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# 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 group of compounds of the terpenic fraction of roasted coffee and account for about 7–20 % (w/w) of the lipid fraction. Several parameters can influence their occurrence in coffee beans and beverages including species and post-harvest processing. Diterpenes in coffee have been studied extensively, but to the best of the authors' knowledge, there is no information in the literature on their stability over time. Coffee is a relatively stable product under optimal temperature, humidity and oxygen conditions. However, during storage it can undergo a series of chemical and physical reactions that alter its flavour and lead to rancidity, mainly due to the oxidative reactions that take place 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. The Results show that the storage influences the internal environment of the capsules with an increase in moisture and a decrease in pH favouring more reactive conditions, especially for Eco capsules. Relative stability over time is observed for cafestol and kahweol. dehydro derivatives show a degradation up to T60 independently on the blends and packaging, which is not related to their precursors. The permeability of packaging and blends affect the modification of these components: while a drastic oxidation process takes place in Arabica eco compatible capsules (PC) when acidity and moisture increase, in Arabica/Robusta eco compatible capsules (IC) as well as in Arabica/Robusta and Arabica standard capsules (IS and PS) the peroxides tend to increase resulting in an autocatalytic propagation.

## 1. Introduction

Coffee is a beverage consumed all around the world becoming an asset of socialization. as part of our daily lives, coffee consumption is widespread not only because of the effects due to its bioactivity but also 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 it is determinant to define its economic value cost. In recent years, we have assisted to 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, Adhikari, & Chambers, 2011).

Roasted coffee quality is correlated with a series of genetic precursors, environmental factors and post-harvest practices, such as depulping, drying fermentation, roasting and storage processes (Folmer, 2017; Kitzberger et al., 2013; Novaes, Oigman, de Souza, Rezende, & de Aquino Neto, 2015; Sunarharum, Williams, & Smyth, 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 with volatilization of aroma compounds, $CO_2$ release, formation of off-notes and rancidity development (Anese, Manzocco, & Nicoli, 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, Zerny, & Schieberle, 2007; Flament, 2002; Manzocco, Calligaris, Anese, & Nicoli, 2016; Yeretzian, 2017). Some sensory features and compounds are

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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 & Alves, 2020a; Novaes et al., 2015).

Lipids are major components of green coffee ranging from 7 to 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 & Alves, 2020a, Moeenfard & Alves, 2020b; Novaes et al., 2015; De Oliveira et al., 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 ($C_{21}H_{30}O_3$) (Fig. 1) (Moeenfard & Alves, 2020a, Moeenfard & Alves, 2020b; Novaes et al., 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 & Alves, 2020a, Moeenfard & Alves, 2020b; Scharnhop & Winterhalter, 2009; Speer & Kölling-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, Beaumesnil, de Oliveira, Elfakir, & Bostyn, 2013; Kitzberger et al., 2013; Moeenfard & Alves, 2020a; Moeenfard & Alves, 2020b).

In roasted coffee, *C. 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 *C. arabica* compared to *C. robusta* (0.003 % to 0.2 % (w/w) (Dias et al., 2010; Kitzberger et al., 2013; Mori et al., 2016). Furthermore, *C. robusta* presents a specific derivative 16-OMC (Mori et al., 2016), although very recently, Gunning et al. (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 & Winterhalter, 2009) along with sinapoyl quinic acid based as biomarkers (Badmos, Lee & Kuhnert, 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 et al., 2010; Moeenfard & Alves, 2020a, Moeenfard & Alves, 2020b; Pacetti, Boselli, Balzano, & Frega, 2012). This partial stability depends on the temperature and time of roasting that could influence the diterpenes profile, with a major reduction in *C. arabica*, due to the higher sensitivity of this specie to roasting (Dias, de Faria-Machado, Mercadante, Bragagnolo, & de Toledo Benassi, 2014; Kitzberger et al., 2013; Kölling-Speer & Strohschneider, 1998;Sridevi, Giridhar, & Ravishankar, 2011; Williamson & Hatzakis, 2019) (Fig. 2).

Diterpenes were widely studied since the 1930 s when kahweol and cafestol were first identified due to their relationship to human health (Kurzrock & Speer, 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 roasted 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. The aim was 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) trying to explain potential reactions which they go through.

