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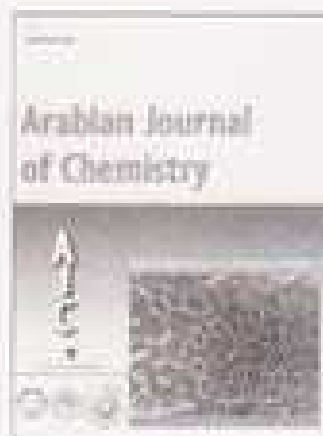
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**PHYSICO-CHEMICAL PROPERTIES OF SOME HONEYS PRODUCED FROM
DIFFERENT PLANTS IN MOROCCO**

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ABSTRACT: Seventy-three Moroccan honey samples were collected between 2005 and 2008. In this study, physicochemical properties of multifloral, honeydew and nine types of unifloral honeys (*Euphorbia resinifera*, *Euphorbia scabra*, *Cercis maculata*, carob, thyme, lavender, Ziziphus and rosemary) were determined of which water content, pH, acidity (free, lactone and total acidity), electrical conductivity (EC), colour, diastase, hydroxymethylfurfural (HMF) and sugar content. The moisture shows values 14.3 and 20.2%, pH between 3.32 and 5.13, the total acidity ranges between 11.94 and 26.03 mg kg⁻¹, hydroxymethylfurfural (HMF) content shows values between 0.09 and 23.38 mg kg⁻¹. Diastase values were between 4.3 and 24.0° Gotic; electrical conductivity between 119.9 and 1741 $\mu\text{S cm}^{-1}$ and fructose, glucose and sucrose values range between 35.07-66.26, 23.7-39.3 and 0.43-2.98%.

A statistical analysis was carried out to classify ten types of honeys, and identify the most significant parameters, using analysis of variance, principal component analysis (PCA) and stepwise discriminant analysis (SDA). PCA showed that the cumulative variance was 74.97%.

and about 88.9% of samples were correctly classified.

The principal aim of this study was to contribute more to the knowledge of the Moroccan honeys by means of the analysis of chemical composition and of physical parameters. Seventy-three Moroccan unifloral, multifloral and honeydew honey samples, including types that have never been studied before, produced in different regions in Morocco (Table 1-2), were analysed to define its main features.

As a consequence, we present data on water content, electrical conductivity, pH, free acidity, lactone acidity, total acidity, diastase, 5-(hydroxymethyl)-2-furaldehyde (HMF) amounts, fructose, glucose and sucrose.

Keywords: Moroccan honey; Physicochemical properties; PCA; SDA

1. INTRODUCTION

Morocco is a valid territory for honey production, due to its melliferous variety sources, deriving above all from cultivations on a large scale, and for the climate. The beekeeping is an old activity and 80% of the productivity are due to traditional beekeeping.

In 2006, honey production has reached 3500 tons of which 2500 tons in the industrial sector and 1000 tonnes in the traditional one, an increase of about 17% over 2005. The number of beekeepers is about 35000 including 26000 traditional and 9000 modern beekeepers. The number of hives is 385000 of which 300000 traditional and 85000 modern hives (Ministère de l'Agriculture et de la Pêche Maritime, Morocco, 2006).

The honey composition depends highly on the type of flowers utilized by the bee as well as climatic conditions (Abu-Tarboush et al. 1991). The Moroccan honey productions regard many types of floral origin (Diet et al. 2004, Terrab et al. 2003a, 2003b, 2003c); some researches about their parameters especially in Southwest of Morocco were carrying out (Belkassi et al. 2008; Nouman et al. 2004, 2005; Terrab et al. 2011, Terrab et al. 2002, 2003a, 2003b, 2003c, Terrab et al. 2003).

In Morocco honey is widely used in traditional medicine, unfortunately, there aren't enough investigation regarding its quality and characterization. and *Euphorbia* arborea, *Ziziphus Avia* and lavender honeys were never studied.

Three races of bees live in Morocco (Hepburn & Radloff 1999) are *Apis mellifera intermedia* (Batal-Rocquet 1906) is present in most regions, *Apis mellifera major* (Bottner 1987) in the Rif mountains in the North, and it is considered as an ecotype not differing from *Apis mellifera intermedia* in behaviour and its taxonomic status, and *Apis mellifera subsaharica* (Baldensiege 1872) in the south.

