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Physico-chemical properties and antioxidant activity of five Algerian honeys and margarines formulated with honey as antioxidant and preservative

Physikalisch-chemische Eigenschaften und antioxidative Aktivität von fünf algerischen Honigsorten und Margarinen, die Honig als Antioxidationsmittel und Konservierungsstoff enthalten

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Summary

The objective of this study was to establish a conceptual approach for developing a margarine enriched with honey free from the synthetic antioxidant „α-tocopherol" and the chemical preservative „potassium sorbate". Five types of honey of different origins were considered. Pollen analysis revealed that four were of monofloral origin (*Fabaceae, Fagaceae* and *Asteraceae*) and one sample was polyfloral. The physico-chemical parameters of honeys and formulated margarines proved compliant with standards. The addition of honey to margarines makes them rich in phenolic compounds. Their antioxidant potential, assessed by Ferric Reducing Antioxidant Power Assay (FRAP) and CUPRAC, revealed values 6.44 (M3) and 28.79 mg GAE/100g (M4), and 22.40 (M5) and 57.32 mg GAE/100g (M2), respectively. The antibacterial power of honeys gave margarines greater microbiological stability. In addition, follow-up assessment of oxidative stability using Schaal, conjugated diene and triene, thiobarbituric acid reactive substances (TBARS) and Rancimat tests over a twelve-week storage period revealed that the honeys used offered statistically significant protection against oxidation of the lipid phase. Margarines M4 and M5 were the most resistant to oxidation, due to the quantity and the quality of the antioxidants present in the honey. Spearmane correlations showed that these Algerian honeys were an excellent source of antioxidants, and that honey flavonoids retard margarine oxidation. Chemometric analysis revealed a single homogeneous group (M1 and M2) and confirmed the correlations obtained. In conclusion, honey could be used as a natural antioxidant and preservative in margarine.

Keywords: Algerian honeys, water-in-oil emulsion, physico-chemical parameters, antioxidant, oxidative stability and Fatty acid profile

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Introduction

Today, agri-food industries are increasingly seeking solutions to innovate food products. Current aims are to transform them into healthy, less impacting the environment, economically feasible, profitable and while meeting the nutritional recommendations ordered by the national and international agencies (Smetana et al., 2020). The current trend of innovations in the formulation of new foods is the use of natural bioactive substances of plant and/or animal origin (Procopio et al., 2022). One of the important processed products in the food industry is margarine, which is considered an alternative to butter and other fat-soluble spreads. Due to its plasticity and great spreadability as well as its unique flavor, it is used in bakeries and cookware (Zhu et al., 2019). Margarine is a water-in-oil emulsion which is divided into two phases (aqueous and oily) and an inter-facial region, the latter being the contact region between the two phases, which represents the critical zone where lipid oxidation occurs (Rafanan and Rousseau, 2019). Oxidation of margarine is influenced by several factors such as the initial concentration of oxygen in the aqueous or fat, the presence of peroxides, the structure of the interface, the presence of oxygen in the packaging and the storage conditions (Silva et al., 2021). The oxidation of lipids causes a loss of quality, generates undesirable aromas, unpleasant odors as well as harmful effects on health, which lead to the rejection of the product by the consumer (Carrizo et al., 2016). This problem can be solved by the addition of synthetic antioxidants, which slow or stop oxidation by acting as radical scavengers, singlet oxygen quenchers or by UV absorption. However, added synthetic antioxidants cause harmful health problems for consumers (Kang et al., 2020). The replacement of synthetic antioxidants by other natural ones is recommended.

Honey can be considered one of the natural sweeteners. Its components (phenolic acids, flavonoids, enzymes, vitamin, minerals…) are responsible for its biological properties (Seraglio et al., 2019). Due to its healthy properties, it is not only used as a sugar substitute in sweets, confectionery and bakery products but also it is added to different foods, such as sausages, beef patties (Poltorak et al., 2018), fruits and vegetables and beverages (Abu Saeid et al., 2021) in order to improve their bioactivity, develop functional foods, and above all extend their shelf life.

To our knowledge, although honey is widely used in food products, no research has been conducted on the addition of honey alone to spreadable margarines as a natural antioxidant and preservative. Therefore, the present study aims to develop spreadable margarines incorporated with different concentrations of honey. This is intended to replace synthetic antioxidants (such as vitamin E) and chemical preservatives (like potassium sorbate). The study will evaluate the effectiveness of honey in preventing fat deterioration through a series of physico-chemical analyses, including pH, acidity, salt content, water content, melting point, and color. Additionally, antioxidant assays will be performed. The study will also assess primary and secondary lipid oxidation by measuring fatty acids, peroxide value, schaal, conjugated dienes and trienes, TBARS, and conducting rancimat tests. Finally, microbiological analyses will be carried out to ensure margarine quality and determine shelf life.

Material and methods

Honey samples

Five samples of honey from four regions of Algeria (El Bayadh, M'Sila, Biskra and Bejaia) were harvested on September 4, 2021 from beekeepers (Table 1). The samples were stored in glass containers, at room temperature and away from light and humidity.