## 2. 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 polypropylene and aluminium. After processing, the commercial samples were subjected to accelerated storage conditions at a temperature of 45 °C and 65 % relative humidity (RH %) in a climate chamber to control temperature and humidity (FDM Environment Makers model CB-CS series, Rome – Italy). Three batches of each condition were analysed at the following time points: T0, T15, T30, T45, T60, and T90 days. The storage conditions were selected taking into account the know-how of the industrial partner in coffee quality preservation, previous studies and knowledge of the raw material tested, and the advices of an external organisation specialised in sensory analysis and shelf life, and of a research group working in the field for many years.

### 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 ($I_S^T$) determination and the reference mixture for FAMEs identification (Supelco 37 components mix) and Sulfuric acid 98 % ($H_2SO_4$) for the fatty acids

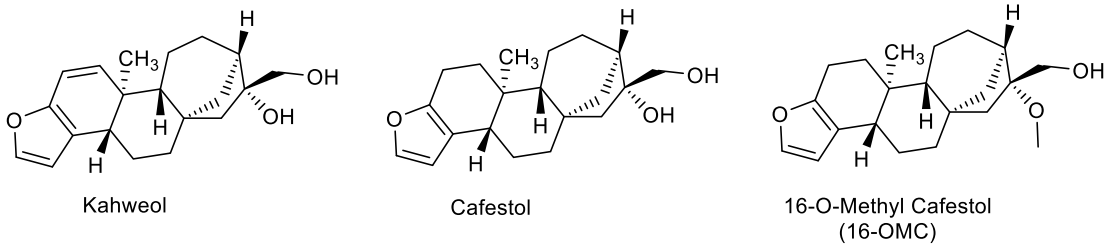


**Fig. 1.** Chemical structure of cafestol, kahweol and 16-O-methylcafestol (16-OMC).

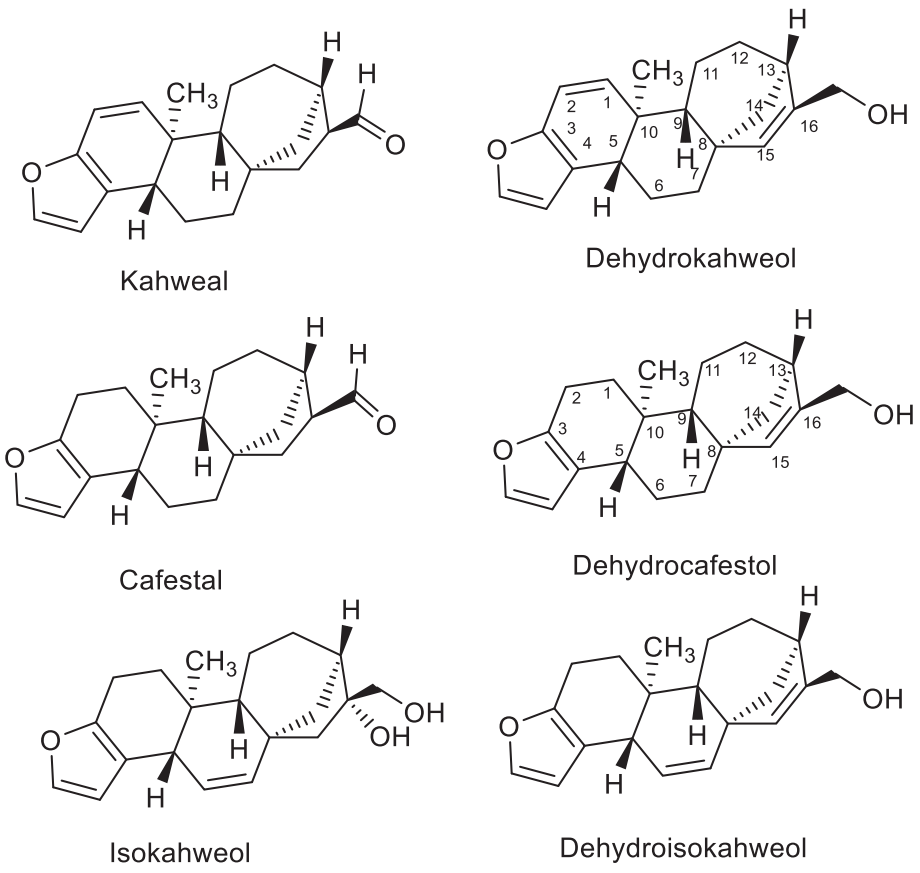


**Fig. 2.** Chemical structure of decomposed and isomerized derivatives of cafestol and kahweol.

transesterification were all from Merck (Milan, Italy).

