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Physicochemical Characteristics, Phenol Content and Fatty Acids of Bitter Almond Oil

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Abstract

In this study, physicochemical properties, thermal analyze, viscosity, phenol content and fatty acid composition were investigated. The plant material used comes from a wild plant growing in the wilaya of Béjaïa (Algeria). Almonds oil is considered functional foods for the presence of polyphenols compounds. The physicochemical parameters of the bitter almond oil were: the acidity values vary from 1.389 to 3.559%, peroxide index 19.538meq of active oxygen/ kg of oil, rancimat test PI=15h, total polyphenols= 0.137mg Gallic acid/mg bitter almond oil. The profile of the viscosity of the oils confirming that oil analyzed was Newtonian in nature. The TG/DTG curves showed bitter almond oil thermally stable consistent with the result of oxidative induction time. The fatty acid composition of bitter almond oil reveals the presence of the following fatty acids: gallic acid; hydrobenzoic acid. Catechic acid, isovanilic acid, vanilic acid, salicylic acid, myricetin, quercetin, anthrone and oxacil acid. Of which gallic acid is the majority. The physicochemical properties of the bitter almond oil indicated that it is promotes use in cosmetics and suggested its suitability for industrial purposes.

1. Introduction

Almond is an edible, nutlike seed of fruit of a tree, *prunus amygdalus*, of the rose family, the Rosaceae **(Aydin, 2003; Rao, 2012; Roncero et al; 2016).**
Almonds begin to bear an economic crop in the third year after the planting of the trees. The fruit consists of an outer hull and a hard shell with the seed ("hut") inside. There are three varieties of almonds, all of which produce nuts, but some are edible and some are not. One almond variety produces the sweet nuts we eat, one produces poisonous, bitter nuts and a third variety produces a mixture of bitter and sweet nuts. Two major types of almonds are grown commercially, which can be categorized as sweet almonds (*Prunus amygdalus dulcis*) and bitter almonds (*Prunus Amygdalus amara*). The sweet almond producing plant and the bitter almond producing plant can be differentiated on the basis of their flowers, since the sweet almond flowers are white in colour, whereas the bitter almond flowers are pink in colour **(Rao, 2012).**
Nuts provide an interesting nutritional supply due to their high nutritive and energetic value. However, their high fatty content makes them unattractive for new consumers demanding "light", low-fatty foods. Among nuts, almonds have a significant economical importance. **(Barku, Nyarko et al. 2012).**
Oils from nut are both edible and non-edible depending on the type. Fats and oils are important food source man, and are also extensively used nutritional, cosmetic, drug dispersant in therapeutics and industrial purposes and are used for supplying essential fatty acids such as linoleic and arachidonic acids.
The Committee on Fats and Oils of the Codex Alimentarius does not describe physicochemical characteristics of bitter almond oil since it is produced at a small scale in few countries like France, Spain and USA **(Sakar et al, 2017)**. Besides, almond oil has long been used in complementary medicine circles for its numerous health benefits. It has been demonstrated that almond oil has several properties including anti-inflammatory, immunity-boosting and anti-hepatotoxicity effects **(Ahmad, 2010)**. It is also used as component of dry skin creams, anti-wrinkle, and anti-aging products in the cosmetic industry as well as for pharmaceutical purposes **(Sakar et al, 2017)**. Other health-promoting compounds in almonds are polyphenols, which have been shown to be protective agents against cancer and cardiovascular disease **(Mandalari et al, 2010)**.
The oil composition bitter almond has not been extensively studied but several authors are interested in sweet almond oils **(Giwa and Ogunbona, 2014; Smeriglio et al, 2016; Delgado-Tobón et al, 2018)**, this study aims to characterize bitter almond oils collected in the region of Béjaïa located in the East of Algeria.

2. Material and methods

2.1 Physicochemical parameters

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Pressing of bitter almond oil (BAO) was carried out at room temperature, Oils were conserved at 4°C. Free acidity (ISO 660), peroxide values (ISO 6320), and UV absorption indices (K_{232}, K_{270}) were determined according to commercial standard methods for olive oil (ISO 3656).
Oxidative stability (OS) was evaluated by Rancimat test (Rancimat model 743), with an air flow rate of 13L/h and temperature of the heating block maintained at 110°C, OS of oils was reported as their equivalent induction times (h).
For rheological analyzes or viscosity, 20 ml of bitter almond oil is placed in an AR2000 rheometer of Coeutte geometry type, Viscosity is the measure of a liquid's resistance to flow.