Elaboration of margarines enriched with honey

The preparation of margarines was produced manually on a laboratory scale at the research, and development laboratory of the „CEVITAL" agri-food complex in Bejaia, Algeria, and carried out according to the method described by Chougui et al. (2015). To find the appropriate amount of honey to introduce, the concentrations of honey added were determined by IC_{50} 2,2-diphenyl-1-picrylhydrazyl (DPPH). The two phases were prepared separately: the fatty phase was prepared with a blend of three vegetable oils (palm oil, sunflower oil and inter-esterified oil (melting point 43 °C)), while the aqueous phase was prepared with a blend of osmosed water, honey, salt (NaCl; flavor enhancer), pasteurized reconstituted milk (protein source), and lactic acid (pH corrector). Finally, an emulsifier (soy lecithin and glyceryl monostearate), and β-

TABLE 1: *Geographic origins, sensory characteristics and pollen spectra of the honey samples.*

Sample	Sensory characteristics		Geographic origin	Dominant pollen (≥45%)		Accompanying pollen (16 - 44%)		Minority pollen (15 – 3%)		Very minor pollen (<3%)	
	Color	Consistency		Type of pollen	Frequency (%)	Type of pollen	Frequency (%)	Type of pollen	Frequency (%)	Type of pollen	Frequency (%)
H1	Dark brown	Liquid	Biskra, (Ouled Djellal)	*Fagaceae*	48	*Fabaceae* *Eucalyptus*	20 18	*Myrtaceae* *Ericaceae*	5 5	*Asteraceae*	4
H2	Brown	Liquid (start of crystallization	M'Sila (Ain Erriche)	*Asteraceae*	56	*Fabaceae*	35	*Fagaceae* *Ericaceae*	5 4	*Absence*	-
H3	Brown	Liquid	El Bayadh	*Fabaceae*	45	*Asteraceae*	25	*Fagaceae* *Eucalyptus* *Lamiaceae*	14 10 4	*Lavender*	2
H4	Black	Liquid	M'Sila (Boussada)	*Asteraceae*	51	*Eucalyptus* *Fabaceae*	20 16	*Fagaceae* *Ericaceae*	9 4	Absence	-
H5	Yellow	Crystallized	Bejaia, (Melbou)	Absence	-	*Asteraceae* *Fabaceae* *Eucalyptus*	37 30 20	*Fagaceae*	11	Tiliaceae	2

carotene are added to prevent separation of the two phases, and to achieve a color close to that of butter, respectively. The two margarine phases, oily and aqueous, were free of synthetic antioxidants (vitamin E), and potassium sorbate (a chemical preservative) respectively. The fatty phase is stirred at 45 ± 2 °C on a stirring hot plate (MS7-H550-Pro) for 15 min. Then, using a Heidolph Hei-TORQUE Overhead Stirrers homogenizer, the emulsion was formed by adding the aqueous phase. Homogenization was carried out at 2 bars for 10 min, then at 3 bars for 10 min. The mixture was cooled to 4 °C to crystallize the fatty acids, and manual agitation ensured that the formulated margarines were smooth and homogeneous. For each honey sample, 500 g of margarine was prepared, packaged in two 250 g trays and stored in a refrigerator at 4 °C.

Pollen profiles

The melissopalynological analysis was carried out according to the method of Louveaux et al. (1978).

Physico-chemical parameters of honeys and margarine enriched with honey

Water content and brix of honeys were determined using an Abbe refractometer (AR 12) at 20 °C. The electrical conductivity was determined by a conductivity meter with a solution containing 20% of the dry matter. HMF was analyzed with a 20% (w/v) honey solution. The proline was dosed with a volume of 500 µL of 5% (w/v) honey (Bogdanov et al., 1997). The protein assay was carried out with a volume of 100 µL of the 50% (w/v) aqueous honey solution (Azeredo et al., 2003), and the results were expressed in mg equivalent of bovine serum albumin (BSA)/100 g of honey with reference to the BSA calibration curve ($y = 1.058x + 0.0244$ $R^2 = 0.9936$). The absorbances were measured using a UV-VIS spectrophotometer (SECOMAM UviLine 9400). pH was measured using a pH meter (BANTE instrument) by introducing the electrodes into a 10% (w/v) honey solution and the aqueous phase of the margarine. The color (L*, a*, b*) measurement was performed with the portable colorimeter NR0 (3NH, Shenzhen, China). The salt content (NaCl) of margarine was analyzed using the Mohr protocol (AOAC, 1990). Water content, acidity, and melting point of margarine were analyzed according to ISO 662 method.

Antioxidant content

The Folin-Ciocalteu method was used to determine the content of phenolic compounds according to the protocol reported by Niathani et al. (2006). The method described by Al et al. (2009) was used to determine the flavonoids content. The results were expressed in mg equivalent of gallic acid / 100 g of honey using the gallic acid calibration curve ($y = 5.29 x + 0.0315$; $R^2 = 0.9918$) and in equivalent mg of quercetin (Q) /100 g of honey using the quercetin calibration curve ($y = 0.368 x + 0.0014$; $R^2 = 0.9867$), respectively.

The antioxidant content of margarines is also determined after extraction of 5 g of margarine, using the protocol described by Longoberdi et al. (2012).

Antioxidant activities

The degradation of DPPH solution was estimated according to the method reported by Noumi et al. (2011) and the IC_{50} was determined by linear regression analysis of the percentage inhibition versus concentration curve. CUPRAC test was performed according to the method of Apak et al. (2004), and the results were expressed in mg equivalent of gallic acid / 100 g of honey using the gallic acid calibration curve ($y = 0.1527 x + 0.0305$; $R^2 = 0.9967$). FRAP test was analyzed according to the method described by Alvarez-Suarez et al. (2010). The results were expressed in mg equivalent of gallic acid /100 g of honey using the gallic acid calibration curve ($y = 5.905 x + 0.0785$; $R^2 = 0.9875$).

