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Research Paper

Physicochemical characterization of honeys sold in the city of Bukavu and the those obtained from production sites in the province of south Kivu in the Democratic Republic of Congo.

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ABSTRACT
Honey is an energy food that humanity has known since the dawn of time. A complex, extremely rich product, has many cosmetic, nutritional and therapeutic properties. Our study is carried out on seven samples of honey harvested in the province of south kivu respectively in its different territories and in its city of Bukavu in order to characterize their physicochemical properties. Eight physicochemical parameters were analyzed : density, pH, water content, electrical conductivity, sugar content, refractive index, polyphenol content, flavonoid content. The results showed significant differences in the physicochemical composition between the various honey samples analyzed. Whit the exception of samples E05 and E06 respectively taken in the commune of Kadutu and commune of Bagira, all other honey samples comply with the established quality standards.

Keywords: Honey, south kivu ; physicochemical characteristics

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I. INTRODUCTION

Hive products include honey, pollen, royal jelly, propolis, beeswax and bee bread. They have always attracted a lot of interest due to their therapeutic and dietary properties. Nowadays, the use of these products by humans for medicinal purposes constitutes a field of study in its own right, which is apitherapy (Fratellone *et al.* 2016). Among these products, honey and pollen are the most studied and used. Thus, honey has been exploited by humans for thousands of years. It has always been appreciated, on the one hand for its taste qualities and on the other hand, for its many therapeutic virtues demonstrated repeatedly by scientific studies. Its empirical uses have spanned the centuries but the advent of modern chemistry has gradually caused them to fall into oblivion (Jones, 2009).

As for pollen, it represents the most valuable product of the hive because it is the main food of the bee. It is rich in nutrients and active substances and is very energetic due to its high content of carbohydrates and proteins. It contains all the essential amino acids that the body cannot synthesize and therefore perfectly compensates for the deficiencies imposed on us by our unbalanced modern diet (Campos et al 2010).

Honey is described as a sweet substance of natural origin, produced by honey bees from flower nectar or honeydew (Codex Alimentarius, 2001). Its composition depends mainly on several elements such as its geographical origin, the season, the health of the colony, the environmental conditions and the beekeeping practices used (El-Metwally, 2015); Leite et al, 2000).

In nutritional terms, honey is a first-class food, with high energy value and certain therapeutic properties. Honey is a living product that undergoes a number of changes over time, leading to the loss of its essential qualities (Amrouche and Kessi, 2003). In addition, the composition of honey depends on the plant species, the climate and the contribution of the beekeeper (Anklam, 1998). Honey from a single-specific flora is more valuable than that from a multi-specific flora. Notwithstanding that the bio-physicochemical characteristics of honey depend on the factors listed above, the falsification of honey by adding water or any

other foreign substance and the dynamics of its properties, which depend on the duration and conditions of storage, are concerns that animate both producers and consumers and affect the nutritional and therapeutic value of honey. However, it should be noted that a significant portion of this product is sold through informal channels without quality control (Moujanni, 2017). This situation calls into question the authenticity of the product and makes it subject to risks of deterioration. And this can lead to a reduction in certain nutritional and medicinal benefits compared to pure honey (Aljohar *et al*., 2018). In this context, the present study aims to analyze the physicochemical composition of seven samples of honey produced in the province of South Kivu, in order to verify their compliance with the standards established by the food code.

II. METHODOLOGY

Collection and extraction of honey samples

This study focuses on seven honey samples from different territories and the city of Bukavu in the province of South Kivu in the DRC, including 4 honey samples from 4 territories, namely the Idjwi, Walungu, Kabare and Kalehe territories (samples collected from large beekeepers) and 3 others from honey sellers in the city of Bukavu at a rate of one sample per commune (Table 1). The extraction of all honey samples collected from beekeepers was carried out under manual pressure and the collected honey was directly filtered using a strainer to remove debris, and transferred to sterile glass jars hermetically sealed and taken directly to the laboratory where they were stored at 4°C in the dark until analysis (Bogdanov, 2001).