2.2 Thermal analyzes: Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (ATG)

Vegetable oils are mainly used for cooking food and cosmetics, so it is important to understand their thermophysical properties during these processes. It is well known that DSC-ATG technology can show the thermal behavior of oils, it is applied in research for their characterization.
By using a thermo-gravimetric analyzer (**SDT Q600)** TGA analyses were performed. Analyses started from 50°C to 500°C with a heating rate of 10°C/min under a nitrogen atmosphere. In the DSC equipment under dynamic nitrogen atmosphere with a flow rate of 50 ml/min at a heating rate of 10°C/min to 500°C, the DSC curves were obtained.

2.3 Total phenol contents

Total phenol contents of samples were determined by using the Folin–Ciocalteu (FC) reagent. Folin–Ciocalteu (0.5 ml) was added to sample (0.5ml) and solution was mixed for five minutes. Afterwards, 0.5 ml to10% Na_2CO_3 was added into solution tubes, and the final volume was completed to 5 ml with distilled water. Absorbance for total phenolic content was measured at 750 nm in a spectrophotometer against gallic acid (0–2 mg/ml) as the standard for calibration curve.
Phenolic compounds of oil extracts were carried out using HPLC system. The mobile phase consisted of H_2O 1% Acetic acid/ methanol. HPLC analyses were conducted using C18 reversed-phase column (C18: 150×4.6mm ,5µl). Almond oils were dissolved in l'hexane and filtered through 0.45 µm membranes. The injection volume is 20 µl.

3. Results and discussion

3.1 Physicochemical parameters

The acid number is a criterion for the quality of the oil. It is used to determine the free fatty acid content, the stability and the purity of the oil. A good quality oil should have a low acid number because it helps give it high stability against oxidation.
The high value of the peroxide value indicates the presence of oxidation of the oil result of contact with the air. This oxidation results in the formation of peroxides, which justifies the high value of the latter.
All the acidity results and the peroxide value make it possible to affirm that the extracted almond oil requires refining before being used.
The acidity values (table 1) of bitter almond oil varies from 1.389 to 3.559% according to **(Martins et al, 2000)** of producted almond in Algarve, Portugal. The acid index of our oil is 6.148 ± 1.212%, this value is higher than those established by the Codex Alimentarius standard 4(mg of KOH / g of oil) which shows that the latter contains significant amounts of free fatty acids. We can conclude that our oils have a high acid number.
Barku et al, (2012) reported acid value of the almond of oil from Indian almond nut was 0.787 mg KOH/g lower than those our values, this may be due to the variation in the moisture contents, solvent extraction and the origin of the seeds.
According to the standard established by Codex Alimentarius the peroxide index of extra virgin almond oil is Max 10 meq of active oxygen / Kg of oil, our oil has a peroxide index of 19.538 ± 4.987, it is higher than standard and therefore this oil is oxidized. Indeed, fatty substances can oxidize in the presence of oxygen and certain favorable factors such as high temperature, photosensitizers, water, enzymes, etc. On the other hand, the extraction of the oil in a short time after the harvest of the almonds and its storage under good conditions lead to obtaining a low peroxide index, this suggests that the oil does not oxidize prematurely and keeps over time **(Tanouti et al, 2011)**.
The low value of the optical density at 270 nm (0.278) shows that the analyzed oil is not degraded because it is freshly extracted.

3.2 Rancimat test

From Fig.1, the induction period of this oil is relatively important (PI=15h).
(Qi et al, 2019) reported the IP values at 110°C of BAO were found to be 6.50 hr, 1.45 hr, and 7.09 hr in three types of almond oil extracted using different techniques,namely, Cold-Press extraction (CP), Hydraulic Press extraction (HP), and Subcritical Fluid Extraction (SFE).
According to the literature, oil that contains a higher content of saturated fatty acids and a lower content of unsaturated fatty acids has a longer induction period.