Fatty acid profile

The fatty acid profile of margarines enriched with honey was performed by gas-liquid chromatography (Agilent 6890N Network GC) with flame ionization detection (GC-FID), equipped with a DB-23 Agilent 122-2362 capillary column. A dissolution of 5 g of the margarine fat phase was performed with 5 mL of hexane, then 0.5 mL of methyl KOH (2N) was added. After 30 s of stirring, the solution was centrifuged at 3000 rpm for 5 min. Two drops of the supernatant were mixed with 1 mL of hexane to analyze the methyl esters obtained by injecting 1 µL of the latter into a gas chromatography column (60 m length, 0.25 mm diameter and 0.25 µm thickness). Temperatures of 260 and 230 °C were set for the injector and detector, respectively. The temperature gradient of the column was programmed to increase from 170 to 230 °C at a rate of 3 °C/min and then maintained for 30 min. The results were expressed in % and the identification of fatty acids was obtained by their retention time compared to a reference chromatogram from a mixture of certified fatty acid methyl esters (37 fatty acids from C4 to C24).

Evaluation of the oxidative stability of margarine enriched with honey

The Oxidation stability was studied by five methods over a 12-week period

Peroxide index (PI) and Shaal test

The peroxide index and the Shaal test, which involves oxidizing the fat in an oven at 30°C, are carried out according to ISO 3960 fourth edition (2017) once a week for twelve weeks.

Conjugated dienes and triens (UV)

Determination of conjugated dienes and triens (UV) was carried out by 0.01 g of margarine. This quantity is dissolved in 10 mL of pure cyclohexane, then the absorbance of the solution obtained is measured by spectrophotometer at two different lengths 233 and 270 nm (Wolff, 1968). The results were calculated using the following equation:

$$K_\lambda = E_\lambda / (C \times S)$$

Where: K_λ: Specific absorbance at wavelength λ; E_λ: Absorbance measured at wavelength λ; VS: concentration of the solution (g/100 mL); S: thickness of the bowl (cm).

TBARS test

Assay of substances reactive with thiobarbituric acid (TBARS) was analyzed with 2 g of margarine which are homogenized in 16 mL of trichloroacetic acid (20%, w/v). Then, the mixture was centrifuged at 4000 rpm for 15 min. A volume of 2 mL of the 20 mM TBA solution is added to 2 mL of the supernatant (the extracted MDA) with stirring for 5 seconds in a vortex. The mixture was placed in a water bath at 95 °C for 10 min. After cooling the mixture, the absorbance was measured at a wavelength of 532 nm (Draper and Hadley, 1990). Results were expressed in mg MDA.Kg^{-1}.

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Rancimat test

Rancimat test was carried out by introducing 3 g of margarine into an air oxidation bottle, and under thermal decomposition conditions (98 °C) with an air flow rate set at 10 L/h (Metrohm743 Rancimat). The degradation products formed were transferred to the measuring cell filled with distilled water (60 mL). The induction time (oxidative stability) was determined using a conductivity meter, and was expressed in hours (ISO 6886, 2006).

Microbiological analysis

Antibacterial activity of honey

The bacterial strains used were *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853. The honey solutions used were 25, 50, 75 and 100 %. The evaluation of the antibacterial activity of honey was carried out by the well method. This method involved mass inoculation of Mueller Hinton (MH) agar using 1 mL of a bacterial suspension. The suspension was prepared in sterile physiological water from a 20-hour fresh culture and contained approximately 10^7 cells/mL. Then, five wells (about 6 mm) were dug in the agar using the upper part of the Pasteur pipette in which a small quantity of agar was poured at the bottom of the well in order to avoid the passage of the honey solution under the agar. Each well was received a volume of 100 µL of each honey solution while the fifth well were received a volume of 100 µL of saline solution. Incubation was carried out at 37 °C for 24–48 h after a diffusion period of 2 h at 4 °C (Brudzynski et al., 2012). The sensitivity profile of the bacteria to honey was determined by measuring the zone of inhibition at the turns of the wells on the Petri plate.

Microbiological analysis of prepared margarines

Samples of prepared margarines stored at a temperature of 4 °C were subject to microbiological analyses once a week for 12 weeks. The different microorganisms analyzed were aerobic germs, total coliforms, yeasts and modls, *E. coli, S. aureus* and *Salmonella* using standard methods (ISO 6579, 2002).

Statistical analysis

The analyzes were carried out in triplicate, the means, and the standard deviations were calculated with Microsoft Office Excel 2016. The statistical analysis of the results was carried out by the STATISTICA 7.1.2 software (Statsoft Co., Tulsa, OK, USA) with the ANOVA Kruskal Wallis app. The significant effect of the time of elaborated margarines was carried out by ANOVA of Friedman, and supplemented by the Wilcoxon test. Spearman correlations and PCA were also performed with STATISTICA 7.1.2.

Results and discussion

Pollen profiles

The results of the pollen analysis showed the presence of four samples of monofloral honeys (the H1 and H3 honeys are from the *Fagaceae* and *Fabaceae* families respectively, the H2 and H4 honeys are from the *Asteraceae* family) while H5 honey is a polyfloral honey. A total of nine pollen families are identified. Monofloral honeys have dominant pollens (> 45%) belonging to three botanical families: *Fagaceae* (H1), *Fabaceae* (H3) and *Asteraceae* (H2 and H4) which have a frequency of 17, 29 and 35%, respectively which indicates the richness of the harvesting regions in these families. Then, *Eucalyptus* family which is presented by H1, H3, H4 and H5 comes in 2nd order with 14%. However, the five remaining pollen families including *Ericaceae* (H1, H2 and H4), *Lamiaceae* (H3), *Lavader* (H3), *Myrtaceae* (H1) and *Tiliaceae* (H5) represent only 4% of the total pollen, and are judged as minority pollens or very minority. Polyfloral honey (H5) contains accompanying pollens including: *Asteraceae, Fabaceae,* and *Eucalyptus* which can be explained by the absence of large-scale monocultures in the harvest region (Bejaia, Algeria). The pollen content of honey depends on the botanical richness of the region, the climatic, and environmental conditions, the distance from the hive to the flower field as well as the strength of the bee colony (Ouchemoukh et al., 2007). The pollen analysis results obtained are similar to those recorded by Otmani et al. (2021) on 26 Algerian honeys.

