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Prunus avium kernel oil characterization: a comparative study of four varieties from Sefrou, Morocco☆

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Abstract – Four varieties of *Prunus avium* (*Burlat*, *Napoleon*, *Coeur de pigeon*, *Van*) kernel oils were extracted using a soxhlet apparatus with n-hexane as solvent. These oils composition was compared amongst them, with fatty acids, phytosterols and tocopherols identification and physicochemical characterization of said oils. Several differences, such as in oil yield were observed, "*Coeur de pigeon*" variety being the highest with a 23.5% yield. Twelve fatty acids were identified in all the varieties with linoleic and oleic fatty acids being the most abundant. β-sitosterol, Campesterol and Δ5-Avenasterol were the major compounds in the sterols assay performed. Also, total tocopherols ranged from 352.22 mg/kg (*Var. Coeur de pigeon*) to 2072.55 mg/kg (*Var. Napoleon*), with γ-tocopherol being the dominant one. These results suggest that these oils have numerous active compounds that can be further exploited.

Keywords: *Prunus avium* / cherry / soxhlet / tocopherols / fatty acids / phytosterols

Résumé – Caractérisation de l'huile d'amande de *Prunus avium* : étude comparative de quatre variétés cultivées à Sefrou (Maroc). Quatre variétés d'huiles d'amandes *Prunus avium* (Burlat, Napoléon, Coeur de pigeon, Van) ont été extraites à l'aide d'un appareil soxhlet avec du n-hexane comme solvant. La composition de ces huiles a été comparée entre elles : identification des acides gras, des phytostérols et des tocophérols, et caractérisation phytochimique de ces huiles. Plusieurs différences, comme le rendement en huile, ont été observées : la variété « Coeur de pigeon » offrait le rendement le plus élevé (23,5 %). Il est intéressant de noter que cette variété a l'indice de peroxyde le plus faible avec 4,4 meq O_2/kg d'huile. Douze acides gras ont été identifiés dans toutes les variétés, les acides gras linoléiques et oléiques étant les plus abondants, suivis par l'acide alpha-éléostéarique, absent dans la variété « Van ». β- sitostérol, Campesterol et Δ5-Avenasterol ont été les principaux composés dans le dosage des stérols effectué. De plus, les tocophérols totaux variaient de 352,22 mg/kg (Variété Coeur de pigeon) à 2072,55 mg/kg (Napoléon), le γ-tocophérol étant le plus important. Ces résultats suggèrent que ces huiles possèdent de nombreux composés actifs qui peuvent être exploités davantage.

Mots clés : *Prunus avium* / cerise / tocophérols / acides gras / phytostérols

1 Introduction

During the processing of fruits and vegetables by the food industries, high rates of loss of nearly 90 million tons of food waste are expelled each year. And in addition to the direct consumption of fruits, these food industries produce large quantities of canned fruit, juice, jam, jelly, compotes, marmalade, syrup and several types of soft drinks (Ferretti *et al.*, 2010; Wink, 2016) at the expense of large amounts of waste in the form of stones and kernels. Some of which are already used as edible oil sources in oil mixtures, in paint and varnish formulation, in surface coatings and as cosmetic oils (Helmy, 1990), while others are still presenting a serious problem of elimination.

"*Prunus avium*" cherries play an important role in markets and processed fruit markets and are used as an ingredient in fruit cocktails, confectionery fodder and maraschino cherries (Gao and Mazza, 1995) and, unfortunately, large amounts of

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cherry stones are discarded without further use, this not only wastes a potentially valuable resource, but also aggravates elimination problems (Mu *et al.*, 2015).

The cherry tree (*Prunus avium* L.) belongs to the *Rosaceae* family, and the *Prunoid* subfamily occupies the subgenus *Cerasus* within *Prunus* genus. The cherries are classified as sweet cherries (*Prunus avium*), tart (*Prunus cerasus*) and dwarf (*Prunus fruticosa Pall*), the first two being ranked among the three most commercially important species in the world (Chockchaisawasdee *et al.*, 2016) and are currently grown in most temperate and Mediterranean countries.

