

Antioxidant activity evaluation and the physico-chemical composition of some Algerian commercial coffees (threatened environment)

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ARTICLE INFO

Article History:

Received : 24/01/2021

Accepted : 23/09/2021

Key Words:

Coffee powder; Antioxidant activity; Sugars; Food fraud; Algerian trade.

ABSTRACT/RESUME

Abstract: The use of different mixtures of products and the addition of cheap raw materials such as chicory, corn and soy alter the sensory and functional characteristics of the beverage based on roasted and ground coffee beans, defects that call into question the quality of the beans imported by the producing countries in Algeria and threaten the environment through the quantities of coffee grounds produced. The objective of our study is the detection of fraud on coffee beans, the differentiation between pure coffee and coffee with sugars, the determination of the antioxidant power of some coffee powders selected in the Algerian trade, and finally to select the best consumable coffee. The physico-chemical analysis of the coffee extracts, gave an acid pH, which varies between 5.71 and 6.19, a moisture content not exceeding 5%, an ash content varying between 4.38 and 5.52%, polyphenols between 11.54 and 34.32 (mg GAE/g MS), an antiradical activity between 26.33 and 81.77% and total sugars varying between 1.156 and 4.38%. At the end of this study, the choice of consumption was made for a type of coffee with superior physico-chemical characteristics and the use of sugar content for their formula below the permitted limit, which results in a low level of regenerated acrylamide with an unpolluted environment.

I. Introduction

The use of synthetic antioxidant molecules is currently being questioned due to potential toxicological risks. New plant sources of natural antioxidants are now being sought [1,2]. Indeed, polyphenols are natural compounds that are widely distributed in the plant kingdom and are becoming increasingly important, particularly due to their beneficial effects on health [3]. Their role as natural antioxidants is attracting growing interest in the prevention and treatment of cancer, inflammatory and cardiovascular diseases [4]. They are also used as additives in the food, pharmaceutical and cosmetics industries [1]. Scientific research has been developed for the extraction, identification and quantification of these compounds from

different sources such as agricultural and horticultural crops or medicinal plants [5, 6].

Coffee is the second most important commodity in international trade after oil. It is one of the oldest existing crops, is one of the most popular beverages in the world, consuming more than 400 million cups per year. The coffee drink can be obtained from blends of roasted and ground coffee beans, both *Coffea arabica* (*C. arabica*) and *Coffea canephora* (*Coffea arabusta*) [7, 8]. The quality of the coffee drink and its sensory properties depend on the form of production [9]. In addition to environmental factors and the harvesting process, the technological processes of roasting and grinding influence the characteristics of the final product [10].

Coffee consumption is very popular in Algeria, but the types of coffee drinks and the modality of consumption are strictly associated with the social habits and cultures of the different regions. Differences in the composition of green beans, roasting conditions and extraction procedures adopted for the preparation of coffee infusions lead to a great diversity in the chemical composition of the final product [11]. Green coffee beans are rich in phenolic compounds and polysaccharides, which undergo deep molecular changes during roasting [12, 13]. The low water activity and high temperature favour the development of the Maillard reaction (MR), with the formation of products between proteins and carbohydrates [14]. When coffee is roasted, it is likely that phenolic compounds also participate in the reaction, forming part of the brown, water-soluble polymers known as melanoidin coffees [15]. Algerians have become easy prey to all carcinogenic products. This is alarming in a context of total impunity for food producers due to the absence of regular controls by the authorities, despite the legislative measures put in place in recent years. Added to this is the lack of awareness among Algerian consumers. Indeed, several national coffee brands do not comply with the standards required by the legislation, said the president of the Algerian Organisation for the Protection and Orientation of the Consumer and his Environment (APOCE). Submitted by the latter to three different Algerian laboratories, the analyses concerned the additional sugar content in coffee and its regulatory labelling for twelve locally produced coffee brands [16]. Thus, the lack of conformity concerns either the high sugar content or the failure to mention the added substances and their levels on the packaging as required by the Executive Decree of February 2017 establishing the characteristics of the coffee and the conditions and modalities of its release for consumption [16]. According to article 19 of the law, producers who incorporate sugar, caramel or starch during the roasting process must visibly and indelibly indicate the name "coffee roasted with sugar" or "ground coffee roasted with sugar". The decree also stipulates that the level of additional matter must not exceed 3% of the final product [17].