### 2.3. Diterpenes extraction

The procedure for the diterpenes extraction was adapted from Dias et al. (2010). Since they are mainly present as esters, 1 g 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 × 3.0 mm 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) 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) (Fig. 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, 5 L/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).

### 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. (2021) to enable a more in-

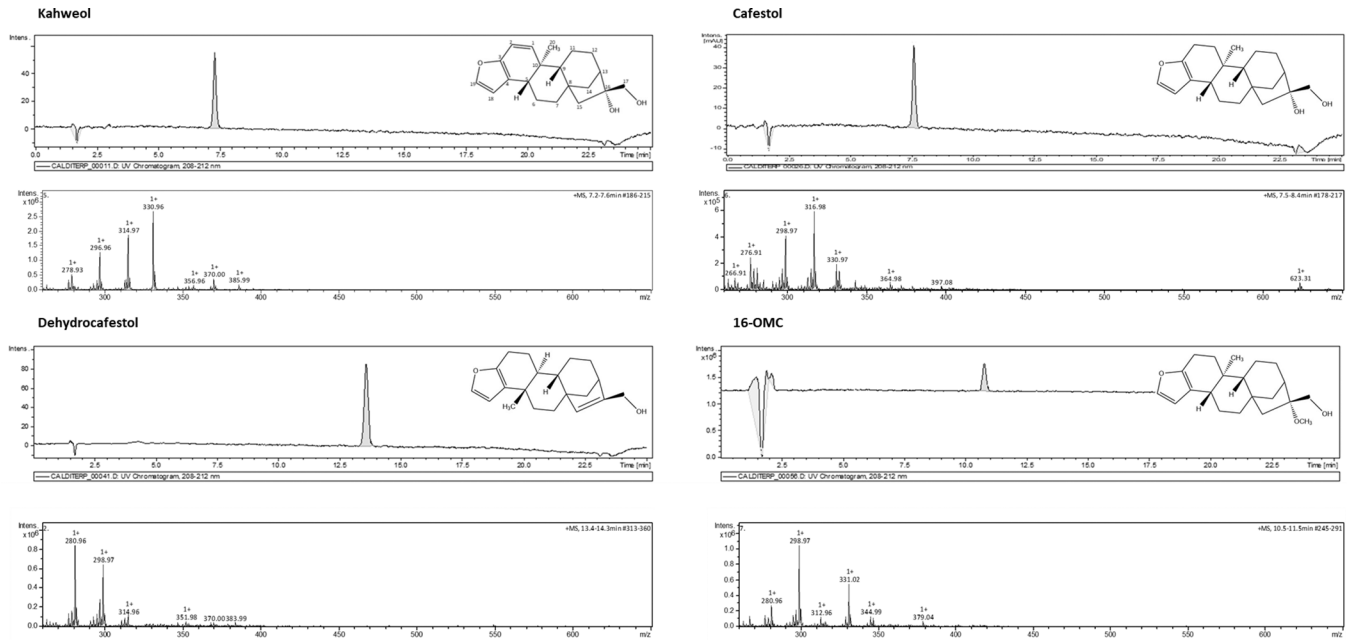


**Fig. 3.** UV signals at 210 nm and the APCI-MS spectrum in positive ion mode of standards at concentration of 0.1 mg/mL.

depth investigation of the evolution of FFAs in the coffee matrix. Total lipids were extracted in duplicate from 1 g of coffee powder with 30 mL 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 2 mL 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 5973 N MSD. A Supelco SLB™-IL 76 (Tri (tripropylphosphoniumhexanamido) triethylaminebis (trifluoromethanesulfonyl) imide) (30 m × 0.25 mm dc, 0.20 μm 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 Appendix 1 Figure A.1. 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 in Appendix 1 Table A.1 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 percentage and pH determination

Moisture content was determined according to the method of Benković and Tušek (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 brew was prepared from the capsules with an espresso Lavazza a Modo Mio machine (model LM800 Tiny, Italy) collecting 25 mL of coffee that was left in agitation to cool at ambient temperature in a water bath before the pH measure. The pH was measured, in duplicate, using the pH 70 portable pH-Mettler, Toledo© (Columbus, Ohio, USA).

### 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.

## 3. Results and discussion

### 3.1. Quantification method

Table 1 reports the Validation parameters of the LC-MS-UV/DAD

**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).

| Compounds | Rt (min) | MS ($m/z$) | LOD (mg/L)[a] | LOQ (mg/L)[b] | RSD (%)[c] | $R^2$ (n = 4)[d] | RSD (%)[e] | Quantification (mg/100 g) 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.

