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ORIGINAL ARTICLE

Physicochemical characterization and antioxidant capacity of the extracted oil from date pits and its effect on storage stability of margarine

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ABSTRACT

Background and aims: The present work deals with the valorization of the date kernel oil of Mech-Degla variety by assessment of its physicochemical and antioxidant properties as well as its use in the formulation of margarine. **Methods:** Kernels' oil was extracted using Soxhlet method and its total phenolic (TP), flavonoid and carotenoid contents and DPPH• scavenging activity were estimated using colorimetric assays. After that, this oil was incorporated into margarine. The determined physicochemical parameters were the pH, the salt content, the solid content, the melting point, and the peroxide index. Finally, the elaborated margarine's oxidative stability was evaluated by the Rancimat test. **Results:** The yield of fat in the extracted oil was 9.84 ± 1.45% and the amount of TP, flavonoids and carotenoids were 112.92 ± 26.57 mg gallic acid equivalent /kg of oil, 15.7 ± 0.7 mg quercetin equivalent/kg of oil and 125.534 ± 0.109 mg β-carotene equivalent/kg of oil, respectively. As regards the anti-DPPH effect, the cold and hot date kernel oil extracts exhibited a moderate capacity by reducing 55.91% and 30%of the free radicals, respectively. After that, table enriched margarine has been industrially elaborated at the Cevital agri-food complex by adding 50 and 100 ppm of date kernel oil. Texture of this margarine was plastic and easy to spread, with an acceptable color and a brilliant and homogeneous appearance. At 37 °C, the solid fat content (SFC) index is lower than 6%, which attested that this margarine melts easily in the mouth. The physicochemical characteristics of the formulated product were also assessed and demonstrated that its pH value was 4.2, its peroxide value was 0.32 meq of O_2/Kg of margarine and its melting point was 36.0°C. It was also revealed using the rancimat test that the margarine enriched in date kernel oil proved to be more resistant to oxidation, than the control one. **Conclusions:** Date kernel oil can be used as a natural additive to enrich table margarine without affecting its properties while improving its oxidation stability.

Keywords: Date kernels oil, antioxidant activity, margarine formulation, physicochemical characteristics, oxidative stability.

ARTICLE INFORMATION

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1 Introduction

The valorization of organic by-products in the food industry has attracted the interest of several researchers for both environmental protection and economic exploitation. For example, some tropical fruits' by-products possess antioxidant activities that retard lipid peroxidation as demonstrated in sunflower oil or food packaging applications [1, 2].

The date palm (*Phoenix dactylifera* L.) is a vital plant for desert regions where it constitutes a basis of survival for their populations. The date palm tree produces globally an approximate of 4.8 million tons (dry weight) where 3.6

million tons are produced in the Middle East only as by-products of pruning, regarded as agricultural waste, which are either land filled or incinerated [3 - 5]. Date palm by-products (leaves, trunk, pits, pedicels, etc.) have various uses for example in the Algerian Saharan regions, pits are used in animal feed [6] and a low-cost agricultural by-product, is a good precursor for production of activated carbon, which is the most popular adsorbent [7].

The volume of date pits (DPs) generated by date processing industries is enormous (represent nearly 30% of the production) and requires special consideration due to the presence of vital bioactive compounds [8].

The date pits, in particular, are used as cattle feed [8, 9] and in traditional medicine for their antimicrobial and antiviral properties [10]. Similarly, several research works are devoted to the valorization of these pits in different forms including activated carbon [11-14], supplement in livestock feed [15], preparation of citric acid and proteins [16].

The characterization of the date pits (*Phoenix dactylifera* L. variety Mech-Degla) revealed their richness in various valuable biochemical and mineral substances namely dietary fibers (22.5 – 94%), proteins (2.3 – 6.4%), ash (0.9 – 1.8%), sugars (5 – 6%), fats (7 – 13%), and phenolic compounds (3102 - 4430 mg/100g) [17, 18]. The oil of date pits, variety Khalas is composed of fatty acids (oleic acid: 56.1%, linoleic acid: 11.6%, lauric acid: 8.3%, myristic acid: 6.0%, and other minor fatty acids) [19] and natural antioxidants: polyphenols, sterols, tocopherols and carotenoids [20].

According to Karleskind [21], margarine is defined as a water-in-oil type emulsion that includes two essential phases: a continuous phase (fat phase) and a dispersed phase (water phase).It also contains additives (lecithin, salt, pigments, antioxidants, vitamins, etc.) distributed partly in the fatty and watery phases [22].

Currently the largest market for margarines is concentrated in North America (dominated by the United States and Canada), followed by Europe, Asia Pacific, South America, and Africa [23]. This market is growing mainly in the Middle East and Africa, with a compound annual growth rate of 2.14% from 2016 to 2025 [23].