II. Materials and methods

II.1. Biological material

Our study covered eight (08) brands of coffee produced in Algeria plus two other samples of roasted and ground Robusta and Arabica coffees, purchased at different points of sale and coded as follows (Table 1).

Table 1. Coffee samples Coding

N°	1	2	3	4	5	6	7	8	9	10
Code	AR	RO	CB1	CF1	CNe	CFac	CB2	C10	CEH	CAr

II.2. Physicochemical analyses

The analyses carried out for the physicochemical characterisation of the coffees are: pH, humidity, total soluble matter, ash, determination of polyphenols, determination of antioxidant and antiradical activity, IC50 and determination of total sugars.

II.2.1. pH determination

The pH coffee samples were determined according to the method described by Ramalakshmi et al., (2000) [18]. A quantity of 03 g of ground coffee was added to 50 mL of hot water. After filtration the extract was cooled to room temperature and the pH was measured using an AD1000 pH/mV/ISE/Temperature Bench Meters.

II.2.2. Moisture content

The moisture content of the coffee samples was determined gravimetrically in the oven at 105 °C and expressed as a percentage by mass [19].

II.2.3. Total soluble matter (TMS)

The total soluble solids of the coffee powder sample were determined by reflowing the coffee powder (2 g) with hot water (200 mL) for 1 h and making up to 500 mL. An aliquot (50 mL) was evaporated to dryness and then heated in a hot air oven at 105 ± 2°C to obtain simultaneous weights and the amount of total soluble solids was calculated [20].

II.2.4. Ash content (AC)

For the Ash determination of the roasted ground samples, about 2 g of sample was weighed into a porcelain capsule and placed in a muffle furnace to be incinerated at 550°C until the organic matter was removed. At the end of the incineration process, the samples were weighed. The results are expressed in percentages [21].

II.3. Extracts preparation

A quantity of 01 g of roasted and ground coffee sample was added to 40 mL of methanol/water (50:50). The pH of this solution is then adjusted to pH 2.0 with a solution of 0.1M HCl acid (Art-chemistry 36%). After stirring for 01 h and centrifugation at 2500g/10min, the supernatant was recovered (extract 01) and the residue added to a volume of 40 mL of acetone/water solution (70:30). The extraction is thus repeated in the same way as for extract 01 (agitation for 01 h followed by centrifugation at 2500g/10min) the recovered supernatant represents extract 02. The mixture of the two extracts (1 and 2) represents the final extract used for the different dosages [22].

II.3.1. Total polyphenols determination

Folin-Ciocalteu's reagent, a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and of phosphomolybdic acid ($H_3PMO_{12}O_{40}$) (Sigma Aldrich chemical) is reduced in the presence of

polyphenols to a mixture of blue oxides of tungsten (W_8O_{23}) and molybdenum (Mo_8O_{23}). The blue coloration produced is proportional to the level of phenolic compounds present in the reaction medium [23]. The total polyphenol content was evaluated according to Vignoli et al., (2011) [24] with some modifications, a volume of 0.1 mL of extract was mixed with 0.3 mL of Folin Ciocalteu reagent, then 0.1 mL of 10% sodium carbonate (Na_2CO_3) solution (Merck, 99.2%) was added. The absorbance is read at 765 nm using a spectrophotometer branded UV-1800 SHIMADZU, Japan.

II.3.2. DPPH free radicals scavenging activity

The principle of the DPPH method is that the antioxidants react with the stable free radical (α -diphenyl- β -picrylhydrazyl) in a dark purple colour and reduce it to α -diphenyl- β -picrylhydrazine, which results in a purple discoloration [22, 25].