3.3 Viscosity

The viscosity of a Newtonian liquid depends only on the temperature and not on the shear rate or on the duration which is confirmed by our result (figure 2,3).
The viscosity profiles were studied at different temperatures to determine their flow behavior and the influence of temperature on the apparent viscosity. The rheological properties were evaluated using a rotary rheometer (AR 2000) on 20 ml of almond oil.
The viscosity of the oils reduced slightly with the shear rate, and reduced considerably with the change in temperature, confirming that the oil analyzed was Newtonian in nature. This information promotes the use of this oil in cosmetics.

3.4 Thermal analyzes: (DSC, ATG)

Figures 5 and 6 show the ATG, DSC and DTG (the ATG derivative) curves of bitter almond oil. The corresponding transformation temperatures determined from the derivative of thermograms (Td: start temperature, Tmax: temperature of the maximum transformation rate and Tf: final temperature), Δw: the observed weight loss and ΔH: transition enthalpy are given in table 2.
As can be seen on the ATG curve the first decomposition step starts from 250 ° C characterized by a peak of 393.9 ° C and the corresponding mass loss is 31.39%. The second stage ends at 450 ° C with a characteristic peak of 461.2 ° C, with a mass loss of 62.57%.
A residue of mass equal to 6.1% was recorded. Regarding the DSC, the first peak occurs at 52.9 ° C which signifies the evaporation of the extraction solvent (hexane) and the volatiles, the second peak occurs at 393.9 ° C which corresponds to decomposition of the majority of the product (residue 6.1%), then the third peak occurs at a temperature of 461.2 ° C, probably corresponding to the impurities.

The cumulative mass loss is represented by the derivative of the thermograms (Fig5), where a temperature equal to 411 ° C was recorded corresponding to the degradation of 93.74% of oil.
(Qi et al, 2019) reported DSC testing revealed three step exothermic effects in almond.
The TG/DTG curves showed bitter almond oil thermally stable consistent with the result of oxidative induction time.

3.5 Total phenolic content

The concentration of total phenolic compounds is determined by referring to the calibration curve obtained using gallic acid as the calibration standard.
The quantitative estimation of total polyphenols (by the Folin-Ciocalteu method) revealed a value of 0.137mg gallic acid /ml bitter almond oil.
Qi et al, (2019) reported the total phenols in AO extracted amount ranged from a minimum of 4.71 mg/100 g to maximum of 11.75 mg/100 g.
Phenolic compounds are responsible for the good oxidation stability of oils.
Referring to the bibliography, several factors must be taken into consideration that may influence the polyphenolic content. There are climatic and environmental factors, genetic factors, experimental factors, the difference between the methods of extraction, evaluation and expression of results between the authors.

3.6 Identification of Phenolic Compounds by High Performance Liquid Chromatography (HPLC)

The lipid content depends mainly on the genotype, and other factors such as climatic conditions and the method of extraction **(Sathe, 1992; García-López et al, 1996).**

Oils and their components are known to have antioxidant activities and could therefore serve as food preservatives, or approved as food additives **(Caillet and Lacroix 2007)**. They are the subject of study for their possible use as an alternative for the protection of food against oxidation **(Ahmad, 2010)**.
According to the results found: the chromatograms recorded referring to the chromatograms of the standards, 17 phenolic compounds appeared, of which 10 compounds could be identified using the peak surfaces and the retention times: gallic acid; hydrobenzoic acid. Catechic acid, isovanilic acid, vanilic acid, salicylic acid, Myricetin, Quercetine, Anthrone, oxacilic acid…
The fatty acid composition (figure 7) of sweet almond (*Prunus amygdalus "dulcis"*) seed oil reveals the predominance of oleic acid (69.7%), linoleic acid (18.2%) and palmitic acid (9.3%) **(Giwa and Ogunbona, 2014).**
Chemical composition of a type of almond, called "Akbadem", cultivated in the Aegean region in Turkey reported by **Nizamlıoğlu and Nas (2017)**, oleic acid, linoleic acid, palmitic acid and palmitoleic acid were 76.11 ± 1.18%, 17.71 ± 1.14%, 6.14 ± 0.05% and 0.04 ± 0.01%.
According to **Ahmed et al, (2019)**, fatty acids showed some differences according to the types of nuts and extraction methods. The oil samples obtained from cold-press had higher contents of fatty acids and tocopherol contents than those extracted from Soxhlet apparatus, probably because of the presence of more impurities in the oil extracted from the Soxhlet apparatus method. The most abundant fatty acid present in both cold-press and solvent extraction oils was linoleic acid, followed by oleic and linolenic acids.