Turkey is the world's largest producer of sweet cherries, followed by the United States, Iran, Italy and Ukraine. In Morocco, cherry cultivation covers about 1600 ha in area producing some 14 100 tons per year. The production basins are mainly in the region of Sefrou, where 4056 tons was produced from 338 hectares in the 2019 campaign. Cherry cultivation is so important in the Sefrou region that a festival has been dedicated to it every year since the early 20th century.

In addition to Cherries fruit consumption in Morocco, stems and kernels are used for the traditional treatment of certain diseases.

The oil contained in the kernels of the sweet cherry fruit is extracted and sold in France and Germany, for cosmetic uses. Such product does not exist in Morocco, even at the cooperatives level.

Previous research has shown that cherry kernels have abundant nutrients and bioactive constituents, especially fat. Cherry kernel oils have been the subject of numerous studies representing approximately 25–30% of the total dry weight (Bak *et al.*, 2010; Siano *et al.*, 2016) and containing more than 80% of unsaturated fatty acids, mainly oleic acid and linoleic acid. Thus, cherry stones are potentially important resources for vegetable oil and it is important to exploit them nationally.

However no study was conducted on the chemical profile of the different types of these fruits kernels in Morocco. For this reason, this investigation focuses on the development of the non-used kernel that results from this product containing active phytochemical ingredients.

2 Material and methods

2.1 Raw material

Three sweet cherry (*Prinus avium*) varieties (*Burlat*, *Napoleon* and *Coeur de pigeon*) and one sour variety (*Van*) were collected from Sefrou city in Morocco. The fruits were cut open to recover the kernels. The kernels were washed with distilled water and then left to dry at room temperature in shade. The kernels were stored in hermetic bags at 4 °C till further use.

2.2 Oil extraction methods

The kernels were brought to room temperature and crushed for the four varieties used in this study. Thirty grams of blended kernels of each variety were crushed to obtain a kernel flour with a granulometry of less than 630 µm. The flour is then placed in a paper thimble and fed into a Soxhlet extractor. The extractor was fitted with a 500 mL round bottomed flask containing 300 mL of solvent under reduced pressure. The oil extraction was carried out with n-hexane on a flask heater at 45 °C for 8 h.

Oils were separated from the solvent using a rotary evaporator (Rotavapor R-100, BUCHI) and kept in sealed brown bottles under refrigeration (4 °C) for further analysis.

2.3 Determination of extraction yield

The extraction yield was obtained by dividing the amount of the extracted oil to the initial amount of kernels flour used in the extraction and multiplying by 100. A 0.0001 g analytical balance was used for accurate weighing.

$$\text{Yield}\,(\%) = \frac{W_f}{W_i} \times 100,$$

Where: W_f is the weight of the extracted oil and W_i is the weight of the initial kernel flour.

2.4 Determination of physicochemical characteristics

The peroxide value was determined according to ISO 3960 (2007) standard method, the free fatty acid (FFA) content was determined using the ISO 660 (2009) standard protocol and the acid value was determined mathematically by multiplying the FFA value by a 1.99 factor.

The saponification value was determined according to ISO 3657 (2002) standard method and the iodine value was determined using Wijs reagent as described in the ISO 3961 (2009) method.

2.5 Gas-Chromatography for fatty acids composition

The fatty acid methyl esters FAME composition was determined following EEC/2568/91 protocol (EEC/2568, 2003), by Capillary gas chromatography – CGC, using a Varian CP-3800 (Varian Inc.) chromatograph equipped with an FID. A split injector was used, and the injected volume was 1 µL. The column used was a CP- Wax 52CB column (30 m × 0.25 mm i. d.; Varian Inc., Middelburg, The Netherlands). The conditions for the chromatographic operations were as follows: The carrier gas was helium and the total gas flow rate was 1 mL/min. The initial and final column temperature was 170 and 230 °C, respectively, and the temperature was increased by steps of 4 °C/min. The injector and detector temperature were 230 °C. Data were processed using a Varian Star Workstation v 6.30 (Varian Inc., Walnut Creek, CA, USA). Results were expressed as the relative percentage of each individual FA presents in the sample.