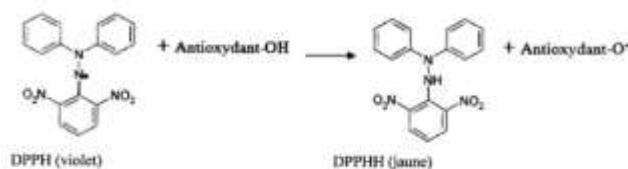


Figure 1. DPPH action.

The determination of the antioxidant activity of DPPH was carried out according to the method used by Madhava Naidu et al., [26], 100 μ L of extract are mixed with 3 mL of DPPH solutions (0.1mM). The absorbance is measured at 517 nm. The results are expressed as % inhibition of the DPPH radical against a control, according to the following formula:

$$\text{DPPHscavenging effect (\%)} = \frac{(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}})}{(\text{Abs}_{\text{Control}})} \times 100$$

Abs: Absorbance.

II.3.3. IC50 Calculation

The IC50 values were determined graphically from logarithmic regression curves of inhibition percentages versus coffee extract concentrations.

II.3.4. Total sugars determination (TS)

The total sugars extraction (Figure 2) was carried out using the Chow and Landhaiser method [27].

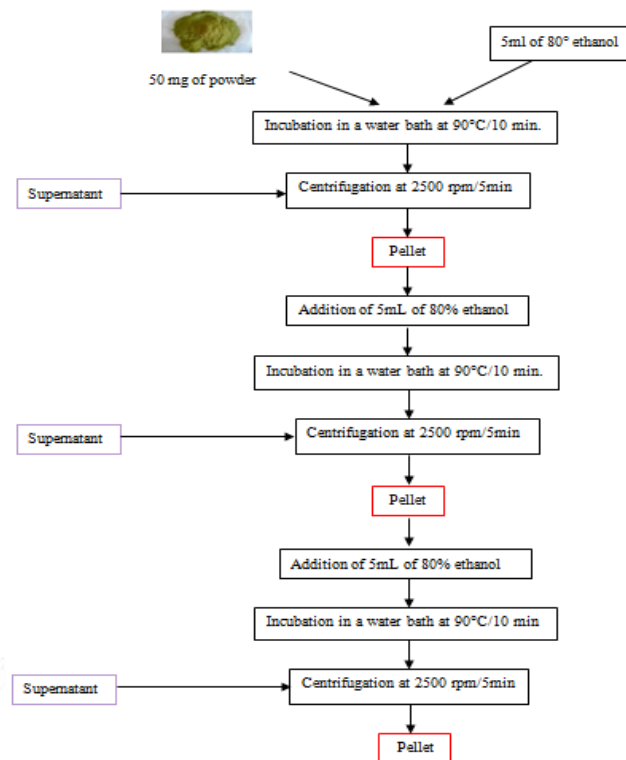


Figure 2. Protocol for the determination of total sugars.

The determination of total sugars is carried out according to the method of Dubois et al., (1956) [28]. In the presence of phenol and concentrated sulphuric acid, the monosaccharides give a yellow-orange colour whose intensity is proportional to the carbohydrate concentration. The optical density is determined at 490 nm [29]. A volume of 1 mL of the supernatant was mixed with 1 mL of phenol (5%), followed by 5 mL of sulphuric acid (98%). The mixture is stirred to homogenise the contents. After 30 min incubation in the dark, the absorbance of the solution is determined at 490 nm against a blank. The sugar content is related to a standard range obtained with glucose used as standard. The results are expressed in mg equivalent of glucose per g of sample (mg Eq/g).

II.4. Statistical analyses

The statistical analyses of the various data were carried out using the software XLSTAT 14. A single criterion analysis of variance (ANOVA 1) was performed and followed by the LSD Fisher test. The significance level was evaluated at the 5% threshold. A Principal Component Analysis (PCA) and a hierarchical ascending classification were carried out in order to highlight a group structure according to physico-chemical parameters.