The inclusion of margarine in this study was supported by the prevalence of Algerians using it to prepare sauces and pastries. On the other hand, oxidation is a process that occurs widely in foods and can be caused by the oxidation of lipids, proteins, and enzymes [24]. Among foodstuffs, margarine is a representative example of lipid oxidation because 82% of its content is made up of fat [21]. Hence, the usefulness of researching natural products to improve margarine stability and avoid its oxidation.

Given the remarkable composition of date kernel oil and its high antioxidant capacity we attempted to use it as an alternative natural food additive to a synthetic additive (α-tocopherol) commonly used as an antioxidant in margarines. Thus, *(i)* firstly the physicochemical and antioxidant characteristics of date pits oil were investigated, after that *(ii)* its effect on the oxidative stability of formulated margarine was achieved.

2 Material and Methods

2.1 Sample collection and preparation

The date variety "Mech-Degla Algerian" from Ouargla province (Algeria) was used, it was harvested in 2012. A batch of 50 kg of the whole date fruit was recovered where a random sampling was adopted. The pits were separated from the pulp manually and were then dried at 50 °C for 48 h until dry weight stabilizes. After that, they were crushed manually with a mortar and pestle, then crushed with an electric grinder to obtain a finer granulometry powder (2 mm) which was kept in the refrigerator (4 °C) until analysis [20].

2.2 Physicochemical characteristics of the date kernels

The morphological characteristics of 12 dates were study. The dimensions of the pits were determined (length and width) using a caliper with a precision of ± 0.1 cm, and their weights using an analytical balance with a precision of ± 0.001 g. Their moisture [25] and fat contents (%) were calculated as reported in [26], respectively [27].

2.3 Extraction and analysis of date pits oil

The extraction of oil was carried out by Soxhlet fat extraction method [28]. Briefly, the dried sample (3 g) was introduced into the Soxhlet extractor and the extraction of the oil with n-hexane (200 mL) was realized during approximately 24 h. Then, the obtained oil was placed in a rotary evaporator to remove all traces of solvent and stored in dark vial.

The evaluated physicochemical and quality parameters of oil were pH, moisture, acidity, acid index, peroxide index [25], iodine index [29], specific absorbance, ultraviolet parameters (K232, K270) [30], and refractive index [31].

The content of fatty acid methyl-esters was evaluated qualitatively and quantitatively using the gas chromatography (GC) method [32]. An aliquot of 0.5 g of oil was dissolved in 5 mL of hexane, and 0.5 mL of a methanolic solution of potassium hydroxide (2 N). The mixture was stirred for 30 s, and then centrifuged at 3000 rpm/min for 5 min. Two drops of the supernatant were taken and mixed with 1mL of hexane. The methyl-esters were analyzed from the supernatant solution by a gas chromatographer Chrompack C 9002 with

injector (SPLIT) for injection of liquids (up to 1 µL). The capillary column was DB 23 of 30 m × 0.32 mm × 0.25 µm. The oven temperature was set at 130 °C, the injector and detector temperature were set at 250 °C. The chromatograms were read in ascending order of number of carbons and unsaturation using "Shimadzu GC solution" for the treatment of the results.

2.4 Phytochemical analysis

2.4.1 Bioactive compounds contents

The total phenolic contents (TPC), of the obtained date pits oil, was determined following the method described by Juntachote et al. [33], using the Folin-Ciocalteu reagent. The absorption of the solution was measured at 760 nm and the results were given as mg equivalent of gallic acid /kg of oil (mg GAE/kg of oil). Total flavonoids content (TFC) was performed by the colorimetric method as described by Kumazawa et al. [34]. The absorbance was read at 430 nm and the results were expressed as mg equivalent of quercetin/kg of oil (mg QE/kg of oil).

Carotenoids' content was determined according to the methodology of Sass-Kiss et al. [35]. Briefly, 20 mL of hexane/acetone/ethanol mixture (2/1/1, *v/v/v*) were added to 5 g of oil. After stirring for 30 min, the upper phase was recovered. Then, 10 mL of hexane were added for a second extraction. The mixture of the two extracts was used for the determination of total carotenoids by spectrophotometry at 450 nm and the results were expressed as mg β-carotene equivalent/kg of oil.

2.4.2 Antioxidant activity

DPPH free radical scavenging activity was measured using the methodology of Blois [36]. An aliquot (0.025 mL) of the fat extract was added to 2 mL of methanolic solution of DPPH. The absorbance was measured at 517 nm after 30 min of incubation in the dark. The free radical scavenging activity was determined according to equation (1):

$$\text{DPPH scavenging effect (\%)} = (A0 - A_1/A_0) \times 100 \ldots\ldots (1)$$

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the sample.

2.5 Incorporation of date kernel oil into margarine

In order to determine the concentration required to be incorporated in the margarine, two concentrations of date kernel oil were tested (50 and 100 ppm). Margarine with 82% lipid phase (palm oil, sunflower oil and equivalent hydrogenated soybean oil) and18% liquid phase (β-carotene (12 mg/kg), aroma (25 µg/g of diacetyl), sodium chloride (0.60%), lactic acid (0.5 mL/kg) and potassium sorbate (300 mg/kg)) was produced at laboratory scale.