Table 1, Description of the honey samples studied

N° sample	Code	Presumed floral origin	Mode extraction	Origin geographical	Harvest Period
01	E01	Multi-floral honey	Manuel	Kabare territory	November 2024
02	E02	Multi-floral honey	Manuel	Ibanda Municipality	November 2024
03	E03	Multi-floral honey	Manuel	Idjwi territory	November 2024
04	E04	Multi-floral honey	Manuel	Walungu territory	August 2024
05	E05	Multi-floral honey	Manuel	Kadutu Municipality	August 2024
06	E06	Multi-floral honey	Manuel	Bagira Municipality	August 2024
07	E07	Multi-floral honey	Manuel	Kalehe territory	August 2024

PHYSICOCHEMICAL ANALYSES

In order to establish a similarity of our honey samples to international standards, we analyzed the most used physicochemical parameters as indicators of honey quality and stability. To do this, the analysis methods established by the Codex Alimentarius Commission as well as those of the International Honey Commission (Ibrahim Khalil *et al*., 2012; Codex Alimentarius, 2001) were used.

pH

The pH was measured according to Codex No. 77-79, (Codex, 1977) on a 10% (w/v) honey solution, using a pH meter calibrated before use using buffer solutions of 7.

Water content

The water content of the honey was measured using a refractometer. The honey to be analyzed was previously well homogenized by shaking. The device provides a direct reading of the index relative to the "D" line of Sodium, but it is possible to operate in natural light or an ordinary lamp to read the value (Younes-Chaouch and Bounsiar, 2018).

Density

The density was determined using a 10 ml pycnometer according to the AFNOR method (1984).

Electrical conductivity

The electrical conductivity was measured using an electrical conductivity cell for a honey solution at 20% (w/v) dry matter and then read the conductance (in milli Siemens) at a temperature of 20 ° C. (International Honey Commission, 2009).

Refractive index

The refractive index of honey was determined using the refractometer. The honey analyzed was previously well homogenized as described by Bogdanov, *at al* (2002).

Total polyphenol content

The determination of total polyphenols was carried out according to the Folin-Ciocalteu colorimetric method (Singleton *et al.* 1999). The amount of polyphenols for each sample was determined by projecting the optical density value at 725 nm on a standard curve of a standard polyphenol (gallic acid) carried out under the same conditions. The total polyphenol content was expressed in mg EAG/100 g of honey.

Flavonoid content

The determination of total flavonoids was carried out according to the method of Arvouet-Grand, *et al*. (1994). The reagent used is a colorless solution of aluminum trichloride ($AlCl_3$ at 20%; w/v). The principle of the method is based on the oxidation of flavonoids by this reagent, leading to the formation of a brownish complex with maximum absorption at 51 0 nm. Flavonoid content was expressed in mg EC/100g.

Sugar content

Sugar content (fructose, glucose, sucrose, and maltose) was performed in duplicate using high performance liquid chromatography (HPLC) with a Scan Type: RID detector. An APS2-HYPERSIL 250X4.6 column with positive polarity was used. The injection volume was 10 µl. at a temperature of 30°C and a flow rate of 1.0 ml/min. (MEHDI; 2016)

STATISTICAL ANALYSIS

The results obtained were presented in the form of tables and graphs.The calculation of means, standard deviations. The PAST software was used for this.