III. Results and Discussion

III.1. Physicochemical parameters

All the samples analysed (Table 2) are acidic. The pH values range from 5.71 for CEH coffee to 6.19 for CNe coffee. The coffee samples studied have pH values higher than 5, which is not in conformity with the standards, this may be due to various parameters such as the purity of the sample, the duration and conditions of conservation and storage. Our values correspond to the values reported in the literature [30]. With regard to the moisture content in coffee powder produced in Algeria, it has been estimated at between 1.20% for CF2 coffee and 3.60% for CF1 coffee, which is important in terms of quality. All these results are in correlation with Executive Decree No. 92-30 of 20 January 1992 relating to the specifications of coffees which show that ground coffee should not have a moisture content higher than 5%. The results obtained are well below the levels reported by Mazzafera, (1999) [30] and Clarke, (1985) [31]. With regard to soluble matter the variation in the level from one sample to another was highly significant and the highest values were observed in the CF1 and CNe samples with values of 20.3 and 19.6%, respectively. Our values are lower than those obtained by Ramalakshmi et al., (2007) [32] on green coffee samples with a range of 29-34%. Determination of the ash content can give us information on the quality of the sample to be analysed. Indeed, only low ash contents of products are acceptable for human consumption. The evaluation of the coffee ash content gave a rate between 4.38% for Arabica coffee and 5.52% for C10. According to these results and to Executive Decree No. 92-30 of 20 January 1992 on the specifications and presentation of coffees, which states that roasted coffee for consumption should not contain more than 6%, our values obtained are in conformity with the standard and slightly higher than those obtained by Kouadio et al [33], who worked on robusta coffee according to the terroirs and cultivation techniques in Ivory Coast and found values ranging from 3.77 to 4.16%.

Table 2. Physico-chemical parameters values.

Coffee type	pH	Moisture (%)	Soluble matter(%)	Ash (%)
RO	5.79± 0.32	2.42 ± 0.18	7.31 ±1.23	5.11 ± 0.18
CF2	6.06 ± 0.21	1.21 ± 0.06	13.62 ± 1.34	5.53± 0.23
CNe	6.19 ± 0.07	1.60 ± 0.35	19.63 ± 2.17	4.82 ± 0.31
CF1	5.93 ± 0.19	3.61 ± 0.33	20.32 ± 2.29	4.55 ± 0.11
CB1	5.91 ± 0.11	1.38 ± 0.10	18.23 ± 2.08	5.50 ± 0.41
AR	5.89 ± 0.14	2.48 ± 0.20	7.95 ± 1.34	4.38 ± 0.25
CAr	6.04 ± 0.27	1.78 ± 0.27	11.15 ± 1.56	4.65 ± 0.10
CB2	6.09 ± 0.24	1.45 ± 0.39	11.84 ± 1.21	4.52 ± 0.25

C10	5.87 ± 0.27	2.00 ± 0.23	16.72 ± 1.48	5.52 ± 0.23
CEH	5.71 ± 0.18	2.01 ± 0.53	12.76 ± 1.38	5.31 ± 0.21

III.2. Total polyphenols determination

The values of polyphenols in different types of coffee are shown in Figure 3.

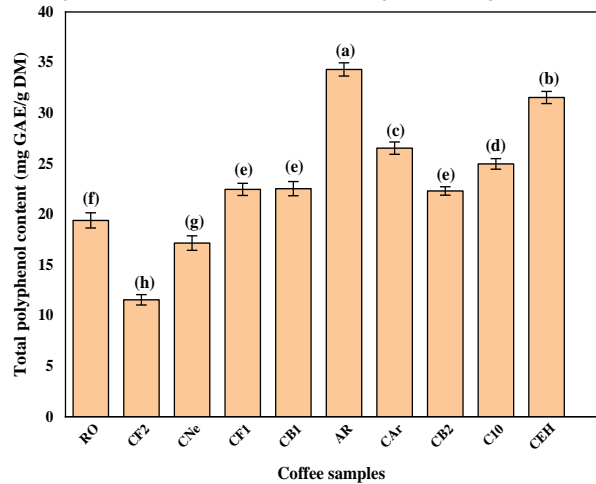


Figure 3. Polyphenols content in different coffee types.