2.6 Tocopherols contents determination

The tocopherol contents of the samples were determined according to the ISO 9936 (2006) standard method using an HPLC equipped with a fluorometric detector (excitation wavelength 290 nm – emission wavelength 330 nm) on a silica column (25 cm × 4 mm). The elution is carried out with a mixture of (isooctane: isopropanol) (99:1) with a flow rate of

Table 1. Oil yield, Peroxide value and antioxidant activity comparison between the different *Prunus avium* varieties tested.

Variety	Peroxide value (meq O_2/kg oil)	Acid value (mg KOH/g oil)	Saponification value (mg KOH/g oil)	Iodine value (g I/100 g oil)	Oil yield (%)
Burlat	8.0 ± 0.7	2.6 ± 0.02	192.1 ± 3.01	121.8 ± 0.01	19.0 ± 1.5
Van	6.0 ± 0.8	2.6 ± 0.02	189.2 ± 2.89	107.4 ± 0.02	23.0 ± 0.5
Napoleon	8.2 ± 0.9	2.3 ± 0.01	191.4 ± 3.11	126.0 ± 0.01	13.7 ± 1.2
Cœur de pigeon	8.0 ± 0.7	2.6 ± 0.02	192.1 ± 3.01	121.8 ± 0.01	19.0 ± 1.5

1.2 mL/min during the analysis time (20 min). Quantification was carried out by external standard curves of four tocopherols and daily reference of quantitative and qualitative tocopherol standards.

2.7 Phytosterols quantification

The phytosterols were quantified according to the ISO 6799 (1991) standard method using capillary gas chromatography CGC on an apolar column (Chroma pack) (30 m × 0.32 mm, DI: 0.25 µm, phase: CPSIL8CB). The VARIAN CP-3800 chromatograph is equipped with a divider injector type 1079 (T: 300 °C) and an FID (T: 300 °C). The carrier gas is helium (flow: 1.5 mL/min).

3 Results and discussion

3.1 Physicochemical characteristics of oil

The physicochemical properties of the extracted oil for each variety were measured. The results displayed in Table 1 show that all oils are conformed to the vegetal oil standards for olive and argan oils. The "Coeur de pigeon" and "Van" varieties are the most abundant in oil with yields of 23.5 and 23%, respectively. "The Coeur de pigeon" recorded also the lowest peroxide value amongst all tested samples with a value of 4.4 meq O_2/kg oil. Several factors are responsible for high peroxide values, such as exposure to air and sunlight.

Few are the investigations that were conducted previously on *Prunus avium.* A study conducted on the sour cherries (*Prunus cerasus*) have reported a similar yield at about 22.5%. Also, the saponification and the iodine values were close to those found by our investigation, with 183 mg KOH/g oil and 122.5 g I/100 g oil. The close values observed are indicative of similarities in the oil composition. However, acid and peroxide values were lower than those obtained here with 1 meq O_2/kg oil and 1.6 meq O_2/kg oil, respectively (Popa *et al.*, 2011).

3.2 Fatty acids composition

Gas chromatography was performed on the obtained oils to determine the fatty acid and phytosterol composition. The results demonstrated that linoleic, oleic and palmitic acids are the most abundant. However, some differences were observed such as the oleic and linoleic percentages in between varieties. The "Van" variety exhibited the highest oleic acid content followed by "Coeur de pigeon". Also, the "Burlat" variety was the most abundant in linoleic acid with a value of 40.14%. For the "Van" and "Coeur de pigeon" varieties, oleic acid has the highest proportion, while for "Burlat" and "Napoleon" it is the linoleic acid that has the highest value. Similar observations were reported previously; the oleic (C18:1) and linoleic (C18:2) were the predominant fatty acids in sour cherry kernel oils (25.25–45.30 and 35.50–46.06%, respectively) (Górnas *et al.*, 2016).