The date pits oil was incorporated in the lipid phase. After obtaining of the two phases, the margarine emulsion was blended together and cooled before being divided into 500 g tray. In parallel, a margarine reference was prepared without date pits oil using the same conditions. The final products were stored at 4 °C.

2.5.1 Physicochemical characterization of margarine

The physicochemical characterization of the formulated margarine was assessed by determining the pH, the solid content [37], the melting point [38] and the peroxide index [39].

2.5.2 Oxidative stability of margarine

The elaborated margarine's oxidative stability was evaluated by the Rancimat test [40]. Briefly, 3 g of samples were kept in a tube at 100°C with 10 L/h airflow under thermal breakdown conditions. In the measurement cell, which was filled with distilled water, the degradation products were transferred (50 mL). By using a conductivity meter, the induction time (also known as the oxidation stability) was calculated and represented in hours.

2.6 Statistical analysis

Using one-way and two-way ANOVA analysis of variance (ANOVA) and a post hoc "LSD test," the means of three independent values were compared. Significant differences were considered at $p < 0.05$ level, by using STATISTICA 5.5 software.

3 Results and Discussion

3.1 Characteristics of the studied date pits

3.1.1 Morphological characteristics

The detailed results concerning the morphological characteristics of date pits of the Mech-Degla variety from Ouargla region (Algeria) which was harvested in 2012 are determined by measuring weights, length, and width of twelve dates (n = 12) which were selected randomly. According to the obtained results, the dimensions of the studied date pits were from 0.7863 to 1.3930 g for the weight, 1.9 to 2.1 cm for the length and 0.5 to 0.7 cm for the width.

According to Abduh et al. [27], who studied 13 varieties of Libyan date pits, the average values of the parameters weight, width and length were 0.7 to 2 g, 0.8 to 1.1 cm and 1.8 to 2.8

cm, respectively. A significant difference has been reported between the trees concerning their diameter, weight and length of the kernel, even if the palms considered come from the same exploitation [41]. These differences can be induced by the types of pollen used by the producers [42]. Indeed, the significant effect of pollen on the morphological characters of the almond has been reported by the same author.

3.2 Moisture and fat composition of the date kernel powder

The detailed results regarding the moisture and fat composition of date pits powder are displayed in Table 1.

Table 1. Moisture and fat content of the date kernel powder

Constituents	Average values (% dry weight basis)
Moisture	5.00 ± 0.03
Fat (boiling)	9.84 ± 1.45

The moisture content is a quality criterion used mainly to estimate the degree of moisture in the date kernel powder, and it provides information on the stability of the product against the risk of deterioration during storage.

The date pits of the Mech-Degla variety from Ouargla region (Algeria) which was harvested in 2012.The moisture content of the date pits powder studied was 5.00 ± 0.03%. This value agrees with that reported (5.19%) by Al-Farsi et al. [43] (4.22 – 4.78%) for Shahal variety. It was, however, higher than the value found by the same authors with Mabseli variety (3.14%). Reversely, Hussein and Alhadrami [15] reported a considerable moister content reaching 7% for the date pits from Al Ain, UAE.

According to Hamada et al. [10], the water content of date pits from the Khalas, Lulu, and Fard varieties ranged between 7 and 11%.

The yield of fat obtained by hot extraction was between 8.59 and 13.16%, which can be compared with that reported in the literature. Indeed, Hamada et al. [10] have reported for 11 varieties of date pits, grown in the Qassim region of Saudi Arabia, value of 12.73% for the Allig variety; these authors have also found 13.2% for the Khalas variety, while Besbes et al. [20] 12.67% for the Allig variety. This rate is relatively high compared to that reported by Al-Farsi Al-Farsi et al. [43] in their study conducted on Tunisian varieties (Mabsili, Um-Salah and Shahal) which was much lower (5 – 6%) than those reported by previous researchers. The marked difference observed in composition of our samples and previous studies may be explained as evidence for the presence of chemical polymorphism due to the influence of environmental and ecological factors [44].

3.3 Date pits Oil quality parameters

The quality parameters of the studied date pits oil are summarized in Table 2.