III. RESULTS AND DISCUSSION

The results of the analyses for the physicochemical parameters (Density, pH, Water content, Electrical conductivity, Refractive index, Total polyphenol content and Flavonoid content) are presented in Table 2 while those for the sugar content are shown in Table 3. Each analysis was carried out in three replicates and the results are reported using the mean ± standard deviation.

pH

The pH of our samples ranges from 3,79 to 6,56 with a mean of 5,166±0,96 (Table 2). The results of samples E01, EO2, E03, E04 and E07 are within the range given by White and Louveaux (1962), who report that the pH of honey varies from 3,42 to 6,10, while samples E05 and E06 are not within the normal range set by the Codex Alimentarius (2001). However, the variability of the pH of honeys is thought to be due to the flora foraged, the salivary secretions of the bee and the enzymatic and fermentative processes during the transformation of the raw material (Louveaux, 1968a). The pH is a measurement that allows the determination of the floral origin of the honey; honeys from nectars have a pH between 3,5 and 4,5, while those from honeydew have a pH between 5 and 5,5 (Gonnet, 1986). Intermediate values often correspond to mixtures of nectar and honeydew as in the case of sample E01 with a pH of 4,741. We note that samples E02, E03, E04 and E07 have a pH not exceeding 4,5; they are therefore honeys from nectars, i.e. flowers.

Water content

The water content of the honeys studied varies from 14,65 to 24,2% with an average of 18,464±3,608 (Table 2). Samples E01, E02, E03, E04 and E07 have values that are well within the standard recommended by the Codex Alimentarius (1993), which recommends a value not exceeding 21% in general, unlike samples E05 and E06, with values of 22.66 and 24,2% respectively, which are not within the standards. The water content is a very important piece of information to know, because it determines the quality of the honey. Indeed, only honeys with a water content of less than 17% are stable during storage and are not at risk of fermenting (Bogdanov *et al*., 2006).

Honey is an aqueous solution; however, microorganisms need water to develop. Too much water in honey constitutes an environment

favorable to the proliferation of these microorganisms: a fermentation phenomenon then occurs (Hoyet, 2005).

The high water content values recorded for samples E05 and E06 can be explained by:

Adding water to the natural product, a practice observed among some sellers to maximize their income. An early harvest of this honey, that is, before its maturation (Cailla, 1927 and

Prost, 1972). Extraction of the honey in a fairly humid environment, which resulted in moisture absorption. According to Bruneau (2005), honey has the capacity to absorb moisture from the air when it is greater than 55%. This will result in rapid fermentation and very often it will undergo defective crystallization making the product unstable (Gonnet, 1993).

Density

The density of the samples studied varies from 1,01 to 1,51 with an average of 1,351±0,214 (Table 2). These results are in accordance with the standard recommended by the food code which is 1,39 to 1,52. With the exception of samples E05 and E06 which have the lowest values of 1,01 and 1,07. This can be explained by their high water contents (22,66% and 24,2%). According to Louveaux (1985), variations in the density of honeys mainly come from variations in water content, a honey harvested prematurely, less ripe will have a lower density. In general, the richer a honey is in water and the less dense it is and vice versa.

Electrical conductivity

The electrical conductivity values of our honey samples vary between 0,493 and 1,21 mS/cm with an average of 0,773±0,283 (Table 2). Samples E01, E02, E03, E04 and E07 comply with the permitted standard of the Food Codex (≤ 0.8 mS/cm). Samples E05 and E06, respectively 1,21 and 1,11 mS/cm, present high values of electrical conductivity that are outside the standards. This can be due either to the involuntary or voluntary addition of mineral substances or other contaminants that can increase the conductivity; a high humidity level in honey can promote the dissolution of minerals and also increase the conductivity. Electrical conductivity is a good criterion for determining the botanical origin of honey (Bogdanov *et al.,* 2004). It provides a valuable indication in defining an appellation.

Refractive index

These results show that the honeys studied have a refractive index varying between 1,476 and 1,5002 (Table 2) with an average value of 1,4902 ± 0,0092. The highest refractive index value is observed in the E03 honey sample, and the lowest in the E06 sample. However, all these refractive index values are within the normal range set by the Codex Alimentarius (2001) which is generally between 1,47 and 1,5. The refractive index makes it possible to determine a very important variable, the water content, much more quickly than for other methods (EMMANUELLE, 1996).