Physico-chemical parameters of honeys and formulated margarine

The results of the physicochemical parameters are summarized in Table 2a and Table 2b.

Water content and Brix

The water content of formulated margarines and honeys ranges from 11.5 (M5) to 15.15 (M4)% and from 13.83 (H2) to 15.52% (H1), respectively. These recorded results

TABLE 2a: *Physico-chemical, phenolic compounds and antioxidant activities of five Algerian honeys.*

	H1	H2	H3	H4	H5
L*	42.16±0.29^{c}	52.36±2.24^{b}	52.47±0.13^{b}	58.72±0.10^{a}	38.19±0.54^{d}
a*	5.72±1.02^{ab}	4.81±0.46^{b}	4.29±0.01^{c}	2.33±0.03^{d}	6.20±0.02^{a}
b*	30.46±0.44^{c}	35.21±1.07^{b}	36.16±0.12^{b}	40.34±0.11^{a}	18.83±0.13^{d}
pH	6.25±0.07^{c}	4.58±0.02^{b}	4.50±0.06^{b}	4.59±0.04^{b}	4.26±0.03^{a}
EC(mS/cm)	1.09±0.01^{d}	1.15±0.01^{c}	0.97±0.00^{a}	1.27±0.02^{ab}	0.79±0.00^{ab}
Brix(%)	82.85±0.25^{d}	84.38±0.13^{a}	83.63±0.13^{b}	83.63±0.13^{b}	83.38±0.13^{c}
Water content(%)	15.52±0.12^{c}	13.83±0.10^{a}	14.65±0.04^{b}	14.65±0.04^{b}	14.88±0.20^{b}
HMF(mg/kg)	1.9±0.17^{a}	4.04±0.40^{b}	4.08±0.23^{c}	8.43±0.46^{d}	8.98±0.45^{d}
Proline(mg/kg)	389.65±2.94^{d}	377.96±0.68^{c}	510.83±1.17^{b}	632.4±1.17^{a}	507.32±1.17^{c}
Protein (mg BSAE/100g)	386.2±0.95^{a}	303.97±0.95^{d}	324.76±0.95^{c}	351.23±0.95^{b}	238.75±0.95^{e}
TPC(mg GAE/100g)	79.3±0.19^{d}	91.97±0.19^{b}	87.05±0.19^{c}	110.03±0.19^{a}	75.08±0.11^{e}
FLA(mg QE/100g)	69.57±2.72^{c}	84.06±1.57^{b}	60.51±1.57^{d}	96.74±2.72^{a}	67.75±1.57^{c}
IC50 DPPH(mg/ml)	1.62±0.00^{b}	2.13±0.00^{d}	1.65±0.00^{c}	2.37±0.00e	1.16±0.00a
FRAP(mg GAE/100g)	65.14±1.03^{b}	85.46±1.70^{a}	52.5±1.35^{c}	85.92±1.03a	66.72±1.35 b
CUPRAC (mg GAE/100g)	64.75±0.15 ^{b}	67.45±0.26^{a}	64.31±0.26^{bc}	64.13±0.15^{c}	59.59±0.26^{d}

EC: electrical conductivity, TPC: total Phenolic compounds, FLA: Flavonoids. Values are mean ± standard deviation. Means followed by the same letter in each line are not different using the ANOVA test (Analysis of one Variance).

TABLE 2b: *Physico-chemical parameters, phenolic compounds and antioxidant activities of margarines enriched with Algerian honeys.*

	M1	M2	M3	M4	M5
L*	70.21±0.96^{a}	68.99±1.39^{b}	68.76±0.81^{c}	67.39±1.61^{e}	67.94±0.24^{d}
a*	3.20±0.20^{e}	3.26±0.13^{d}	3.92±0.17^{c}	4.10±0.19^{b}	4.85±0.33^{a}
b*	23.37±0.32^{d}	22.38±0.63^{e}	26.60±0.42^{c}	27.08±0.37^{b}	31.60±0.70^{a}
pH	3.77±0.01^{a}	3.54±0.08^{a}	3.61±0.01^{a}	3.53±0.03^{a}	3.60±0.01^{a}
Water content (%)	12.58±0.14^{a}	13.27±0.70^{a}	14.43±0.02^{a}	15.15±0.28^{a}	11.50±0.00^{a}
Acidity (%)	0.27±0.00^{a}	0.28±0.00^{a}	0.28±0.00^{a}	0.27±0.00^{a}	0.28±0.00^{a}
Melting point (°C)	36.33±0.15^{a}	36.07±.21^{a}	36.97±0.06^{a}	36.77±0.25^{a}	36.17±0.55^{a}
Salt content (%)	0.20±0.01^{a}	0.20±0.00^{a}	0.23±0.01^{a}	0.21±0.00^{a}	0.29±0.00^{a}
TPC(mg GAE/100g)	22.72±0.29^{d}	34.69±0.76^{b}	30.28±0.29^{c}	53.15±0.50^{a}	21.64±0.50^{e}
FLA(mg QE/100g)	21.56±1.57^{d}	32.43±4.15^{b}	14.31±4.15^{d}	51.45±1.57^{a}	26,991.57^{c}
FRAP(mg GAE/100g)	09.60±0.39^{d}	11.40±0.39^{c}	06.44±0.68^{e}	28.79±0.68^{a}	10.50±0.68^{b}
CUPRAC (mg GAE/100g)	24.49±0.26^{c}	57.32±0.40^{a}	24.23±0.26^{d}	24.93±0.40^{b}	22.40±0.26^{e}

TPC: total Phenolic compounds, FLA: Flavonoids. Values are given as mean standard deviation. Different letters indicate a significant difference ($p < 0.05$) on the same line. Samples with the same letters indicate no significant difference ($p < 0.05$).

are below the maximum limit (< 18%) for margarine, and within the limit set at less than 20 % by European Honey Commission Directive 2014/63/EU, indicating that formulated margarines are stable, and honeys are ripe, of good quality, and suitable for long-term storage.