Table 2. Physicochemical characteristics of the date pits oil

Parameters	Average contents
Iodine index (g iodine /100 g of fat)	53.00 ± 0.19
Acidity (%)	0.37 ± 0.17
UV extinction (%)	K_{232}: 1.528 ± 0.060 K_{270}: 0.381 ± 0.016
Refractive index at 40° C	1.4563 ± 0.0002
Peroxide value (meq O_2 / kg of fat)	3.66 ± 0.68

The determination of the peroxide value expressed in meq of O_2/kg of fat of a fatty substance gives information on its alteration by oxidation (AFNOR, 1988). The oxidation process of the oil studied is monitored by determining the peroxide value (PI). The fats are essentially altered by oxidation, a chemical phenomenon involving very different reaction mechanisms leading to oxidative or hydrolytic rancidity [45]. The obtained result (3.66 ± 0.58 meq O_2/kg of fat) is in accordance with the standard of refined oil (< 5). It also confirms the stability and resistance of date kernel oil to oxidation. This value was higher than that found by Abdel-Nabey [46] for six varieties of Egyptian date kernel oil (1.54 meq O_2/kgof fat). The marked difference observed in composition of our samples and previous studies may be explained as evidence for the presence of chemical polymorphism due to the influence of environmental and ecological factors [44].

The studied date kernel oil had an iodine value of 53 g of iodine /100 g of fat. This value was similar to that reported by Besbes et al. [20] for kernel oil of six Libyan date varieties (54.8 g iodine/100 g GC) and almost similar to the value (50.92 g iodine / 100 g) advanced by Abdel-Nabey [49] for kernel oil of six Egyptian date varieties.

The iodine value is a simple and rapid measure of total unsaturation, but it does not provide any indication of the fatty acid profile of oils. The iodine value experiment is based on the fact that fatty acids, corresponding to the oil, can bind as many iodine molecules as they have double bonds. Indeed, the higher the iodine value, the more unsaturated the oil is.

The refractive index of a substance is meant the ratio between the speed of a light of determined wavelength in the air and the speed of this same light in this substance. It is used to measure the purity of a sample.

The refractive index obtained at 40 °C was 1.4563, this value was similar to that determined at 40 °C by Besbes et al. [20] for the date kernel oil of Deglet Nour variety (1.457).

The value of the refractive index of date kernel oil is similar to those of the values of edible vegetable oils [29] which allows us to conclude the purity of oil.

The measurement of ultraviolet absorbance is one of the methods of measuring the state of oxidation of the oil. It allows following the evolution of the peroxidation and knowing the content of the secondary products of oxidation.

The results of the specific absorbance of the studied oil are comparable with those obtained by Besbes et al. [20] from the varieties Deglet Nour (K_{232}: 1.2) and Allig (K232: 2.5), as well as the value of K_{270} found for both varieties was 0.5. Our results are different compared to those of Codex [47] standard for olive pomace oil, which specifies a wavelength of 232 nm, a maximum specific extinction of 3.5 for virgin olive oil and a maximum specific extinction of 5.5 for olive pomace oil.

We noted that these values are lower than those set by the standard, which indicates that the studied oil contains a very low quantity of oxidation products.

By comparing the result of the iodine index and the specific absorbance it can be concluded that the date pits oil was composed with a high level of saturated fatty acid, a value that makes it resistant to oxidative.

3.4 Fatty acid profile of date-pits oil

The results of the fatty acid composition of the analyzed date kernel oil are recorded in Table 3. The fatty acids detected are palmitic, lauric, myristic, stearic, oleic, linoleic acid.

The oleic acid represented 44.90% of the studied date kernel oil; the majority fatty acids of the oil give information about its properties; they are monounsaturated and of type ω-9. This result is in agreement with the findings of Besbes et al. [20] where the major fatty acid found in date pits oil of two date palm (*Phoenix dactylifera* L.) cultivars, Deglet Nour and Allig, from the Degach region (Tunisia) was oleic acid, ranging from 41.3% for Deglet Nour seed oil to 47.7% for Allig seed oil.

The major fatty acid found in those cultivars was oleic acid, ranging from 41.3% for Deglet Nour seed oil to 47.7% for Allig seed oil. This is in agreement with previous reports.

Table 3. Fatty acid composition of date pits oil

Fatty acid	Content (%)
Lauric acid (C12:0)	19.53
Myristic acid (C14:0)	11.53
Palmitic acid (C16:0)	12.20
Stearic acid (C18:0)	3.87
Oleic acid (C18 :1) n 9 cis	44.90
Linoleic acid (C18 :2)	7.96

This fatty acid is also the most abundant in olive oil. Quantitatively, the second fatty acid encountered in this oil was lauric acid with a rate of 19.53%, followed by palmitic acid at 12.20%; myristic acid at 11.53%; linoleic acid at 7.96%; stearic acid at 3.87%. Therefore, the studied oil can be considered as oleic-lauric oil [48].

Besbes et al. [20] also proved that the kernel oil of Deglet Nour variety was considered as oleic-lauric oil, while the kernel oil of Allig variety was oleic-linoleic oil. On the other hand, Al-Hooti et al. [49] proved that date kernel oils were oleic-linoleic or oleic-palmitic type, this classification is based on the predominance of fatty acids in the composition of the seed oil studied.

It has been reported by Besbes et al. [20] that some varieties contained up to fourteen fatty acids, but generally, date kernel oil is characterized by the presence of five important fatty acids (C18:1, C18:2, C16:0, C14:0, C12:0) with oleic acid (C18:1) as the most abundant one [26].