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 = 3.3σ/S and LOQ = 10σ/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 percentage and pH evaluation

Preliminarily the moisture % 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 moisture

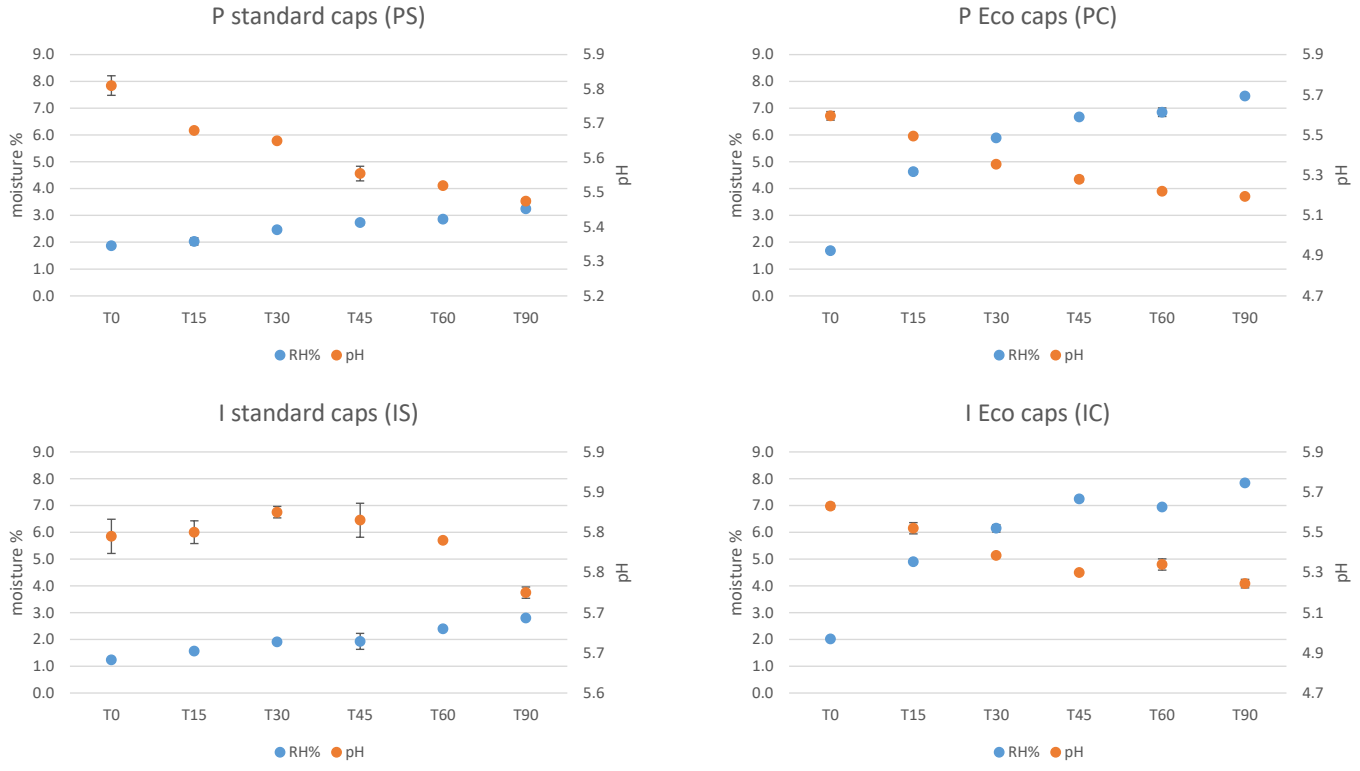


**Fig. 4.** Variation in time from T0 to T90 days of moisture % and acidity (pH) in analysed samples. Mean of two measures for each samples. PS, PC, I S and IC capsules.

could be caused by slow hydrolysis of chlorogenic acids releasing free caffeic acid during storage (Jaiswal, Matei, Golon, Witt & Kuhnert, 2012). For the two Eco-capsules (IC and PC), the moisture % value increases faster than for the standard packaging (IS and PS) (i.e. polypropylene/aluminium copolymer), because of the higher permeability of the former (Fig. 4). This behaviour can promote degradative processes over time on coffee components, such as diterpenes.