The polyphenol content in the different types of coffee (Figure 3) varies from 11.54 (mg GGE/g DM) for CF2 coffee to 34.32 (mg GGE/g DM) for AR coffee. This is similar to the result of Panusa et al., (2013) [34], who showed that the total phenol in the coffee sample was between 17.1 and 35.5 mg GAE/g DM. However, this is slightly higher than the value found by Mussato et al., (2011) [35], which is 16 mg AEG/g total phenol content in the methanolic extract of coffee. The statistical analysis ANOVA followed by the Fisher test reveals a significant difference between the values obtained, which is not the case between the two types of coffee CB1, CF1 and CB2.

III.3. DPPH free radicals scavenging activity

DPPH is a stable free radical with a violet color in solution, this color disappears quickly when it is reduced to diphenyl dicryl hydrazine by a compound with anti-free radical properties resulting in a light yellow coloring (DPPH-H). The intensity of the color is proportional to the capacity of the antioxidants present in the medium to give protons and is measured with a spectrophotometer [36].

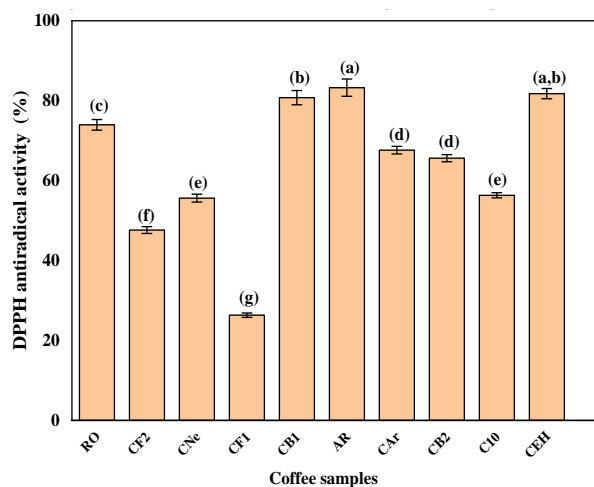


Figure 4. DPPH Antiradical activity of different coffee types.

Antiradical activity (ARA) (Figure 4) ranges from 26.33 for CF1 coffee to 81.77% for CEH coffee. Our results are similar to those obtained by Vanida Chairgulprasert et al., (2017) [37]. However, they are significantly higher than those obtained by Katarzyna Janda et al., (2020) [38], for coffee beverages obtained by brewing using five different coffee preparation techniques: Aeropress, drip, espresso machine, French press, and simple infusion and they found a range from 31 to 42 %. Statistical analysis followed by the Fisher test revealed a significant difference between the coffee samples.

III.4. IC50 determination

The coffee effective concentration (Figure 5) varies between 0.46 (mg/mL) for CB1 coffee and 1.42 (mg/mL) for CF1 coffee. When comparing these coffees with respect to ascorbic acid (0.47mg/ml) and BHA (0.37mg/ml), it can be seen that AR and CEH coffees have the best antioxidant power:

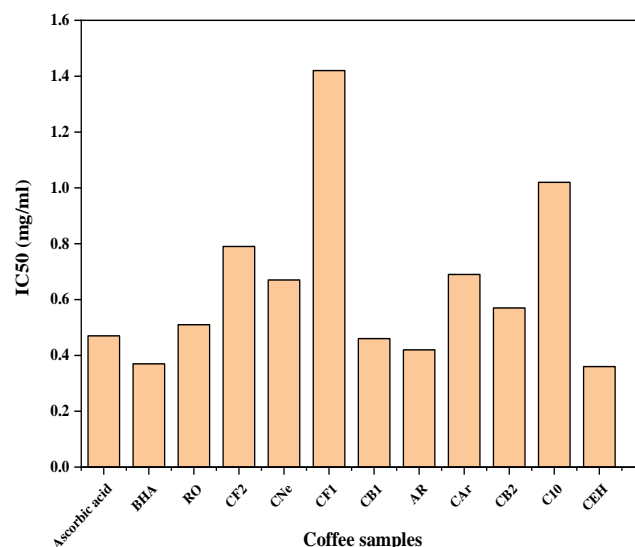


Figure 5. Effective IC50 concentration values for different types of coffee.