The degree of saturation shows that the obtained date pits oil was composed of 47.14% of saturated fatty acids, a value that makes it resistant to oxidative rancidity, which is in agreement with the low result of the iodine index in the date kernel oil (53 g of iodine /100 g of fat), because the iodine index tells us about the overall degree of unsaturation of fatty substances. The degree of unsaturation was 52.86% of unsaturated fatty acids, of which 44.90% were monounsaturated and 7.96% were polyunsaturated. These results confirm the fluid aspect of the oil.

In a study conducted by Besbes et al. [20] on two cultivars of date kernel oil, oleic acid ranged from 41.3% for Deglet Nour kernel oil to 47.7% for Allig kernel oil. However, Al-Hooti et al. [49] found a higher content of oleic acid (53.3 – 58.8%) in date kernel oil extracted from Emirate varieties.

The fatty acid composition of date pits oil seems to vary slightly with cultivars. This may be due to the different physiological state of the pits and the influence of climatic factors in the studied regions [20].

3.5 Minor components

The compositions of bioactive compounds namely total phenolics, total flavonoids and carotenoids which are considered minor compounds of date kernel oil are summarized in Table 4.

Table 4. Composition in total phenolics, flavonoids and carotenoids of date–pits oil

Compounds	Content (mg/kg of oil)
Total phenolics	112.92 ± 26.57
Flavonoids	15.7 ± 0.7
Carotenoids	125.53 ± 0.11

The total phenolic content (polar and nonpolar) was 112.92 ± 26.57 mg GAE/kg of oil. It has been reported that the average of the TPC of date seed oil ranged from 0.64 to 1.27 mg/g and this content varies by cultivar [50]. Indeed, Besbes et al. [20] have reported 520.8 mg caffeic acid Eq/kg in Deglet Nour seeds oil and 220.3 mg caffeic acid Eq/kg in Alligone. Moreover, Ourradi et al. [48] have reported significant difference in phenolic content among eight Moroccan varieties (58.04 ± 0.30 to 181.03 ± 0.75 mg GAE/100 g). It is not appropriate to compare our results with those reported in the literature because the operating conditions were different, namely the storage conditions of the date kernel oil, which may affect the polyphenol composition [51], the extraction conditions (time, temperature, solvent used) and the type of the variety analyzed.

The flavonoid content of the studied date pits oil was 15.7 ± 0.7 mg QE/kg oil; this value is very low compared to those reported by Ourradi et al. [48] which ranged from 22.81 ± 0.08 to 53.41 ± 0.72 mg RE/100 g oil.

The content of carotenoids present in the studied kernel oil was 125.53 ± 0.11 mg/kg, this significant amount supports the various authors' interpretation of the yellow color of date pits oil as being due to these pigments. This value is significantly higher than that reported by Nehdi et al. [52] in a study conducted on the oil of the date stone of the species *Phoenix canariensis* (55.1 ppm). Additionally, Ourradi et al. [48] in their study on eight Moroccan varieties recorded low values ranged from 12.35 ± 0.03 to 17.57 ± 0.02 mg/kg oil. Surprisingly, Mrabet et al. [50] reported very low values ranged from 2 to 5 mg/kg oil. Indeed, Uzzan et al. [53] have reported values ranged from 5 to 10 ppm. Carotenoids in oils are found in varying concentrations depending on the variety, maturity level, technical system used for oil extraction, age of the oil [54], and genetic factors [55].

3.6 Free radical scavenging activity

Concerning the antioxidant activity, the cold-extract of the date kernel oil showed a higher anti-radical activity than hot extract, they reduced 55.91% of the free radicals and 30%, respectively. This difference is due to the oxidation of the hot extracted oil (temperature being a factor of oxidation) and the characteristic state of the oil. This result cannot be compared with other reports because there are not expressed in the same way. The reducing capacity of the DPPH radical of the date kernel oil of eight Moroccan varieties varied from 17.28 ± 1.06 to 47.37 ± 1.05 mg ascorbic acid equivalent/100 g Ourradi et al. [48] and that of two Algerian date palm (*Phoenix dactylifera* L.) cultivars expressed in terms of IC_{50} value was ranged from 0.140 ± 0.005 to 0.330 ± 0.005 mg/mL) [56].

3.7 Physicochemical properties of margarine

Table 5 lists the physical and chemical properties of studied margarine enriched with date pits oil.

It must be noted that the moisture content of the margarine control was statistically ($p < 0.05$) different from the two margarines elaborated with date kernel oil; it was about 13.85% for MF1 (control) while for the margarines prepared with 100 ppm (MF2) and 50 ppm (MF3) of date kernel oil the values were 15% and 15.06%, respectively. These results correspond to the criteria fixed for the elaboration of this product; they are in conformity with the standard [57] which sets the water content at 16%.