This oil contains a high percentage of unsaturated fatty acids. Linoleic and oleic acids represents major unsaturated fatty acids present in most plant based oils. Peanut, cotton and corn oils display higher content of unsaturated fatty acids than saturated ones.

It is estimated that polyunsaturated fatty acids (PUFAs) contribute to 7% of total energy intake and 19–22% of energy intake from fat in the diets of adults. These values are in accordance with the recommended dosage for adults (Kris-Etherton *et al.*, 2000).

The presence of oleic acid in the skin grants it an antioxidant property that has been evaluated previously by DPPH essay.

These findings are different than those obtained by Straccia *et al.* (2012), where sweet cherry fruits from Campania region in Italy were studied. The investigation reported that the arachidic acid is the major fatty acid (~41%), followed by linoleic acid (~32%) and palmitoleic acid (~8%) (Straccia *et al.*, 2012).

On another study on the sour cherries, a closely similar composition in Oleic, Linoleic and palmitic acids was reported, with values of 42.9, 38.2 and 11%, respectively (Popa *et al.*, 2011) (Tab. 2).

Another interesting observation in the obtained results is the presence of Alpha-eleostearic acid in all varieties. The presence of this fatty acid was previously reported for sour cherry kernel oils such as the "van" variety in our case (Górnas *et al.*, 2016). Alpha-eleostearic is a particularly interesting fatty acid as it was previously reported to hold antitumor properties (Zhuo *et al.*, 2014).

3.3 Phytosterols composition

For sterols, Beta-Sitosterol is by far the most abundant sterol with values ranging between ~77 and ~83%, with "Coeur de pigeon" recording the highest percentage.

Besides Beta-Sitosterol, Sitostanol, Δ5-Avenasterol and Δ7-Stigmasterol are the most abundant compounds, with an advantage for Δ5-Avenasterol for all varieties except "Van", where Δ7-Stigmasterol is the most abundant.

Table 2. Fatty acids composition of the different oil varieties.

Fatty acids		Burlat	Van	Napoleon	Cœur de Pigeon
Myristic	C 14 : 0	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
Palmitic	C 16 : 0	7.63 ± 0.41	7.84 ± 0.39	7.72 ± 0.52	7.46 ± 0.36
Palmitoleic	C 16 : 1	0.46 ± 0.03	0.50 ± 0.03	0.58 ± 0.03	0.5 ± 0.03
Heptadecanoic	C 17: 0	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
Heptadecenoic	C 17 : 1	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
Stearic	C 18 : 0	3.21 ± 0.14	2.83 ± 0.10	2.22 ± 0.09	2.97 ± 0.11
Oleic	C 18 : 1	37.53 ± 0.95	42.91 ± 0.92	37.9 ± 0.93	44.08 ± 1.01
Linoleic	C 18 : 2	40.14 ± 1.05	37.33 ± 0.89	39.7 ± 0.94	34.62 ± 0.91
Linolenic	C 18 : 3	0.32 ± 0.03	0.20 ± 0.03	0.2 ± 0.02	0.2 ± 0.02
Alpha-eleostearic	C 18 : 3	8.31 ± 0.04	6.62 ± 0.35	10.15 ± 0.06	8.35 ± 0.05
Arachidic	C 20: 0	1.59 ± 0.12	1.18 ± 0.02	0.9 ± 0.09	1.19 ± 0.11
Eicosenoic	C 20 : 1	0.57 ± 0.03	0.41 ± 0.03	0.42 ± 0.03	0.42 ± 0.03

Table 3. Sterols composition of the different oil varieties.