2. MATERIALS AND METHODS

2.1. Honey samples

Seventy-three unifloral, multifloral and honeydew honey samples were collected from beekeepers in different regions of Morocco between 2005 and 2008, during different seasons of the year depending on floral sources (*Euphorbia*, *Cistus*, *sacalypus*, *coron*, *cytus*, lavender, *Ziziphus* and rosemary). The informations about samples are presented in Tables 1 and 2.

2.1. Analytical procedures

Water content (moisture) was determined by an Abbe-type refractometer reading at 20° C, according to the relationship between honey water content and refractive index. (Bogdanov 2002, Chatway 1932).

pH was measured by means of a potentiometric pH-meter (Hanna Instruments) in a solution containing 10 g of honey in 75 ml of CO₂ free distilled water. Free, lactic and total acidity were determined by a titrocaric method as follows: the addition of 0.05 M NaOH is stopped at pH 8.5 (free acidity), immediately a volume of 10 ml 0.05 M NaOH is added and, without delay is back titrated with 0.05 M HCl to pH 3.3 (lactic acidity). Total acidity results are obtained by adding free and lactic acidities (Bogdanov et al. 1997).

The detection method of Hydroxymethylfurfural (HMF) was based in the original work of Juring & Kupper (1980) suggested by European Honey Commission (Bogdanov et al. 1997). HMF was determined in a clear, filtered, aqueous honey solution using reverse phase HPLC (High Pressure Liquid Chromatography) equipped with UV detection. Separation was performed on an octadecylsilane C18 column 150 mm x 4.6 mm, 5 µm particle size. The signal was compared with those from standards of known concentration.

Electrical conductivity was measured at 20 °C in a conductimeter, the sample solution was prepared using ultra pure water (Vorwald 1964).

Dextrose was measured using Benedict method based on procedure of Siegenhaker (1975), modified by Bogdanov (1984) and harmonized by the European Honey Commission (Bogdanov et al. 1997). Absorption was determined using a spectrophotometer UV/VIS at $\lambda = 620$ nm.

Sugar content was determined by HPLC with RI (Refractive Index) detector and analytical stainless-steel column in polar amyropolysilane (1-SiH₃) (µpor) 250 x 4.6 mm. In a 100 ml volumetric flask, containing 25 ml of methanol, 5g of honey dissolved in water were transferred and filled up with water. The solution was filtered through a 0.45 µm syringe filter (Boydston et al. 1997, Boydston & Hausman 1988).

Colour was measured according to Pfund colour scale, using the Lovibond comparator; the reading is expressed in millimetres.

2.3. Statistical analysis

Statistical Package for Social Science (SPSS) was used to establish the difference between the ten honey types by means of their physicochemical parameters. The results are expressed as mean values, range of values and standard deviation (SD) using analysis of variance (ANOVA). In order to check if the correlation matrix can be presumed to be the identity; Bartlett test of sphericity and the KMO test (Kaiser-Meyer-Olkin measure of sampling adequacy) were performed. We proceeded to carry out a study of the bivariate correlations between all the variables, detecting which of them were significant. With the aim of evaluating which of the main factors identified will explain most of the variability, the data matrix was submitted to principal component analysis (PCA), using the covariance matrix. A step-by-step discriminant analysis (DA) technique was performed in attempt to classify the honey samples.

3. RESULTS AND DISCUSSION

3.1. Physicochemical parameters

The means, ranges of values and standard deviations of water content, acidity, hydroxymethylfurfural, electrical conductivity, colour and sugars content are listed in Tables 3 and 4.

The moisture, parameter related to maturity, degree of honey and temperature. In the present study moisture values are between 14.3 and 20.2%. One sample with 20.2% exceeded the limit (20%) allowed by European Community regulations (The Council of the European Union, 2002). Moisture values were within the values found in Algerian honeys (between 14.64 and 19.04%) Ouchemouh et al. (2006) and less than those found in Northwest Moroccan honeys (between 14 and 24.1%) (Tertilt et al. 2002), which confirm that the moisture content is also affected by climatic conditions (Narda et al. 2003).

Acidity of honey due to presence of organic acids, pH values were between 3.52 and 5.13, according with the values found in Algerian honeys (Ouchemouh et al. 2006). Values for free acidity ranged from 8.56 to 44.09 mg kg^{-1} ; the lactic acidity ranged between 2.68 and 14.17 mg kg^{-1} , while the total acidity ranges between 11.94 and 58.03 mg kg^{-1} . Values for free acidity were below the allowed limits (50 mg kg^{-1}) (The Council of the European Union, 2002), showing the absence of undesirable fermentation.