Concerning the pH of the aqueous phase for the three products it was about 4.2, 4, and 4.1, respectively. The pH of the aqueous phase of the three products was too close; therefore, the pH values of the two emulsions were stable. According to Karleskind and Wolff [58], it is preferable to control the pH of the aqueous phase, to avoid the growth of microorganisms at low value. Generally, the pH is set between 4.0 and 5.5 (in some margarines it can be up to values of 3.0 to 3.5).

The melting points for the three margarines MF1, MF2 and MF3 are shown in table 5, ranging from 35 to 36°C, they correspond to that chosen for the recipe selected.

The standard for the melting point of spread margarine varies between 33 and 37◦C, which means that margarine can quickly melt in mouth and be firm at room temperature to withstand mechanical work during its spreading [59].

Table 5. Physicochemical characteristics of the elaborated margarine enriched with date pits oil

Parameters	Contents		
	MF1	MF2	MF3
Humidity (%)	13.85 ± 0.13[b]	15.00 ± 0.15[a]	15.06 ± 0.19[a]
pH of aqueous phase	4.20 ± 0.012[a]	4.00 ± 0.01[a]	4.00 ± 0.018[a]
Meting point (°C)	35.80 ± 0.15[a]	35.2 ± 0.19[a]	36.00 ± 0.16[a]
Peroxide index (Meq O_2/Kg MG)	0.32 ± 0.01[a]	0.30 ± 0.02[a]	0.32 ± 0.01[a]

MF1: Control margarine; MF2: Margarine with 100 ppm of date kernel oil; MF3: Margarine with 50 ppm of date kernel oil.
Each value is the average of three replicates ± standard deviation. In each line, different letters indicate significant difference ($p < 0.05$).

According to Belitz et al. [60], the melting point depends on factors attributed to the structure of triglycerides. It must be fixed in such a way that the margarine is melting in the mouth but also plastic at room temperature to support the mechanical work during spreadability. The results are within the range of melting temperatures of 15 Turkish margarines (33.0 and 36.9 °C) [61].

The first products formed by oxidation are peroxides or hydroperoxides which then evolve into more stable structures: volatile and non-volatile products [62]. The peroxide value is a very useful and sensitive criterion for assessing the early stages of oxidative deterioration [32].

We noticed that the value of the peroxide index is almost the same for the three margarines, it is clearly lower than the standard used by the company which is 10 meq O_2/Kg MG (NE.1.2.98.88) and lower than 5 meq/kg, maximum required by the standards [47].

This preliminary study shows that date pits oils could easily be conserved due to their high oxidative stability.

3.8 Texture analysis by NMR

The Solid Fat Content (SFC)index refers to the percentage of fat that is solid at different temperatures. The results obtained for SFC of the three studied margarine samples are presented in Figure 1.

From the obtained results, it can be seen that the formulated margarines were plastic and easy to spread, at 37°C. The SFC index was lower than 6%, and therefore it can be considered that the margarine can melt easily in the mouth [63]. The information obtained from the solid curves (SFC) allows predicting the compatibility of the fat, as well as the final characteristics of the finished product. The solids content at different temperatures provides good indications of the general behavior of the fat on one hand, and information that is primarily used for formulation and the development of new products on the other. In fact, it is well known that each type of margarine (cooking, spreading, creaming, puffing) corresponds to a certain type of solid curve [58, 64].

According to Figure 1, a decrease in the solid going from low temperatures (5°C) to high temperatures (40°C) can be seen, even tending to zero in the case of MF1. Lumor et al. [65] studied the properties of rapeseed oil blend and palm mid-fraction at different mass percentages, in terms of solid content (SFC) and crystal morphology. According to their results, a good margarine for spreading is the one with a SFC of at least 7.6% at 10°C, necessary to maintain the crystalline structure, thus ensuring a good spreadability of the margarine once removed from the refrigerator and melting completely in mouth. The total melting in mouth of this one allows a better release of the flavor and gives a sensation of softness more increased. Oils and fats with moderate SFC between 7.6 – 13% at 10°C and a steep solid curve at non-refrigeration temperatures are suitable for the formulation of spreadable margarines. Compared to the results of Lumor et al. [65] at 10°C the margarines MF1, MF2 and MF3 had this minimum of 7.6% in SFC, with average values of 28.3%, 29.7 and 22.8%, respectively, which proves their acceptable spreadability.

These results are also in agreement with the recommendations of Ribeiro et al. [66], whose SFC of margarines at 10°C should not exceed 32% for the spreadability to be guaranteed at refrigeration temperatures. According to Ribeiro et al. [66], Karleskind et al. [21] the amount of solid present at different temperatures during crystallization and also inversely during melting is undoubtedly a key parameter to consider and will immediately specify the fat phase.