Sterols	Burlat	Van	Napoleon	Cœur de pigeon
Cholesterol	0.11 ± 0.01	0.3 ± 0.02	0.11 ± 0.01	0.14 ± 0.01
Campesterol	3.29 ± 0.24	3.26 ± 0.23	3.03 ± 0.19	3.17 ± 0.21
Beta-Sitosterol	83.47 ± 1.5	77.92 ± 1.2	83.21 ± 1.48	83.72 ± 1.6
Sitostanol	4.69 ± 0.22	2.45 ± 0.16	1.99 ± 0.11	2.53 ± 0.17
Δ5-Avenasterol	5.24 ± 0.29	4.63 ± 0.23	4.81 ± 0.26	5.37 ± 0.3
Δ5,24-Stigmastadienol	0.77 ± 0.04	0.48 ± 0.03	0.74 ± 0.04	0.69 ± 0.03
Δ7-Stigmasterol	1.06 ± 0.11	7.43 ± 0.32	2.43 ± 0.16	2.52 ± 0.14
Δ7-Avenasterol	0.43 ± 0.03	1.83 ± 0.09	0.81 ± 0.06	1.05 ± 0.08

Table 4. Tocopherol content of the different oil varieties.

Tocopherols (mg/kg)	Burlat	Van	Napoleon	Cœur de Pigeon
α-tocopherol	51.84 ± 0.12	80.53 ± 0.18	152.87 ± 0.21	32.49 ± 0.08
γ-tocopherol	482.87 ± 1.51	952.30 ± 1.87	1851.80 ± 2.14	301.52 ± 1.23
δ-tocopherol	38.82 ± 0.06	39.52 ± 0.07	67.87 ± 0.15	18.19 ± 0.05
Total tocopherols	573.53 ± 2.81	1072.41 ± 3.31	2072.55 ± 3.82	352.22 ± 2.32

It was previously reported that sweet cherry oil from Italy contained only about 0.6% of Beta-Sitosterol and about 0.025% of Campesterol (Straccia *et al.*, 2012).

Phytosterols are important ingredients in a wide range of cosmetic products, such as surfactant, emulsifiers, thickeners and other formulations (Folmer, 2003). Vegetale oils extracted from tropical fruits have been previously studied and used to obtain unsaponifiable fractions (Esuoso *et al.*, 2000). The sterol composition of sweet cherry oil (Sitostanol, sigmasterol, Δ5-avenasterol, β-sitosterol...) grants it a rich unsaponifiable fraction that can be used in functional foods and cosmetic uses (Bernardo-Gil *et al.*, 2001) (Tab. 3).

3.4 Tocopherols composition

Liquid phase chromatography was performed on the obtained oils to determine the tocopherols composition.

γ-tocopherol is the major type of tocopherol found in all varieties, with "Napoleon" having the highest content with a value of 1851.8 mg/kg, followed by "Van" (952.3 mg/kg), "Burlat" (482.87 mg/kg) and "Coeur de pigeon" (301.52 mg/kg) (Tab. 4).

4 Conclusion

The study of cherry kernel oil from three sour and one sweet varieties gave out important results. The kernels, though being hard to obtain, have important oil yields reaching 23.5%. The phytochemical values were also adequate, with the peroxide value being 8.2 meq O_2/kg oil at most. This oil is linoleic in nature, with alpha-eleostearic acid being the second most important fatty acid. The studied oil also contains a great amount of beta-sistosterol that has numerous important uses.

The total tocopherol amounts were important, while varying from one variety to another, with a maximum of 2072.55 mg/kg for the Napoleon variety.

Sweet cherry kernel oil hold within it active ingredient that can be used in several field such as food and cosmetic industries. The composition suggests that the four varieties have a great nutritional value, cosmetically active compounds, while rich in antioxidants in the form of tocopherols. This study could be a scientific testimony for the development of new sweet cherry based products in Morocco.

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