Hydroxymethylfurfural (HMF) content, an indicator of honey freshness (Ichida et al. 1958), shows values between 0.09 and 53.38 mg kg^{-1} ; four samples with values between 50.26 and 783 mg kg^{-1} exceeded the limits established by European Community regulations (The Council of the European Union, 2002) due to excessive heating.

Dioxane shows values between 4.3 and 34.6^o Grabe, four samples exceeded the limits of European Community legislation (The Council of the European Union, 2002) with values less than 5^o Grabe and HMF content more than 12 mg kg⁻¹.

Electrical conductivity, closely related to the concentration of mineral and organic acids, shows great variability according to the floral origin. Values were between 119.9 and 2741 $\mu\text{S cm}^{-1}$ and within values found in Algerian (mean ranged between 210 and 1810 $\mu\text{S cm}^{-1}$) (Ouchermoulh et al. (2006) and Northwest Moroccan honeys (between 240 and 1134 $\mu\text{S cm}^{-1}$) (Terrat et al. 2002).

Fructose, glucose and sucrose values range between 35.07-46.26, 23.7-36.3 and 0.42-2.99%, being within the values found in Northwest Moroccan ranges: 29-41, 24-35 and 0-1% (Terrat et al. 2002), French (29.56-42.9, 22.25-42.4 and 0-5.3%) (Devillers et al. 2004) and Spanish (31.9-40.6, 22.7-37.8 and 0.02-1.2%) honeys (Matao & Bosch-Reig 1998). The maximum value of sucrose, present in all honey samples, is below the maximum found in the last studies (Terrat et al. 2002, Devillers et al. 2004 and Matao & Bosch-Reig 1998) and within the limit (<5%) allowed by the European Community requirements (The Council of the European Union, 2002).

3.1.1. *Euphorbia* (*Euphorbia resinifera*, *Euphorbia echinus*) honeys

Euphorbia resinifera and *Euphorbia echinus* are both Moroccan endemic plants, occurring on the slopes of the Atlas Mountains. *E. resinifera* is more widespread in the surroundings of Tadmra-Azilah, while *E. echinus* is more present in the Agadir and Tiznit surroundings. This type of honey is lacking studies of physicochemical properties; *E. resinifera* honey has strong

antimicrobial activity on bacterial strains (Nouman et al. 2004); it has a pungent flavour, very appreciated by Moroccan consumers and highly used in traditional medicine.

Appearance: liquid or crystallised, the colour can be from gold yellow to dark amber.

Taste: sweet, pinch in the throat with a typical light bit back flavour.

The mean value of water content was 17.3%, free acidity 19.93 meq kg⁻¹ and HMF 14.43 mg kg⁻¹. The value of diastase 13.98° Grabe was relatively higher than in the other samples and electrical conductivity showed a mean value of 460 µs cm⁻¹; previous values are within the results found by Narnan et al. (2005). The *E. scabius* honey shows values of colour, free acidity, HMF, diastase and electrical conductivity higher than those of *E. resinifera* honey.

3.1.2. Thyme (*Thymus* spp.) honeys

Appearance: crystallises spontaneously after a few months, the crystals are often irregular, aroma: intense, distinctive, floral and spicy at the same time, the dried flowers, cloves and aromatic herbs. The colour is always more or less amber. **Taste:** Normally sweet with typical spicy flavour.

The antimicrobial activities of this type of honey were similar to those of Euphorbia honeys (Nouman et al. 2004). In the Mediterranean area, the thyme honeys are mainly produced in Greece, Italy, Morocco and Spain (Baccanelli D'Albore, 1998).

In this study, thyme honey shows higher value of electrical conductivity (535 µs cm⁻¹) than the values found in Italian (Perrano Oddo et al. 2000), Spanish (Terrab et al. 2004) and Moroccan (Narnan et al. 2005) honeys; (390, 243 and 295 µs cm⁻¹ respectively).

Water content 16.69% and pH 4.14 were within those found in Italian (Perrano Oddo et al. 2000), Spanish (Terrab et al. 2004) and Polish (Buczekal 2009) honeys, Narnan et al. (2005) in

Moroccan honeys found a relatively high values of water (between 19.89 and 21.8%) and pH (between 4.42 and 4.5), however moisture 18.27 Gofat was very low compared with values found in the same studies (Perrone Oddo et al. 2000, Terrah et al. 2004 and Naman et al. 2005).

3.1.3 Rosemary (*Rosmarinus officinalis*) honeys

Appearance: crystallised a few months after harvest, often fine-grained, color from pale yellow to almost colorless when liquid, white to ivory when crystallised.