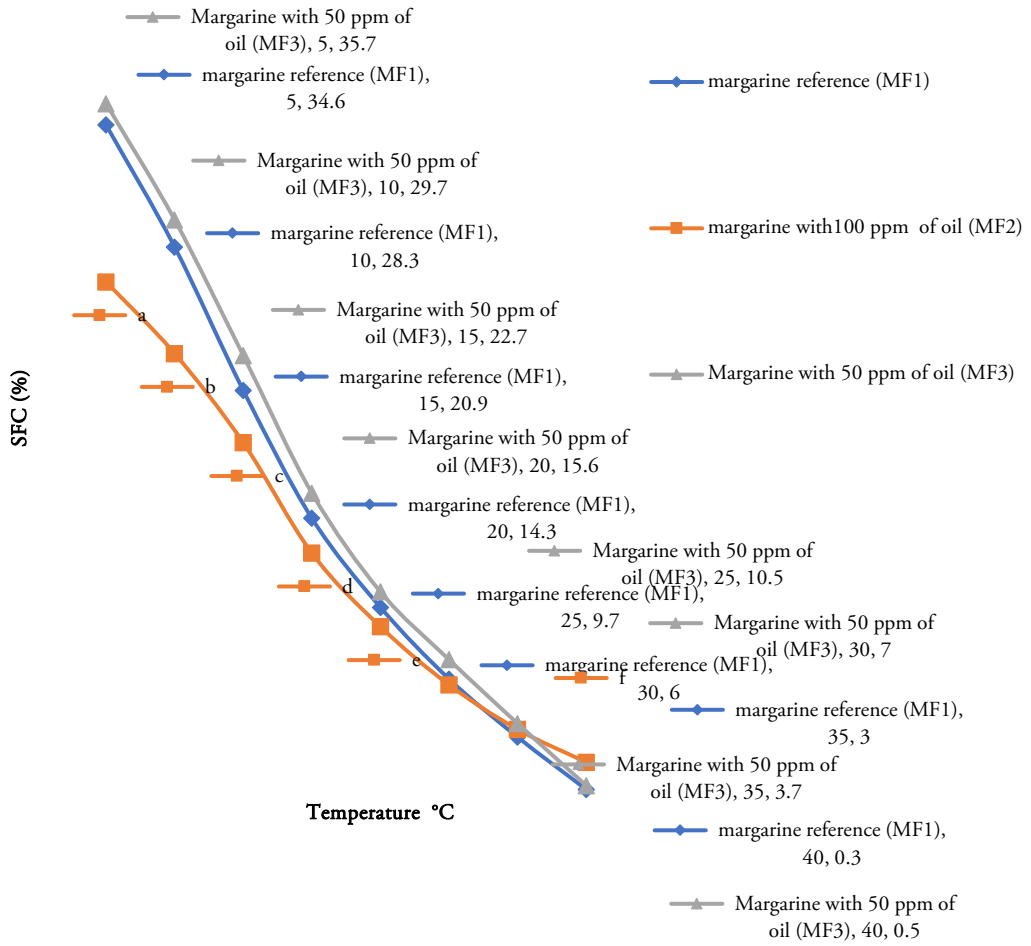

Figure 1. Solids' content (SFC) of margarines enriched with date pits oil.

Means with different letters were significantly different ($p < 0.05$); Values represent the mean ± standard deviation; $n = 3$.

3.9 Rancimat test

Lipid oxidation of foods is a growing problem in food processing. The Rancimat test can predict the oxidative stability of the oil as well as its shelf life [67]. To estimate the stability or susceptibility of margarine to oxidation, the samples were subjected to an accelerated oxidation test (Rancimat). The results of the analysis of the MF1, MF2 and MF3 samples are shown in Figure 2. They are presented in the form of graphs (parabolic function) representing the induction time as a function of the conductivity.

According to the results obtained in Figure 2, the induction time of the control margarine (MF1) is 17.67 hours followed by margarine enriched with 100 ppm of date pits oil (MF2) with a rate of 19.90 hours and finally margarine enriched with 50 ppm of date pits oil (MF3) with a value of 22.56 hours.

According to Arain et al. [68], this oxidative phenomenon is explained by the fact that the volatile degradation products are trapped in the distilled water, thus increasing the conductivity. The induction period is determined from the inflection point of the conductivity curve [40]. Accelerated oxidability tests on Rancimat were thus carried out at