### 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 (Fig. 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 (Carlos et al., 2014; Scharnhop & Winterhalter, 2009).

More in detail, principal component analysis (PCA) reveals the discrimination in terms of blends rather than on ageing over time. Fig. 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 well 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 and formed during the high-temperature process (Dias et al., 2010; Moeenfard & Alves, 2020a, Moeenfard & Alves, 2020b; Pacetti et al., 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.

In particular, as shown in Fig. 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

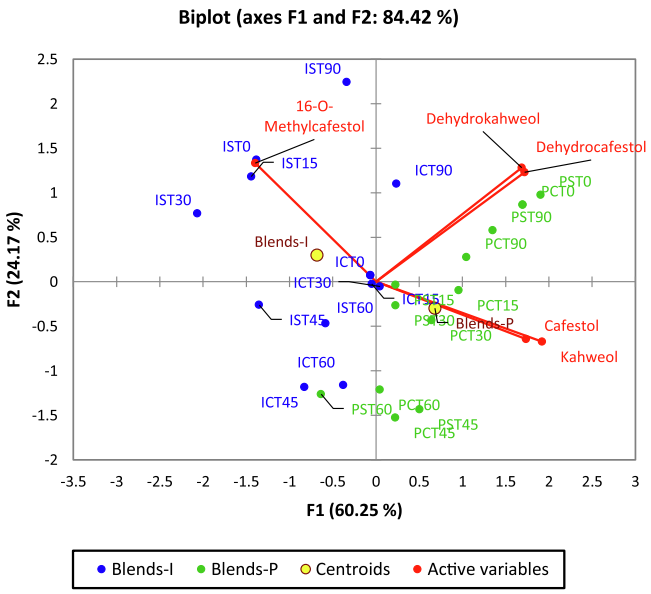


**Fig. 6.** PCA Biplot on the investigated samples (PS, PC, IS and IC capsules from T0 to T90 days). Data were pre-processed by autoscaling.

cafestol in all samples even if they are not significant at alpha = 0.05 as confirmed by the ANOVA test.

In both packages, Eco and standard, the trends of these three derivatives over time are quite similar with a common decrease between 45 and 60 days due to potential oxidative reactions occurring in the presence of oxygen, acidic environment (pH) and high moisture %. As depicted in Fig. 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). Similar decreasing trends over time starting from day 15 (in orange) are displayed for the P samples both in