Table1 bitter almonds oil analysis

	Acidity (%)	**peroxide values (mèq O2/Kg)**	**UV absorption indices λ232**	**UV absorption indices λ270**
Valeur	6.148±1.212	19.538±4.987	1.869±0.569	0.278±0.088

Table 2 thermal analyzes results

Paramèters DSC						**Paramèters ATG**
sample	**Pics**	**T_0 (°C)**	**T_p (°C)**	**T_c (°C)**	**ΔH (mw /mg)**	**PM en %**
bitter almonds oil	1	34.6	52.9	95.1	0.1153	31.39
	2	378.4	393.9	402.6	0.4419	62.57
	3	479.82	560.00	515.20	0.1959	

T_0 = Initial temperature; Tp = peak temperature; Tc = Final temperature. ΔH = Transition enthalpy. PM: loss of mass.

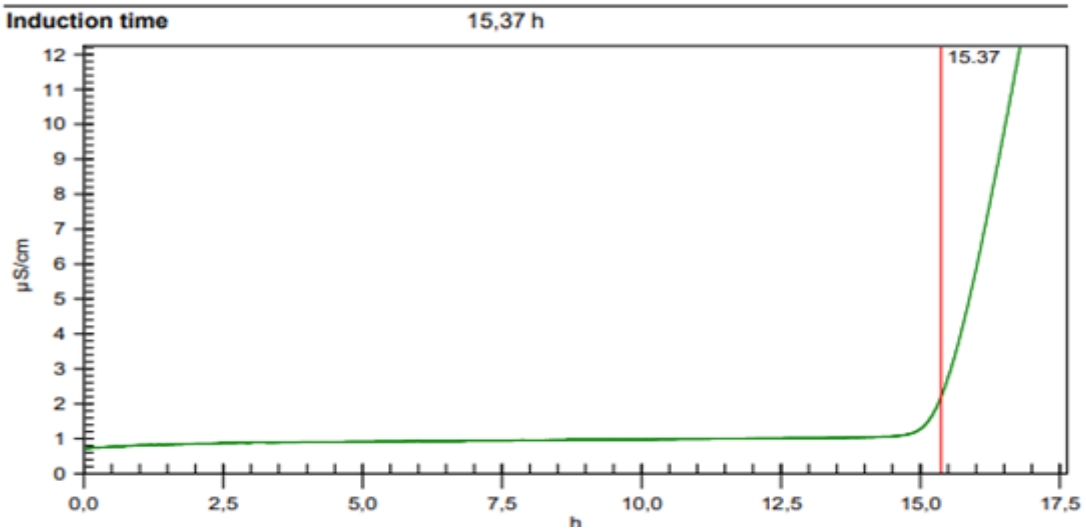

Figure1 Oxidation stability curve (Rancimat test) of bitter almond oil

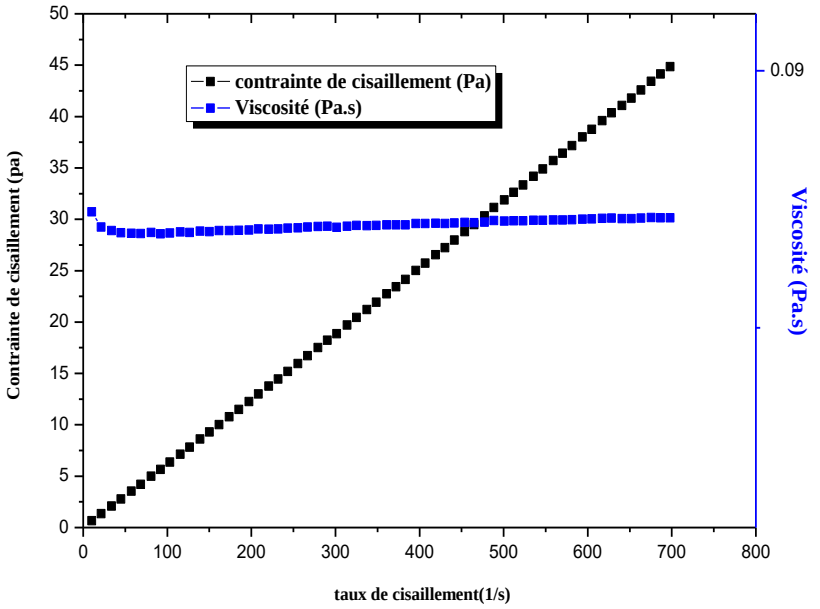

Figure2 Viscosity and constraint of bitter almond oil at room temperature

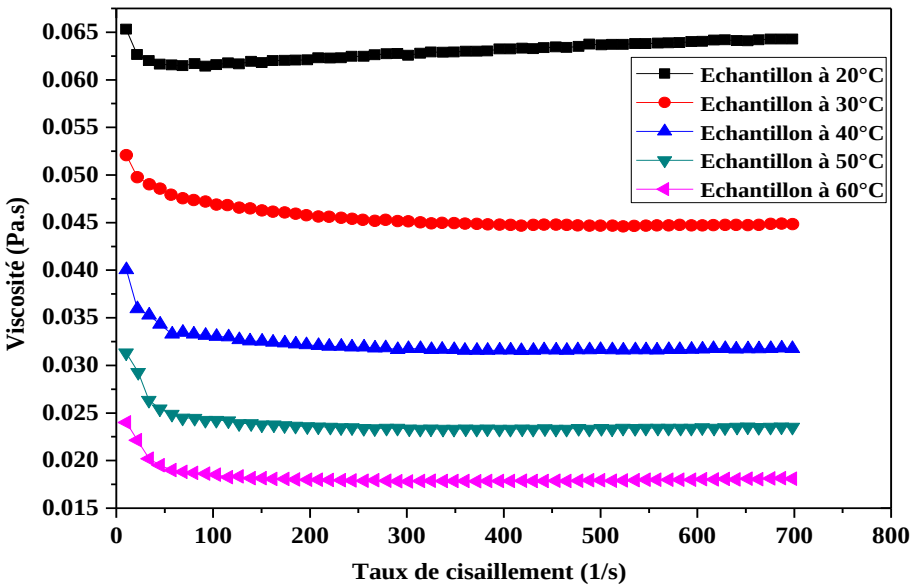

Figure3 Viscosity of bitter almond oil at different temperatures

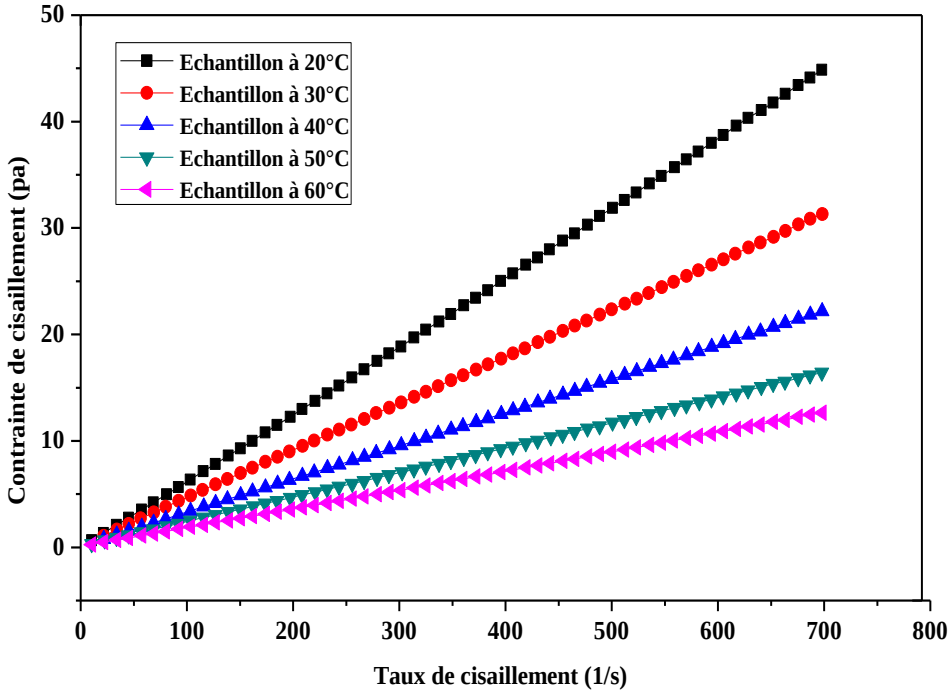

Figure 4 Constraint of bitter almond oil at different temperatures

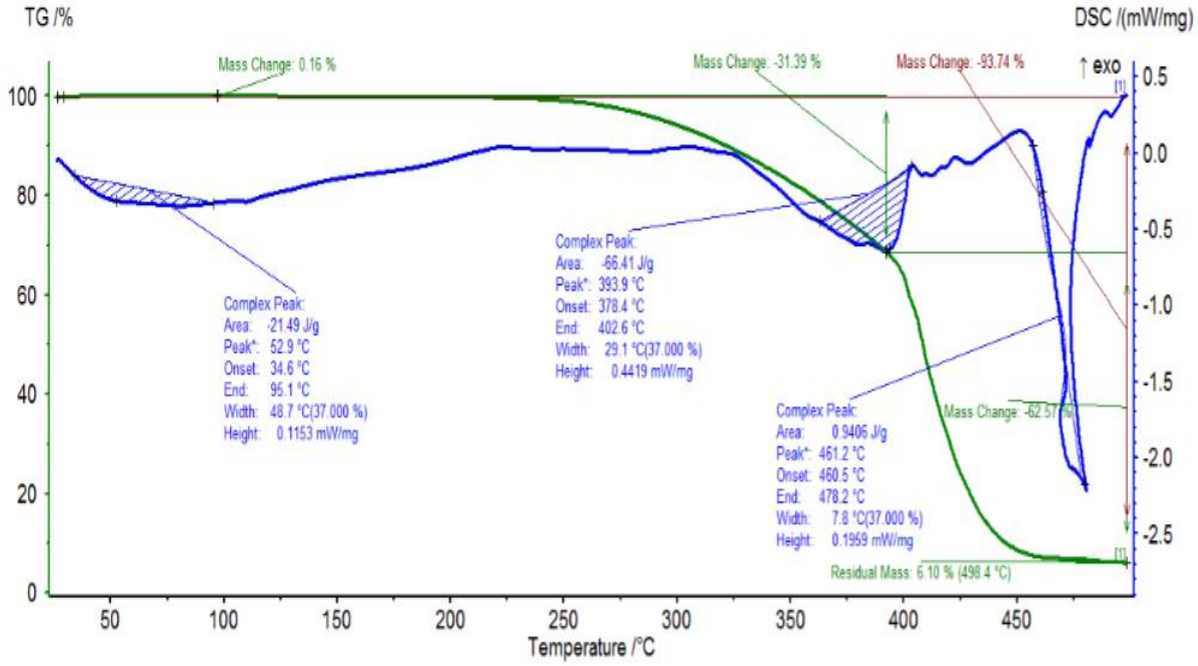