Total polyphenol content

The values of the total polyphenol contents vary from 6,971 to 73,2 mg of Galic Acid Equivalent /100g of honey. The average polyphenol content of our samples is 41,525 ± 23,93 mg EAG/100 g of honey (Table 2). These results are similar to those obtained by Ibrahim Khalil et al. (2012) on 14 honey samples from Tualang in Malaysia. They are close to those obtained by Buba *et al*., (2013), on 18 honey samples obtained from the northeastern sub-region of Nigeria. Many other previous works on the composition of honey in polyphenols have shown variable contents depending on the region and the botanical origin of honey. The work carried out by Ibrahim Khalil et al. (2011) on honey from tropical regions indicates that the average of phenolic compounds is 166,97 ± 3,12 mg EAG/Kg. However, there are some differences between our results and those observed in

Chilean honey, with a total phenolic content ranging from 0,0 to 8,83 mg EAG/100g of honey (Muñoz and Copaja, 2007). Another study on 5 samples of Yemen honey shows total polyphenol contents ranging from 56.32 to 246,21 mg EAG/100g (Al-Mamary *et al*., 2002). While honey samples from Slovenia have contents ranging from 44,8 to 241 mg GAE/100g of honey (Bertoncelj *et al*., 2007). Phenolic compounds are secondary metabolites of plants. They exist in the form of a significant diversity and belong to various chemical groups (alkaloids, terpenes, flavonoids, etc.). Polyphenols present in honey are mainly flavonoids (quercetin, luteolin, kaempferol, apigenin, chrysin, galangin), phenolic acids and phenolic acid derivatives (caffeic acid, coumaric acid, ellagic acid, gallic acid). Heat treatments can influence these polyphenolic compounds and alter the quality of honey, in particular by increasing the concentration of Hydroxy-methul-furfural (HMF) (Amiot *et al*., 1989).

Flavonoid content

Flavonoid contents range from 7,11 to 13,5 mg EC/100g of honey, with an average of 11,192±2,4385 mg EC/100g (Table 2). The flavonoid content of seven honeys from the South Kivu province is similar to those obtained by Perna et al. in 2012. The latter indicate close values in total flavonoids ranging from 5,09 to 14,05 mg EC/100g of honey for 78 samples from southern Italy. A later study shows that the flavonoid content of honeys from Chile ranges from 0,014 to 13,8 mg EC/100g of honey (Muñoz and Copaja, 2007). However, our results are higher than those obtained by Ibrahim Khalil *et al.* (2012) on Tualang honeys where flavonoid contents range from 4,02 to 8,64 mg EC/100 g of honey. Also, the flavonoid content of honeys from Burkina Faso was from 0,17 to 8,35 mg EC/100 g of honey, while the values for those from Rio de Janeiro and Minas Gerais in Brazil range from 2,94 to 10,91 mg EC/100 g of honey (Pérez-Pérez *et al*., 2013). This variation in flavonoid content in honey from one region to another in the world suggests that the environment of the hive is a determining factor on its composition. The flavonoid content was higher in samples produced during a dry season with high temperatures (Kenjeric *et al*., 2007), this is the case for samples E04 (honey sample from Walungu territory) and E07 (honey sample from Kalehe territory) which recorded high flavonoid content values (i.e. 13,5 and 13,01 mg EC/100g of honey). The phenolic and flavonoid compounds contained in honey are responsible for its odor. The majority of these molecules come from the plant. However, some are added to the honey by the bee itself. Among these molecules, we find the large families of polyphenols and flavonoids. Thus, phenolic substances intervene, more or less directly, on the color through flavonoids likely to contribute to the yellow coloring (Harbone and Smith., 1978); which is the case among the honeys studied, samples E04 and E07 with a yellow color and a high content of flavonoids, i.e. 13,5 and 13,01 mg EC/100g of honey.