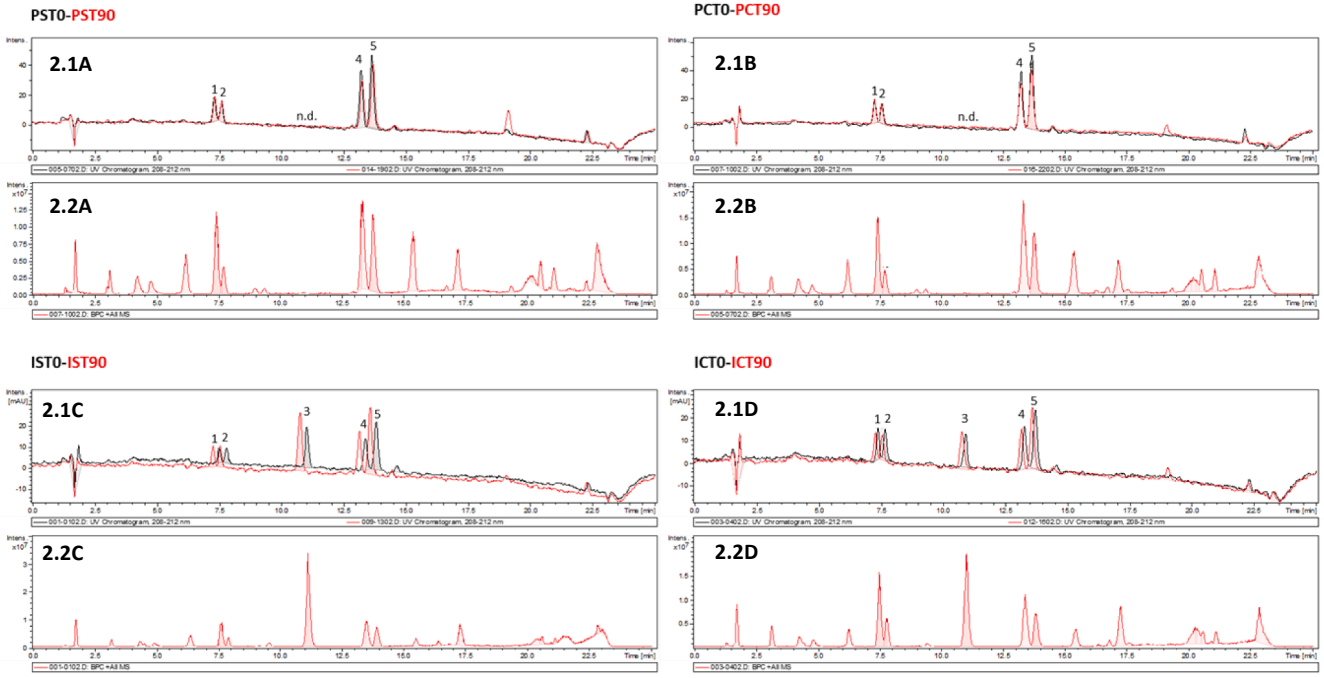


**Fig. 5.** In 2.1A-D are reported the UV signals 208–212 nm spectrum of diterpenes in R&G coffees at of T0 (black) overlaid at T90 (red. The peaks corresponding to the diterpenes are indicated as (1) kahweol, (2) cafestol, (3) 16-OMe and (4) dehydrokahweol and (5) dehydrocafestol. 16-OMe are not detected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

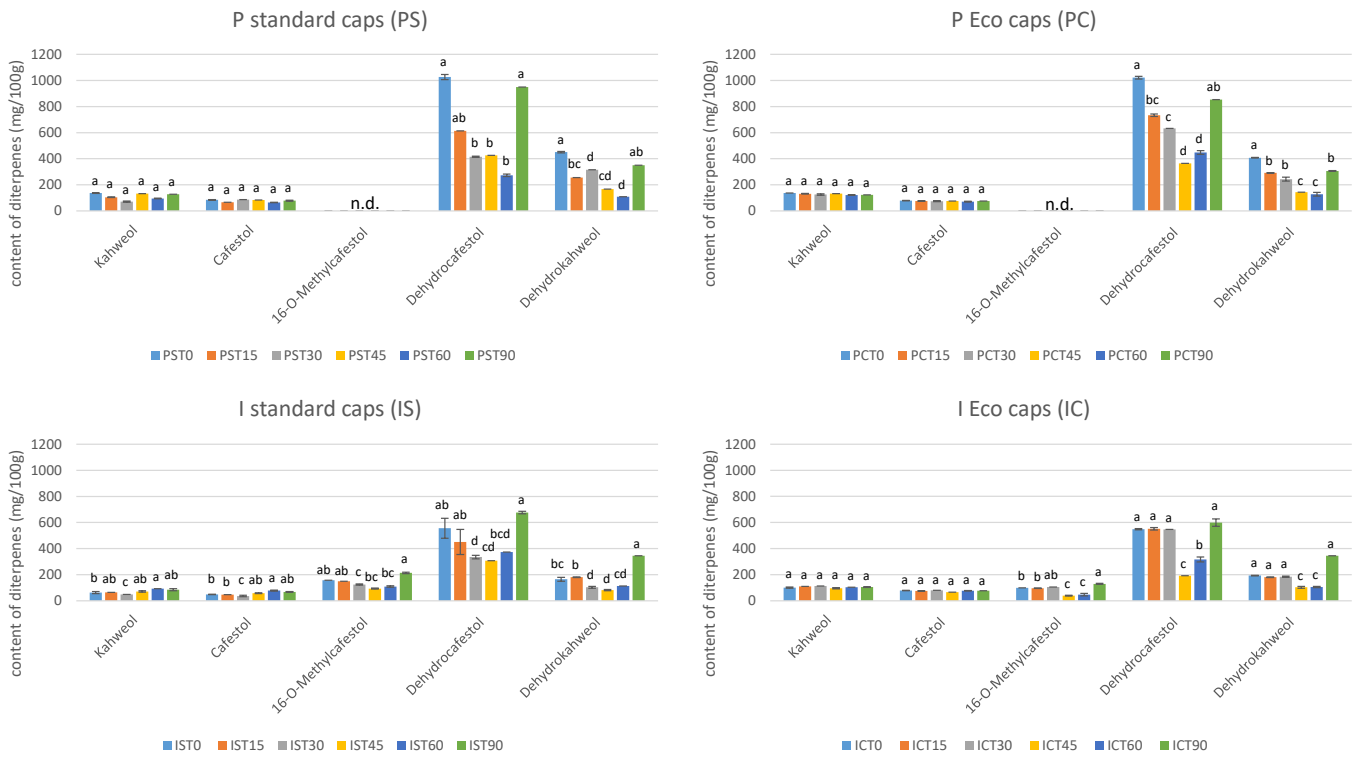


**Fig. 7.** Variation in time from T0 to T90 days of diterpenes in analysed samples. Mean of two extractions and two analytical replicates for each extract. PS, PC, IS and IC capsules. Same letters correspond to no variation (p -value > 0.05).