Figure5 DSC-ATG thermogram of bitter almond oil

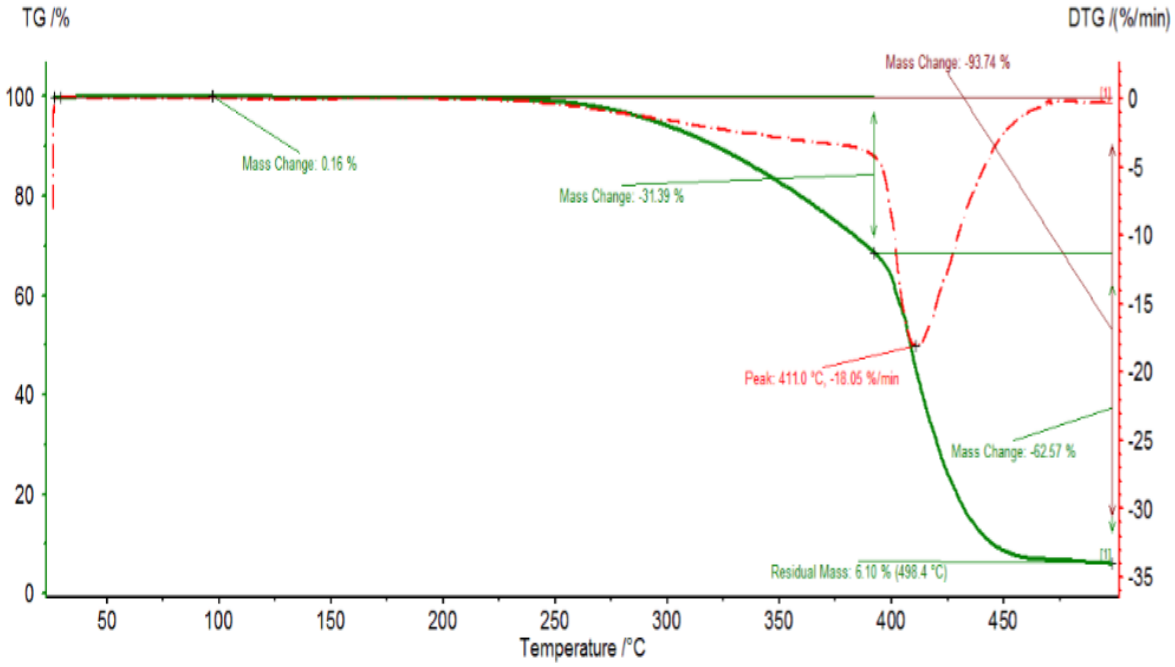

Figure 6 DTG-ATG thermogram of bitter almond oil

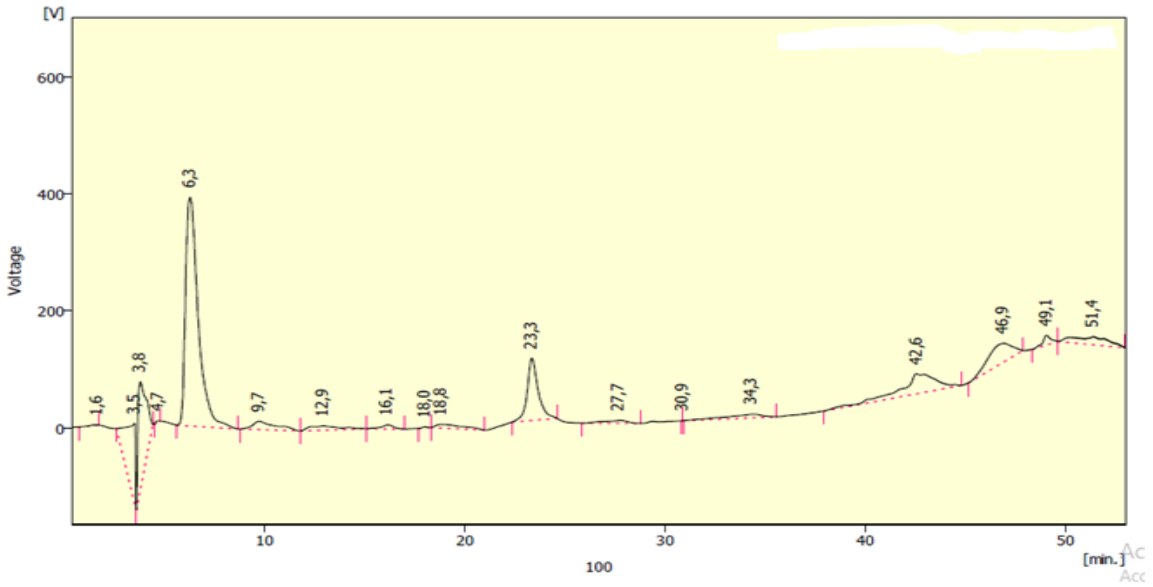

Figure 7 HPLC chromatogram of the methanolic extract of almond oil

4. Conclusion

The analysis of the extracted oil allowed for a contribution to a better knowledge of this oil. The physicochemical properties of bitter almond oil have been studied for their cosmetics and commercial applications.

The TG/DTG curves showed bitter almond oil thermally stable consistent with the result of oxidative induction time. The present study has shown that almond oil also contains significant amounts of phenolic acids which improve oil stability without using synthetic antioxidants.

The chromatograms obtained confirm these results and referring to the chromatograms of the standards, 10 compounds could be identified using the peak surfaces and the retention times.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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