The margarines M2, M3 and M4 produced from honeys H2, H3 and H4, respectively, have close water contents, meaning that the water content of the honey can influence that of the margarine. ANOVA reveals a single homogeneous group composed of H3, H4 and H5, and Kruskal Wallis ANOVA shows that all margarines are significantly different ($p < 0.05$), and that time has no significant effect on moisture content over three months.

The Brix values of the honey samples varied from 82.85 (H1) to 84.38% (H2). The latter shows the highest Brix value which is due to its richness in carbohydrates. These results are close to those reported by Yebou et al. (2021) on honeys from Ivory Coast (78.60 to 83.80%). H3 and H4 honeys show no significant difference ($p < 0.05$).

Electrical conductivity (EC)

The EC values varied from 0.79 (H5) to 1.27 mS.cm^{-1} (H4). These results suggest that all honeys studied are probably mixtures of nectar and honeydew with the exception of honey H5 (< 0.8 mS.cm^{-1}) which comes from, nectar. These values obtained are included in the range reported by Mouhoubi et al. (2018) on Algerian honeys (0.417 to 1.412 mS.cm^{-1}). The ANOVA test reveals the existence of two homogeneous groups (H3, H4 and H5) and (H4 and H5).

HMF

The HMF content of the honey samples varied from 1.90 (H1) to 8.98 mg.kg^{-1} (H5), far from the maximum limit of 40 mg.kg^{-1}. These low HMF values indicate that honeys are fresh. The results are included in the range (2 to 9 mg.kg^{-1}) obtained by Bouhala et al. (2020) on Algerian honeys, and they are lower than the results (10.18 to 166.14 mg.kg^{-1}) reported by Yeboue et al. (2021) on honeys from Ivory Coast. Both H4 and H5 honeys do not show any significant difference ($p < 0.05$).

Proline

The proline content of honey is between 377.98 (H2) and 632.40 mg.kg^{-1} (H4), above the minimum value of 180 mg.kg^{-1} recommended for quality honey, which confirms the maturity of honeys. The results are lower than those reported by Amessis-Ouchemoukh et al. (2021) on Algerian honeys (731.27 and 3730.90 mg.kg^{-1}). All samples are significantly different ($p < 0.05$).

Protein content

The results vary from 283.75 (H5) to 386.20 mg BSAE/100g (H1). These values are included in the intervals obtained by Bouhala et al. (2020) and Milek et al. (2021) with Algerian honeys (35 to 900 mg/100g), and Polish honeys (10 to 475 mg BSAE/100 g), respectively. All samples are significantly different ($p < 0.05$).

pH

All margarines record acid pH values between 3.53 (M4) and 3.61 (M3). The values comply with the standard (3.5–5.5). All honeys used except H1 are also acidic (4.26 (H5), 4.59 (H4)), these results indicate that the honeys H2, H3 and H4 are a mixture of honey of nectar, and honeydew (pH> 4.5) on the other hand H5 honey is of nectar origin (pH< 4.5), confirming the results obtained by EC. However, margarine M1 made with honey H1 also registers an acidic pH of 4.77, while honey alone has a near-neutral pH of 6.25, which can be explained by biochemical degradation following poor harvesting or storage conditions. Biluca et al. (2016) demonstrated that pH values of honey oscillate between 3.3 and 6.6 for Brazilian honeys. The ANOVA test reveals no significant difference between the H2, H3 and H4 honeys and between M2, M3 and M5 for pH and time has no significant effect on pH over the three months.

The conformity of the results indicates control over the quantities of pH corrector (lactic acid) added, and good monitoring of pH during production. The presence of honey and lactic acid ensures a stable pH and thus protects the margarine from any possible contamination or microbial development.

Color intensity L a* b**

The color of margarines and honeys is characterized by shades of red and yellow, as the coordinates a* and b* have positive values. Luminosity (L*), which reflects the reflection of translucent light when values are close to 100 and opaque light when values are closed to 0, varies from 67.39 (M4) to 70.21 (M1) and from 38.19 (H5) to 58.72 (H4) indicating that honeys and margarines are translucent. All the margarines, statistically different ($p<0.05$), recorded close L* values despite the fact that the honeys were of different colors, which means that honey color has no influence on margarine color, which represents an important index due to its direct relationship with the consumer's visual experience. The brightness results (L*) of the margarines obtained are lower than those reported by Martínez-Girón et al. (2022) on margarines enriched with red bell pepper oleoresin and yellow tomato juice (78.34–88.91).

Salt content

The salt content (NaCl) of prepared margarines is between 0.20 (M1 and M2) and 0.29 (M5)%. These results

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comply with the standard (0.2–0.4%). The statistical analysis revealed two homogeneous groups (M1, M2 and M4) and (M3 and M4) which are not significantly different, and time has no significant effect on the salt content.