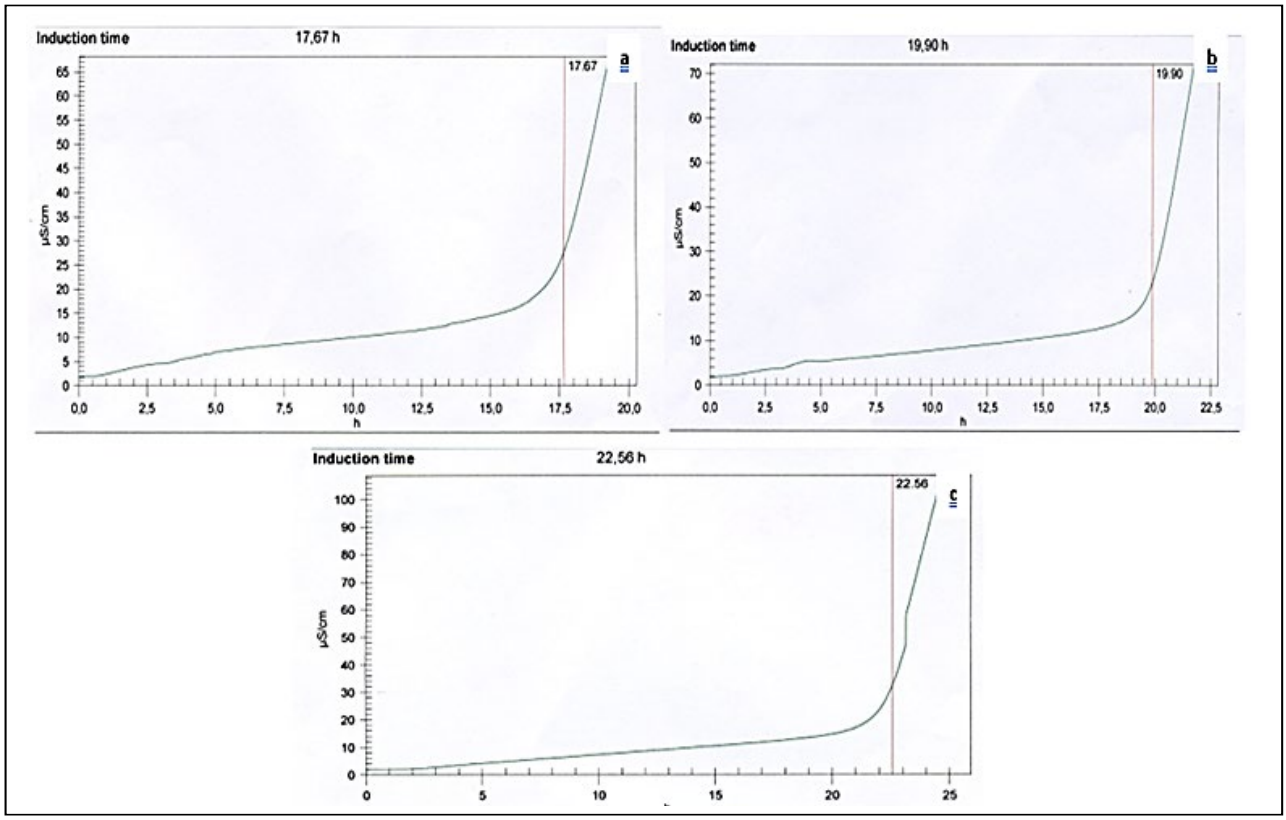

Figure 2. Oxidative stability curve in the Rancimat test of a) MF1 (control), b) MF2 (produced margarine with 100 ppm date kernel oil and c) MF3 (produced margarine with 50 ppm date kernel oil.

concentrations of 100 and 50 ppm of date kernel oil in the table margarine.

The margarine enhanced in date kernel oil at 50 ppm resisted oxidation after 22 hours, but the one at 100 ppm reacted after 19 hours. It confirms that the addition of the natural antioxidants can improve the results of oxidative stability showed a trend toward high oxidation in case of margarine containing 100 ppm of the date kernel oil. The prooxidant properties of some antioxidants at high concentrations could explain this phenomenon [69, 70]. It is well established that in the fat systems such as margarine, the addition of the antioxidant at low concentration is highly essential for their oxidative stability [23]. Margarine with synthetic antioxidant exhibited a short induction time, and therefore, showed less prevention against lipid deterioration. Antioxidants in the fat extend its oxidation resistance, resulting in greater stability. Synergistically reacting antioxidants in date kernel oil are stronger than synthetic -tocopherol.

The date pits have a higher antioxidant content (α-tochopherol and phenolic compounds) which are well known as food lipid antioxidants [6].

4 Conclusion

The current study aimed to valorize date kernels which are generated in large quantities in Algeria by the date processing industries. This involves extracting their oils and using them as natural ingredients in the preservation of margarine which is too prone to oxidation. Analyzes of date kernel oils revealed that they comply with standards, their richness in polyunsaturated fatty acids beneficial for human health, particularly oleic acid with proportion exceeds 40%. In addition, this oil contains minor compounds with strong antioxidant power including total phenolics, flavonoids and carotenoids. So, the kernel date oil exhibited an interesting effect in reducing the DPPH radical. Interestingly, the addition of this oil to margarine did not significantly impact its physicochemical properties; on the contrary it increased its stability to accelerated oxidation compared to the control. However, it would be interesting to complete this work by carrying out a sensory analysis in order to have the consumer opinion.

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