Eco and standard packaging (PC and PS). The stability of cafestol and kahweol indicates that they have not a direct correlation with the modification of dehydro derivatives over time (Fig. 7).

The diterpene change in concentration between T0 and T90 is reported in Table 1.

Dehydrocafestol and dehydrokahweol are 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, Tripodi, Merlino, Verzera, & Condurso, 2020; Flament, 2002; Toci, Neto, Torres, & Farah, 2013). Based on the results reported in Fig. 7 it can be hypothesised that the isolated double bond in position 15–16 (Fig. 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 precursors (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, (Fig. 4) we hypothesized that the environment can be responsible for hydrolytic 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, Sodvat, Vivan & Kuhnert, 2010; Jaiswal, Matei, Ullrich & Kuhnert, 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 Fig. 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. PS samples (Fig. 8a) shows a low inverse correlation between dehydro derivatives with peroxides in blue (dehydro derivatives decrease and peroxides tend to slightly increase), while for PC (Fig. 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, Grosch, & Schieberle, 2009). In the same way, IS (Fig. 8c) presented a similar behaviour to PS (Fig. 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 (Fig. 8d) presented a similar trend for peroxides and dehydro derivatives (strong inverse correlation), but similarly to PC (Fig. 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.

## 4. Conclusions

This study is a development in the investigation of the temporal

| PS | Decaf | Dekahw | Moist % | pH | Kahw | Caf | ΣFFA | Perox |
|---|---|---|---|---|---|---|---|---|
| Decaf | **1.0000** | 0.8774 | **-0.8987** | **0.9435** | 0.5961 | 0.3156 | -0.6451 | -0.0111 |
| Dekahw | 0.8774 | **1.0000** | -0.8536 | **0.9675** | 0.2178 | 0.5611 | -0.2126 | -0.1972 |
| Moist % | **-0.8987** | -0.8536 | **1.0000** | **-0.9512** | -0.2989 | -0.1298 | 0.4330 | 0.3239 |
| pH | **0.9435** | **0.9675** | **-0.9512** | **1.0000** | 0.3085 | 0.3646 | -0.3702 | -0.1922 |
| Kahw | 0.5961 | 0.2178 | -0.2989 | 0.3085 | **1.0000** | 0.1740 | **-0.9495** | 0.2440 |
| Caf | 0.3156 | 0.5611 | -0.1298 | 0.3646 | 0.1740 | **1.0000** | 0.0888 | -0.2104 |
| ΣFFA | -0.6451 | -0.2126 | 0.4330 | -0.3702 | **-0.9495** | 0.0888 | **1.0000** | -0.2377 |
| Perox | -0.0111 | -0.1972 | 0.3239 | -0.1922 | 0.2440 | -0.2104 | -0.2377 | **1.0000** |

| PC | Decaf | Dekahw | Moist % | pH | Kahw | Caf | ΣFFA | Perox |
|---|---|---|---|---|---|---|---|---|
| Decaf | **1.0000** | **0.9860** | **-0.9664** | **0.9479** | 0.6040 | 0.6554 | 0.4486 | 0.8648 |
| Dekahw | **0.9860** | **1.0000** | **-0.9669** | **0.9792** | 0.6951 | 0.7535 | 0.3473 | 0.8499 |
| Moist % | **-0.9664** | **-0.9669** | **1.0000** | **-0.9512** | -0.7608 | -0.7768 | -0.4633 | **-0.8993** |
| pH | **0.9479** | **0.9792** | **-0.9512** | **1.0000** | 0.7717 | 0.7894 | 0.1889 | 0.7593 |
| Kahw | 0.6040 | 0.6951 | -0.7608 | 0.7717 | **1.0000** | **0.9627** | 0.0534 | 0.6217 |
| Caf | 0.6554 | 0.7535 | -0.7768 | 0.7894 | **0.9627** | **1.0000** | 0.1314 | 0.7150 |
| ΣFFA | 0.4486 | 0.3473 | -0.4633 | 0.1889 | 0.0534 | 0.1314 | **1.0000** | 0.7418 |
| Perox | 0.8648 | 0.8499 | **-0.8993** | 0.7593 | 0.6217 | 0.7150 | 0.7418 | **1.0000** |

