguardo. Vorrei ringraziare, anche i miei nonni che sono stati i miei secondi genitori, i pilastri della mia infanzia, della mia adolescenza e della mia maturità (se si può dire che alla maturità io ci sia arrivata). Ringrazio anche i miei genitori che mi hanno sempre aiutata e sostenuta nelle decisioni più insolite e difficili. Siete stati un esempio di cosa significa essere una famiglia unita. A te mamma, che sei una grande donna, mi hai insegnato che se la vita colpisce duro, tu sai sempre come reagire e che se qualcuno ha bisogno tu rimani la persona più disponibile che io conosca, anche quando non se lo merita. A te papà che sei e sarai per sempre il mio primo principe azzurro, la spalla su cui contare, il mio aggiusta tutto, il mio insegnante di matematica e soprattutto la persona a cui chiedere aiuto sempre per qualsiasi cosa. A Lorenzo chiamato in ogni modo possibile tranne che con il suo nome, per essere stato il mio compagno di avventure immaginarie e non solo, per aver giocato alle barbie anche quando non volevi, per aver compiuto scontri eroici di wrestling e per essere stato il mio complice e il mio "bimbu" oltre che mio fratello. Grazie alla famiglia intera e agli amici che sono stati come una famiglia, per essere stata accolta così come sono, per essere stata una nipote non sempre gentile, una figlioccia non sempre amorevole, una cugina non sempre bonacciona e una "figlia" non troppo gentile. Grazie per avermi fatta giocare, riempita di regali, amata e soprattutto viziata. Grazie al mio compagno di banco del Liceo, al mio principino. Ci siamo incontrati, ci siamo "picchiati", ci siamo divertiti, ci siamo persi e poi ritrovati. Simo, mi hai insegnato la cosa più importante che una persona possa fare: la "cazzimma". Al mio mentore, Eli, che mi ha seguita quando ancora non avevo finito l'università, che mi ha insegnato un mestiere e soprattutto che ha creduto in me, prima ancora che io credessi in me stessa. Non sei solo un'insegnante, ma una amica e mi hai fatto entrare nella tua vita e nella tua famiglia e in tutto ciò che da sola hai costruito. Alla mia futura testimone, Fedina, che sa sempre cosa dire e come ascoltarti con una bontà mai vista prima. Sei capitata nel posto giusto e nel momento migliore. Ai miei colleghi di lavoro, ai miei nuovi amici. Al mio compagno di banco, Simone, che si arrabbia sempre al suono trionfale del suo

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