Our values are significantly higher than those obtained by Vanida Chairgulprasert et al., (2017) [37], for robusta coffee where IC50 values are between 0.19 and 0.46%.

III. 5. Total sugars determination

The values of total sugars in the different types of coffees are shown in Figure 6.

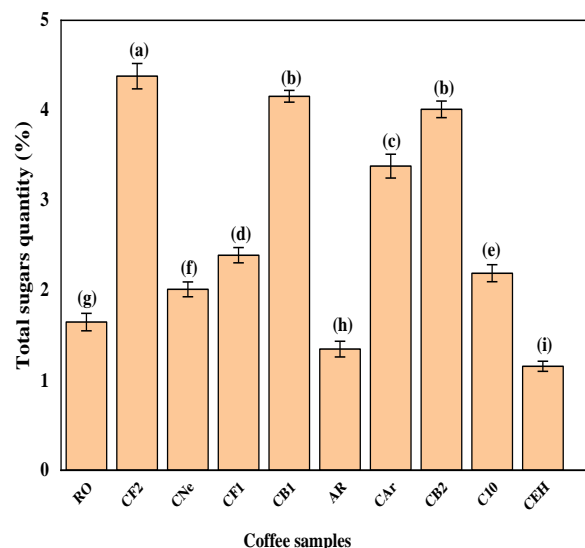


Figure 6. Total sugars quantity in the different coffee types.

The values of the total sugars obtained (Figure 6) vary between 1.156% for CEH coffee and 4.38% for CF2 coffee, firstly, we see that the other types of coffee contain caramelized sugar and secondly, there are four types of coffee (CF2, CB1, CAr and CB2) which exceed the limit tolerated by Algerian regulations in terms of adding caramelized sugar

which is 3% according to Executive Decree No. 17-99 of 29 Joumada El Oula 1438 corresponding to February 26, 2017 fixing the characteristics of the coffee as well as the conditions and modalities of its release for consumption. Therefore, these coffees are fraudulent, the statistical analysis shows a significant difference between the different types of coffee. Our values obtained are similar to those found by Djemaoun [39], for five types of Arabica and Robusta coffee and she found a range from 2.56 to 4.59%.

III.6. Principal Component Analysis (PCA)

The results of the principal component analysis are reported in Figure 7.

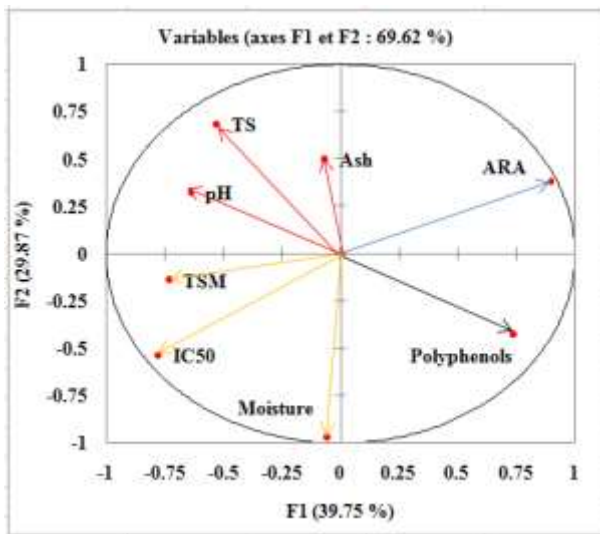


Figure 7. Correlations circle.

The representation of coffee samples on the first 2 main planes (Figure 7) of the PCA shows a group structure of physico-chemical parameters according to the type of coffee.

According to the correlation circle we notice that there is a link between polyphenols and antiradical activity, on the other hand there is a negative relationship between polyphenols and other parameters such as total sugars, this finding was detected by G. J. Nemlin et al., (2009) [40] on robusta coffees in ivory coast to see the influence of terroirs on the physicochemical quality of the coffees.