Smell: generally weak, not very characteristic, finely aromatic, herb, slightly floral.

Taste: normally sweet. Aroma: light, floral, bitter almonds, not very persistent.

Water content, pH, electrical conductivity (16.37%, 3.98 and 130 $\mu\text{s cm}^{-1}$, respectively) and the sugar content values were within those found in Italian (Perrone Oddo et al. 2000) and Spanish (Males and Bosch-Reig (1998) honeys, however free acidity 10.69 meq kg⁻¹ and moisture 7.37 Gofat showed sensitively lower values than the last studies, on the other hand HMF 23.88 mg kg⁻¹ was higher than the values found in the same studies. Electrical conductivity, pH, moisture, acidity, sucrose and fructose/glucose values were within the values found in Spanish rosemary honey (Pérez-Arquillat et al. 1994).

3.1.4 Orange (*Citrus spp.*) honeys

Orange honey is the most popular honey produced in Morocco, Appearance: often crystallised quickly after production. The colour is pearly light yellow depending on honey crystallisation. Taste: sweet gently acidulous, with fruits and flowers flavour.

This type of honey showed a low HMF content (7.16 mg kg^{-1}) and light colour (26 umpfund). Water content, pH, and free acidity (17.47%, 3.91 and $16.52 \text{ meq kg}^{-1}$ respectively) were within the values found in Italian (Perrano Oddo et al. 2000), Northwest Moroccan (Terrah et al. 2003), Andalusian (Serrano et al. 2004) and Algerian (Chafrouh et al. 2007) honeys.

Sugars and diastase values are low than those found in Italian honeys (Perrano Oddo et al. 2000); the electrical conductivity value, $313 \mu\text{s cm}^{-1}$, was relatively higher than the value found in the previous studies.

3.1.5. *Eucalyptus (Eucalyptus spp.)* honeys

Appearance: usually crystallized. The colour is amber with yellow gray reflections. Taste: normally sweet, with a very peculiar flavour. Light backflavour typically salad; remainder liquorice.

Water content, fructose and glucose (17.01%, 39.37%, 32.62%) were within the values found in Italian (Perrano Oddo et al. 2000), Northwest Moroccan (Terrah et al. 2003) and Andalusian (Serrano et al. 2004) honeys, pH (4.24) was within the values found in Italian and Andalusian honeys and relatively higher than the value found in Northwest Morocco honeys, electrical conductivity value ($768.78 \mu\text{s cm}^{-1}$) was in accord with the last study (Terrah et al. 2003) and higher than the other ones (Perrano Oddo et al. 2000; Serrano et al. 2004).

3.1.6. *Ziziphus (Ziziphus lotus)* honeys

This type of honey is poorly studied; it has been characterized by a high value of electrical conductivity ($673 \mu\text{s cm}^{-1}$). Water content (16.65%) and diastase (15.63° Gøtha), were similar to those found in Pakistani ziziphus honeys (Asif et al. 2002), however pH (4.45), HMF (8.71 mg kg^{-1}) and sucrose (11.61%) are very lower than those found in the last study.

3.1.7. Carob (*Ceratonia siliqua*) honey

The values of water content, pH, free acidity and HMF (18.59%, 4.29, 19.76 mg kg⁻¹ and 17.8 mg kg⁻¹ respectively) agree with the results found by Terrab et al. (2003), the electrical conductivity value (90) $\mu\text{s cm}^{-1}$ is higher than the one found in the last study (679 $\mu\text{s cm}^{-1}$).

3.1.8. Lavender (*Lavandula* spp.) honey

Appearance: normally crystallized. The colour is from very light to amber. Taste: sweet and sour at the same time with a typically bitter backflavour.

This honey type is characterized by its high values of colour, electrical conductivity and free acidity (110.5 mEq/litre, 433 $\mu\text{s cm}^{-1}$ and 30.74 mg kg⁻¹), values higher than those found in French (33.6 mEq/litre, 221.2 $\mu\text{s cm}^{-1}$ and 14.86 mg kg⁻¹) (De-Blere et al. 2004) and Spanish (156 $\mu\text{s cm}^{-1}$ and 14 mg kg⁻¹) (Pérez-Anguita et al. 1995) honeys. On the other hand water content, pH, fructose and glucose are within the values found in the previous studies.

3.1.9. Honeydew honey

Appearance: often remains liquid for a long, dark amber color if rapidly pitch black, becomes when crystallization.