Acidity

The acidity of margarine is between 0.27 (M1 and M4) and 0.28% (M2, M3 and M5), far from the maximum limit (≤0.3%). Statistical analysis indicated the existence of three homogeneous groups (M1, M3 and M4), (M2 and M5), and (M3, M4 and M5). The results did not change during the three months, indicating that the formulated margarines remain stable during storage, and time has no significant effect.

Melting point

The melting point of margarines ranges from 36.1 (M2) and 37.0 °C (M3), which means that all margarines formulated are easily melting in the mouth (37°C). The results are included in the fixed interval (32 and 38 °C). The results of the ANOVA analysis showed three homogeneous groups (M1, M2, M4 and M5), (M4 and M5) (M3 and M4), and time has no significant effect.

Antioxidant contents

Margarine M4 has high levels of polyphenols and flavonoids (53.15 mg GAE/100g and 51.45 mg QE/100g, respectively) following the addition of honey H4, which also records higher levels of these phenolic compounds (110.30 110.30 mg GAE/100g and 96.74 mg EQ/100g). The ranking of margarines in terms of polyphenols is M5<M1<M3<M2<M4, with average concentrations of 21.64, 22.72, 30.28, 34.69, and 53.15 mg GAE/100g, respectively. The polyphenol results obtained fall within the range obtained by Lopes et al. (2014) on margarines enriched with spices (17.1 to 92.3 mg GAE/100g). All margarines are significantly different ($p < 0.05$). The source of antioxidant in the formulated margarines is due to the presence of honey. The difference in phenolic compound content between margarine formulations is attributed to the different proportions of honey added, which confirms the richness of M4 margarine in antioxidants compared with other margarines, and also indicates the richness of Algerian honeys in natural antioxidants, which can be used to enrich fatty products such as margarines.

Antioxidant activities

The results of antioxidant activities are shown in Table 2a and Table 2b.

The IC_{50} DPPH values of the honeys presenting the concentration which caused 50% trapping of free radicals vary from 1.16 (H5) to 2.37 (H4) mg/ml. These values are lower than those obtained by Bouhala et al. (2020) in Algerian honeys with values that oscillated from 4.2 to 17.92 mg/mL. All honeys are significantly different ($p < 0.05$).

Iron-reducing activity (FRAP) and antioxidant capacity values measured by the CUPRAC method ranged from 52.50 (H3) to 85.92 mg GAE/100g (H4), and 6.44 (M3) to 28.79 mg GAE/100g (M4), and from 59.59 (H5) to 67.45 mg GAE/100g (H2), and 22.40 (M5) to 31.13 mg GAE/100g (M2), respectively. Margarine M3 recorded low antioxidant activities values despite the richness of added honey H3 in antioxidants, compared with margarine M4 produced by honey H4, which is also rich in bioactive substances. This difference could be due to the quantity of added honey and the nature of the natural antioxidants present in honey. ANOVA reveals that all margarines are significantly different ($p < 0.05$).

In addition, Guenaoui et al. (2024) demonstrated that M5 margarine revealed the best antioxidant activities in terms of DPPH, ABTS and Ferrozine tests.

Fatty acid profile

The fatty acid profile of margarines presented in Table 3 reflects the fatty acid content of the vegetable oils used to make the margarines. The main fatty acids present in all the margarines are lauric (C12:0), myristic (C14:0), palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acids. Caprylic (C8:0), and capric (C10:0) acids were detected in all the margarines produced, with the exception of margarine M3, while arachidic (C20:0) and behic (C22: 0) are only detected in margarines M2, M4 and M5 at low levels, which indicates the richness of the honeys used (H2, H3, H4 and H5) in these fatty acids because the fat phase used during the formulation of the margarines is the same. The two oils palmitic and oleic are the predominant fatty acids in all the margarines with contents of 31.39% (M5) to 32.34% (M3), and 31.51% (M2) to 31.91% (M3), respectively. Anwar et al. (2006) have shown that a diet rich in oleic acid can reduce the incidence of cardiovascular disease and lower LDL cholesterol. The linoleic acid content, a classification parameter for margarines, is between 20 and 40%, indicating that all formulated margarines are semi-molasses. Guenaoui et al. (2024) reported that all margarines recorded low hardness values, indicating that they are more spreadable and don't require much energy to spread on a surface (bread, teeth and tongue).

TABLE 3: *Fatty acid profile of margarines enriched with honey for 1 and 12 weeks at 4 °C.*