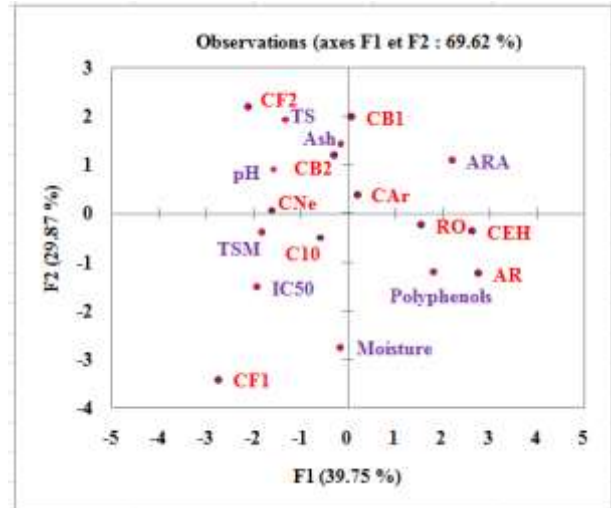


Figure 8. Observations graph observations.

According to the observations graph (Figure 8), there is a relationship between sample-sample, parameter-parameter and a relationship also between sample-parameter. The individuals on the right are important in terms of physicochemical properties to those on the left. This analysis has made it possible to create the distances of the coffee types expressing their distance or proximity to each other.

III.7. Ascending hierarchical classification

The results of the hierarchical ascending classification are reported in Figure 9.

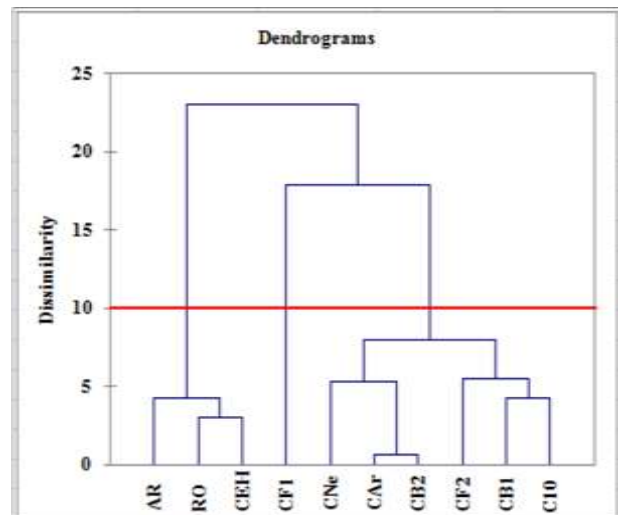


Figure 9. Dendrogram of different coffee types.

The hierarchical ascending classification allowed us to group the coffee samples into classes with different segments, we find that the class containing the coffee types (AR, RO, CEH) is the best class in terms of physicochemical properties. Our results are different from those obtained by Wael Taha Kasem and Atta E. M., (2015) [41], for 11 robusta coffee cultivars (Saudi Arabia) which found two classes and two sub-classes.

Table 3. Hierarchical bottom-up classification of coffee types according to their physicochemical properties.

	Class 1	Class 2	Class 3	Class 4
Coffee type	AR	CF1	CNe	CF2
	RO	-	CAr	CB1
	CEH	-	CB2	C10

IV. Conclusion

For most coffee samples, the caramelised sugar percentage is not available on the packaging. This affairs state constitutes a fraud because on the one hand the use of the robusta species is frequent among producers because its price which is cheaper than arabica and on the other hand the declaration of the species used is obligatory according to the decree fixing the coffee caractéristiques as well as the conditions and modalities of its release for consumption.

According to this preliminary study, our choice of consumption would be CEH and CNe or CF1 coffee which use sugar levels in their formulas below the permissible limit, resulting in a low level of regenerated acrylamide. This study should be extended over years and over several coffee samples for the identification and optimal use of food additives in coffee powder in Algeria. In addition, the growing coffee sector raises the historical problem of environmental pollution. The non-treatment of coffee waste (green or roasted) is a major source of environmental pollution.

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Please cite this article as:

Djeziri M., Belfadel O., Boudriche L., Antioxidant activity evaluation and the physico-chemical composition of some Algerian commercial coffees (threatened environment). ***Algerian j. Env. Sc. Technology*, 9:2 (2023) 3101-3108**