Flavour: medium intensity, vegetable / fruit, cured fruit. Taste: not too sweet, sometimes a little pung, medicine syrup

This type of honey showed the following mean values: water content 14.84%, pH 4.92, free acidity 21.40 mg kg⁻¹, HMF 1.87 mg kg⁻¹, electrical conductivity 1119 $\mu\text{s cm}^{-1}$, diastase 19.1° Gorte and colour 124.5 mEq/litre.

Values of pH, diastase and sugars are higher than those found in Northwest Moroccan honeydew honey (Terrab et al. 2002 and Diaz et al. 2004). Authors in the same studies found

values of water content, free acidity, HMF and electrical conductivity higher than those found in the present study; however water content and pH values are within the values found in Turkish honeys (Kayacier & Karaman 2008).

3.2. Statistical analysis

From the KMO (0.588) and Bartlett ($p < 0.001$) tests it can be concluded that there is a significant intercorrelation between the variables represented by the different analysed parameters, and the data matrix can be proceeding for factorial analysis. In order to classify the ten types of honeys by their physicochemical properties, a standardized PCA was used. From Table 5 it can be concluded that 74.97% of the variation existing in the data can be explained by five factors.

Fig. 1 shows the groups formed by different unifloral honeys. Honeydew honeys are difference from the other honey types; these results agree with Terrah et al. (2002).

Table 6 lists the percentage of the variance explained for each factor. Variables which load highly in the first factor based on acidity, viscosity, HMF and diastase. The variables which correlate highly on second factor are pH, electrical conductivity and lactic acid/free acidity, the addition of the first and second one agree with the result of Sane et al. (1995) who classified honeys basing in their acidity, pH, electrical conductivity, ash, HMF and diastase, however Terrah et al. (2002) established water content, free acidity, lactic acid, and protein as classification factors on the other hand electrical conductivity, free acidity, protein and pH found by Krizan & Zabrowski (1991) as classification factors. From the study of Pons-Cussac et al. (1993) we can conclude that water content and acidity were as classification parameters. The third and fourth factors are formed by sucrose and fructose respectively. The variables that load higher to the fifth factor are related to glucose and water content.

In order to test the homogeneity of covariance matrices the Box's M Test was used, it was significant, it be concluded that the covariance matrices of the group differ.

The variables selected by stepwise discriminant analysis were pH, lactone acidity, total acidity, diastase, electrical conductivity and colour. This fact is corroborated by the Wilks test being significant ($p < 0.001$). The ten samples were 87% correctly classify.

4. CONCLUSION

The types of honey analysed constitute the main Morocco melliferous production. *Euphorbia echinata*, *Euphorbia hirta* and lavender Moroccan honeys have been investigated for the first time about their physicochemical parameters.

In general, the results of the present study were within those found in previous studies about physicochemical properties of Moroccan honeys (Tarrab et al. 2001; 2002; 2003a; 2003b; 2003c; Diaz et al. 2004 and Naimen et al. 2005).

The cumulative variance is approximately 74.56%, showing that the ten honey types are not well distinguished by their physicochemical parameters. Microscopological analyses, being the best method to distinguish honey botanical and geographical origin and to obtain their characterization (Ferrero & Ferracis, 2008), will be applied to the examined samples, but physicochemical parameters are very important to determine the honey quality.

As regard to the basic parameters warrant the honey quality, water content and HMF previously comply with values proposed by Bogdanow et al. (1999) and The Council of the European Union (2002); a deeper professional education would be necessary to promote Moroccan beekeepers vocational training.

The knowledge of physicochemical features of Moroccan honeys is very important in order to set up certification marks and improve the local beekeeping, also for a possible export.

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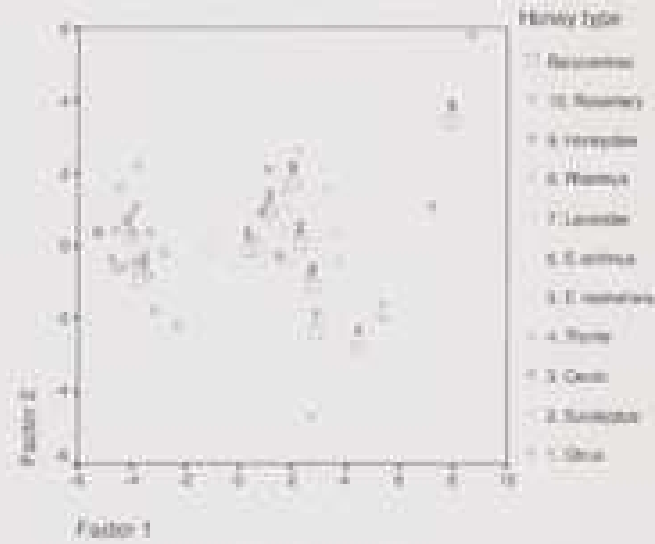