Fatty acid composition%		M1		M2		M3		M4		M5	
		Week1	Week 12	Week1	Week 12	Week1	Week 12	Week1	Week 12	Week1	Week 12
C8: 0	**Caprylic acid**	0.28±0.03^{a}	0.28±0.02^{A}	0.26±0.02^{b}	0.26±0.02^{B}	-	-	0.26±0.02^{b}	0.26±0.02^{B}	0.26±0.02^{b}	0.26±0.02^{B}
C10: 0	**Capric acid**	0.26±0.02^{a}	0.26±0.02^{A}	0.24±0.01^{b}	0.24±0.02^{B}	-	-	0.24±0.00^{b}	0.24±0.00^{B}	0.24±0.00^{b}	0.24±0.00^{B}
C12: 0	**Lauric Acid**	3.65±0.02^{a}	3.62±0.02^{A}	3.40±0.02^{b}	3.40±0.03^{B}	3.33±0.02^{c}	3.32±0.02^{C}	3.41±0.02^{b}	3.40±0.02^{B}	3.36±0.02^{bc}	3.36±0.02^{BC}
C14: 0	**Myristic acid**	1.93±0.00^{a}	1.92±0.00^{A}	1.80±0.00^{b}	1.79±0.00^{B}	1.81±0.00^{b}	1.80±0.00^{B}	1.79±0.00^{c}	1.79±0.00^{C}	1.78±0.00^{c}	1.78±0.00^{C}
C16: 0	**Palmitic Acid**	32.24±0.02^{b}	32.22±0.01^{B}	31.79±0.02^{c}	31.77±0.02^{C}	32.34±0.02^{a}	32.32±0.02^{A}	31.44±0.02^{d}	31.43±0.02^{D}	31.39±0.02^{d}	31.39±0.02^{D}
C18: 0	**Stearic Acid**	4.16±0.00^{d}	4.15±0.02^{D}	4.42±0.02^{a}	4.40±0.02^{A}	4.28±0.02^{b}	4.26±0.02^{B}	4.20±0.02^{c}	4.20±0.02^{C}	4.21±0.02^{c}	4.21±0.02^{C}
C18:1	**Oleic acid**	31.80±0.02^{b}	31.77±0.01^{B}	31.51±0.02^{e}	31.50±0.02	31.91±0.02^{a}	31.90±0.02	31.61±0.02^{d}	31.60±0.02	31.73±0.02^{c}	31.72±0.02
C18:2	**Linoleic acid**	25.70±0.02^{c}	25.65±0.00^{C}	25.86±0.02^{c}	25.82±0.02^{C}	26.33±0.02^{a}	26.29±0.02^{A}	26.15±0.02^{b}	26.13±0.02^{B}	26.14±0.02^{b}	26.12±0.02^{B}
C20: 0	**Arachidic acid**	-	-	0.45±0.02^{b}	0.45±0.02^{B}	-	-	0.63±0.02^{a}	0.63±0.02^{A}	0.63±0.02^{a}	0.63±0.02^{A}
C22: 0	**Benehic acid**	-	-	0.27±0.02^{a}	0.27±0.02^{A}	-	-	0.27±0.02^{a}	0.27±0.02^{A}	0.27±0.02^{a}	0.27±0.02^{A}
Saturated fatty acids %		42.52±0.01^{a}	42.52±0.02^{C}	42.45±0.02^{b}	42.63±0.02^{B}	42.31±0.02^{c}	41.76±0.02^{A}	41.70±0.02^{a}	42.24±0.02^{D}	42.22±0.02^{d}	42.14±0.02^{E}
Monounsaturated fatty acids %		31.80±0.01^{b}	31.80±0.03^{B}	31.77±0.02^{e}	31.51±0.02^{E}	31.50±0.01^{e}	31.91±0.02^{A}	31.91±0.01^{a}	31.61±0.02^{D}	31.60±0.02^{d}	31.73±0.02^{C}
Polyunsaturated fatty acids %		25.70±0.01^{b}	25.70±0.02^{D}	25.65±0.02^{d}	25.86±0.02^{C}	25.82±0.02^{c}	26.33±0.02^{A}	26.29±0.02^{a}	26.15±0.02^{B}	26.13±0.02^{b}	26.14±0.02^{B}
Unsaturated / Saturated		1.35±0.02^{a}	1.35±0.02^{A}	1.36±0.02^{a}	1.36±0.02^{A}	1.40±0.02^{a}	1.40±0.02^{A}	1.37±0.02^{a}	1.37±0.02^{A}	1.37±0.02^{a}	1.37±0.02^{A}
(C18:2 / C16:0)		0.80±0.00^{a}	0.80±0.00^{A}	0.81±0.00^{a}	0.81±0.00^{A}	0.81±0.00^{a}	0.81±0.00^{A}	0.83±0.00^{a}	0.83±0.00^{A}	0.83±0.00^{a}	0.83±0.00^{A}

Values are given as mean standard deviation. Samples with the same letters indicate no significant difference ($p < 0.05$). a-e: statistical comparison between margarines during week 1. A-E: statistical comparison between margarines at week 12.

By comparing the quantities of fatty acids before, and after storage, a noticeably low decrease in unsaturated fatty acids is recorded, which is particularly indicated for the types of acids most prone to oxidation, such as linoleic acid. The latter showed a very slight decrease in all the margarines, which means that the formation of primary, and secondary products is very low. In addition, the C18:2/C16:0 ratio, used as an indicator of the level of degradation, did not decrease over 90 days. The results obtained are better than those reported by Ouahrani et al. (2022) on margarines enriched with Moringa oleifera leaf extract. The net protection of margarine is due to the addition of honey and its antioxidants, which appear to be beneficial and can reduce or stop fatty acid oxidation reactions during storage.

Evaluation of the oxidative stability of prepared margarines

Oxidative stability is an important factor for margarines due to the fact that oxidative deterioration can negatively affect organoleptic, nutritional, sensory properties and therefore consumer acceptance.

Peroxide index (PI) and Schaal test

The peroxide and Schaal test values of margarine (Figure 1) range from00.00 Meq O_2.kg^{-1} fat for all the samples during the first three weeks to 0.15 (M1 and M2), and 0.22 (M5) Meq O_2.kg^{-1} fat, and from 0.43 (M3) to 0.76(M5) Meq O_2.kg^{-1} fat during the twelfth week, respectively. The results are too far from the maximum limit (≤ 10Meq O_2.kg^{-1} fat), the proportional increase in PI from the 4th week is observed with the increase in storage time, and temperature, which corresponds to the formation of peroxides from unsaturated fatty acids during hydrolysis. The results of PI and Schaal test are lower than those obtained by control margarine (from 0.02 to 0.35 Meq O_2.kg^{-1} fat, and from 0.39 to 1.18 Meq O_2.kg^{-1} fat for weeks 5 to 12, respectively), and those reported in a study on the oxidation stability of margarine wrapped in chitosan/graphene oxide composite films (Han Lyn et al., 2021), and another study of the oxidative stability of margarine enriched with grape essential oil as a natural antioxidant (Kaanin -Boudraa et al., 2021), respectively. Statistical analysis revealed the existence of a significant difference from 4th week and 5th week, which means that time has a significant effect.