| IS | Decaf | Dekahw | Moist % | pH | Kahw | Caf | ΣFFA | Perox |
|---|---|---|---|---|---|---|---|---|
| Decaf | **1.0000** | 0.8675 | -0.7707 | -0.6222 | -0.1217 | -0.1778 | 0.6524 | -0.6197 |
| Dekahw | 0.8675 | **1.0000** | -0.6894 | -0.5558 | -0.1738 | -0.2580 | 0.2895 | -0.7240 |
| Moist % | -0.7707 | -0.6894 | **1.0000** | 0.0521 | 0.6191 | 0.6547 | -0.5610 | 0.0784 |
| pH | -0.6222 | -0.5558 | 0.0521 | **1.0000** | -0.6875 | -0.6337 | -0.1039 | **0.9260** |
| Kahw | -0.1217 | -0.1738 | 0.6191 | -0.6875 | **1.0000** | **0.9956** | -0.3806 | -0.5282 |
| Caf | -0.1778 | -0.2580 | 0.6547 | -0.6337 | **0.9956** | **1.0000** | -0.3687 | -0.4528 |
| ΣFFA | 0.6524 | 0.2895 | -0.5610 | -0.1039 | -0.3806 | -0.3687 | **1.0000** | -0.0013 |
| Perox | -0.6197 | -0.7240 | 0.0784 | **0.9260** | -0.5282 | -0.4528 | -0.0013 | **1.0000** |

| IC | Decaf | Dekahw | Moist % | pH | Kahw | Caf | ΣFFA | Perox |
|---|---|---|---|---|---|---|---|---|
| Decaf | **1.0000** | **0.9645** | -0.6969 | 0.7703 | 0.7376 | 0.8002 | -0.3125 | -0.3227 |
| Dekahw | **0.9645** | **1.0000** | -0.7617 | 0.8049 | 0.6121 | 0.6652 | -0.4693 | -0.5096 |
| Moist % | -0.6969 | -0.7617 | **1.0000** | **-0.9764** | -0.0330 | -0.4937 | 0.4730 | 0.1161 |
| pH | 0.7703 | 0.8049 | **-0.9764** | **1.0000** | 0.1679 | 0.5172 | -0.5409 | -0.0939 |
| Kahw | 0.7376 | 0.6121 | -0.0330 | 0.1679 | **1.0000** | 0.6600 | 0.0150 | -0.2767 |
| Caf | 0.8002 | 0.6652 | -0.4937 | 0.5172 | 0.6600 | **1.0000** | 0.3004 | 0.1040 |
| ΣFFA | -0.3125 | -0.4693 | 0.4730 | -0.5409 | 0.0150 | 0.3004 | **1.0000** | 0.5303 |
| Perox | -0.3227 | -0.5096 | 0.1161 | -0.0939 | -0.2767 | 0.1040 | 0.5303 | **1.0000** |

*Values in bold are different from 0 with a significance level alpha=0.05*

**Fig. 8.** Pearson correlations between Peroxides value, the principal free fatty acids normally esterified with the diterpenes, dehydrocafestol and dehydrokahweol, moisture %, pH, kahweol and cafestol. a) PS, b) PC, c) IS and d) IC capsules. Data were pre-processed by autoscaling.

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 moisture 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 moisture and acidity, the degradation reactions are observed with reaction rates that depend principally on the 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 peroxides have a tendency to degrade due to lysis reactions resulting from very strong oxidative processes. Different is for IC which behaves more similarly to standard samples (PS and IS) with initial formation peroxides attesting the primary oxidation state. However, the behaviour of the latter components after 60 days under stress conditions has still to be clarified and that deserves further investigation.

## CRediT authorship contribution statement

**Giulia Strocchi:** Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Anja B. Müller:** Formal analysis, Investigation. **Nikolai Kuhnert:** Methodology, Writing – review & editing. **Katia Martina:** Data curation, Writing – original draft, Writing – review & editing. **Carlo Bicchi:** Writing – review & editing. **Erica Liberto:** Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

The authors do not have permission to share data.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2023.113577.

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