FIG. 1. PLOT OF THE FIRST FACTOR VERSUS SECOND FACTOR, FOR CLASSIFICATION OF TEN UNIFLORAL HONEYS

TABLE I.
UNIFLORAL HONEY SAMPLES

N Samples	Honey Type	Production region	Production year
1	Euphorbia schima	Senou Massou	2005
1	Euphorbia schima	Senou Massou	2006
2	Euphorbia schima	Senou Massou	2007
1	Euphorbia musifera	Tadla Azzilal	2006
9	Euphorbia musifera	Tadla Azzilal	2007
1	Citrus	Senou Massou	2007
3	Citrus	Tadla Azzilal	2007
2	Citrus	Tadla Azzilal	2008
2	Citrus	Temsit Al Haouz	2006
6	Citrus	Temsit Al Haouz	2007
1	Citrus	Temsit Al Haouz	2008
1	Eucalyptus	Chaouia	2005
1	Eucalyptus	Gharb	2007
1	Eucalyptus	Oriental	2007
1	Eucalyptus	Tadla Azzilal	2007
3	Eucalyptus	Temsit Al Haouz	2007
2	Carob	Gharb	2007
1	Carob	Tadla Azzilal	2006
2	Thyme	Gharb	2007
1	Thyme	Tadla Azzilal	2007
1	Lavender	Gharb	2007
1	Lavender	Fallahet	2007
1	Ziziphus	Gharb	2007
1	Ziziphus	Oriental	2007
1	Ziziphus	Senou Massou	2007
1	Ziziphus	Temsit Al Haouz	2007
1	Rosemary	Oriental	2007
1	Rosemary	Gharb	2007

TABLE 2.
MULTIFLORAL AND HONEYDEW HONEY SAMPLES

N Samples	Honey Type	Production region	Production year
2	Multifloral	Chaonia	2006
1	Multifloral	Chaonia	2007
2	Multifloral	Ghazb	2007
1	Multifloral	Oriental	2007
2	Multifloral	Tadla Azilal	2007
1	Multifloral	Tadla Azilal	2008
3	Multifloral	Tafilalet	2006
1	Multifloral	Tafilalet	2007
3	Multifloral	Tensift Al Haouz	2006
1	Multifloral	Zacr	2007
1	Honeydew	Tensift Al Haouz	2007
1	Honeydew	Oriental	2007

TABLE 5.
TOTAL VARIANCE EXPLAINED

Component	Initial Eigenvalues			Fraction sums of squared loadings		
	Total	% of variance	Cumulative %	Total	% of variance	Cumulative %
1	3.93	27.17	27.17	3.93	27.17	27.17
2	2.41	18.53	45.70	2.41	18.53	45.70
3	1.46	11.22	56.92	1.46	11.22	56.92
4	1.28	9.85	66.78	1.28	9.85	66.78
5	1.06	8.19	74.97	1.06	8.19	74.97
6	0.88	6.74	81.70			
7	0.79	6.06	87.77			
8	0.57	4.39	92.15			
9	0.49	3.76	95.91			
10	0.35	2.72	98.63			
11	0.16	1.22	99.85			
12	0.01	0.10	99.95			
13	0.01	0.05	100.00			

TABLE 6
COMPONENT MATRIX

	Component				
	1	2	3	4	5
Total acidity	0.927	-0.245	0.197	0.094	-0.052
Free acidity	0.920	-0.109	0.292	0.109	-0.131
Lactone acidity	0.724	-0.550	-0.171	0.033	0.209
Color	0.666	0.378	-0.347	-0.115	0.144
HMF	0.532	-0.263	-0.171	-0.221	0.295
Diastase	0.525	0.324	-0.262	0.448	-0.039
pH	0.067	0.870	-0.231	-0.174	0.001
Electrical conductivity	0.409	0.595	-0.082	-0.539	0.008
Lactone acidity / Free acidity	0.342	0.563	0.558	0.050	-0.406
Sucrose	-0.003	0.128	0.751	-0.042	0.320
Fructose	-0.009	0.270	-0.032	0.734	-0.005
Glucose	-0.062	0.198	0.354	-0.010	0.656
Water content	0.087	-0.401	0.161	-0.363	-0.441