Table 2: values of the physicochemical parameters of honey studied

Physicochemical parameters	Honey samples							Statistical analysis	
	E01	E02	E03	E04	E05	E06	E07	M	σ
pH	4,741	4,043	3,79	4,17	6,41	6,56	4,251	5,166	0,961
Water content (%)	16,16	15,8	14,65	17,91	22,66	24,2	17,87	18,464	3,608
Density	1,48	1,48	1,51	1,45	1,01	1,07	1,46	1,351	0,214
EC (µS/cm)	0,493	0,671	0,8	0,59	1,21	1,11	0,54	0,773	0,283
Refractive index	1,4961	1,4971	1,5002	1,4915	1,479	1,476	1,492	1,4902	0,0092
Polyphenol content (mg EAG/100 g)	48,62	53,4	73,2	52,54	6,971	11,257	44,688	41,525	23,93
Flavonoid content (mg EC/100g)	12,11	12,58	11,58	13,5	7,11	8,46	13,01	11,192	2,4385

Sugar content

Four sugars were determined in our honey samples; including two monosaccharides (fructose and glucose) and two disaccharides (sucrose, maltose) (Table 3).

The fructose content ranges from 15,4 to 37,6% with an average of 27,071%±8,106 and the glucose content of our samples ranges from 20,3 to 25,1% of honey sugars with an average of 22,585%±2,081. According to Codex Alimentarius (2001), the sum of fructose and glucose content (sum of both) of honeydew honey, mixtures of honeydew honey and nectar honey, should be at the limit ≥45%. In our study, the sum of the fructose and glucose contents of samples E01, E02, E03, E04 and E07 are within the standards while those of samples E05 and E06 are outside the standards.

The sucrose content varies between 0,01 and 39,8% with an average of 12,57%±18,083. According to the standards, the sucrose content of honey must be at the limit ≤ 5% (International Honey Commission, 2002). In this study, the sucrose contents of samples E01, E02, E03, E04 and E07 are within the standards while those of samples E05 and E06 are outside the standards.

The maltose content of our samples varies between 0,01 and 2.3% with an average of 0,85%±8,082. According to Codex Alimentarius (2001), the maltose content of honey varies between 0 and 5,4% for multi-floral honeys. In our study, the maltose contents of all our samples are within the standards. These results are the same as those of Makhloufi *et al*., (2010) and those of Ouchemoukh, *et al*., (2010). Fructose is the main sugar in honey samples followed by glucose, then sucrose and maltose with lower values. In reality, the composition of honey sugars depends strongly on the type of flowers visited by bees, as well as the foraging region and climatic conditions (Ouchemoukh *et al.*, 2010). The presence of glucose and fructose is the result of the action of an enzyme on sucrose: invertase. The presence of other sugars seems to depend on the plants that have been foraged (Hoyet, 2005).

Table 3: Sugar content of honey studied

setting	Honey samples							Statistical analysis	
	E01	E02	E03	E04	E05	E06	E07	M	σ
Fructose	33,6	25,8	37,6	32,3	15,4	18,4	26,4	27,07	8,106
Glucose	20,5	20,3	25,1	23,7	23,5	24,5	20,5	22,585	2,081
Sucrose	2,7	2,3	3	0,01	38,2	39,8	2,01	12,574	18,083
Maltose	0,24	0,18	0,01	2,01	2,3	1,01	0,18	0,847	8,082

IV. CONCLUSION

This initiative is part of a contribution to the valorization of honey from the region. The study of physicochemical parameters is a good criterion of honey quality, often used in routine control. The results on the physicochemical characterization of certain samples of honey from the province of South Kivu were found to comply with all international quality requirements described by the Codex Alimentarius and the European Union. The main parameters representative of the quality and good conservation of honey, namely: pH, water content, electrical conductivity, density, refractive index, polyphenol content, flavonoid content and sugar content were found to be within the regulatory limits with the exception of samples E05 and E06, respectively the honey samples taken in the commune of Bagira and in the commune of Kadutu.

Future studies will be considered to analyze other physicochemical and microbiological parameters of honey produced in the region. In order to preserve the nutritional and therapeutic quality of this product, it will be important to implement a quality control of honey before its marketing.

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