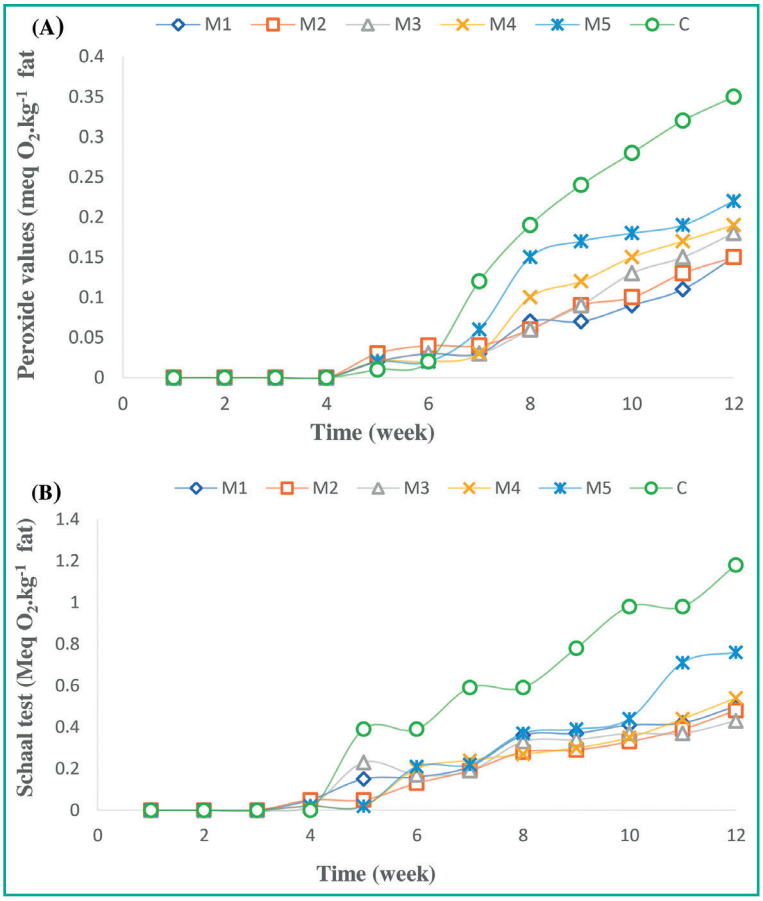

FIGURE 1: *Monitoring the peroxide index and Schaal test of formulated margarines. (A): Vertical bars represent the Peroxide index (Meq O_2.kg^{-1} fat) and the horizontal arm represent the time (week). (B): Vertical bars represent the Schaal test (Meq O_2.kg^{-1} fat) and the horizontal arm represent the time (week).*

The low results confirm that honey offers a good protection to margarine, which remains stable suring storage by preventing the formation of peroxides, even when stored at 30 °C.

Conjugated dienes and trienes (UV)

The quantity of conjugated dienes (K_{233}) at 4 °C, shown in Table 4, varies from 0.122 (M4) to 0.215 (M1), and from 0.232 (M4 and M5) to 0.262 (M1) over the 1st and 12th weeks. On the other hand, values at 30 °C vary from 0.240 (M4) to 0.258 (M5), and from 0.249 (M5) to 0.290 (M1) for weeks 1 and 12th week. The greatest degradation was observed in the control from 0.404 to 0.540 (4 °C), and from 0.436 to 0.447 (30 °C), indicating that honey added as a natural antioxidant ensures good margarine oxidative stability, unlike the commercial antioxidant used in the control margarine. Both formulated margarines M4 and M5 with honey H4 and H5 show better oxidative stability, which could be explained by the quantity of honey H4 added to margarine M4 compared with honey H5, and by the nature of the antioxidants contained in honey H5, which may exert a better effect. The evolution of conjugated trienes (K_{270}) in all formulated margarines showed the same trend as that of conjugated

TABLE 4: *Conjugated dienes and trienes of margarines stored for 1 and 12 weeks at 4 and 30 °C.*

	Conjugated dienes K_{233}				Conjugated trienes K_{270}			
	4 °C		30 °C		4 °C		30 °C	
	Week1	Week12	Week1	Week12	Week1	Week12	Week1	Week12
M1	0.215±0.01^{Ba}	0.262±0.02^{Bb}	0.256±0.01^{Ba}	0.290±0.02^{Bb}	0.124±0.04^{Ca}	0.299±0.03^{Bb}	0.167±0.00^{Ba}	0.237±0.02^{Bb}
M2	0.154±0.22^{Da}	0.235±0.02^{Db}	0.256±0.09^{Ba}	0.265±0.02^{Db}	0.152±0.04^{Ba}	0.260±0.03^{Cb}	0.112±0.00^{Ea}	0.212±0.02^{Cb}
M3	0.174±0.03^{Ca}	0.249±0.02^{Cb}	0.255±0.01^{Ba}	0.273±0.01^{Cb}	0.115±0.01^{Da}	0.185±0.03^{Db}	0.139±0.00^{Ca}	0.214±0.01^{Cb}
M4	0.122±0.01^{Fa}	0.232±0.02^{Db}	0.240±0.01^{Ca}	0.263±0.02^{Db}	0.090±0.01^{Fa}	0.186±0.02^{Db}	0.123±0.00^{Da}	0.198±0.02^{Db}
M5	0.141±0.01^{Ea}	0.232±0.03^{Db}	0.258±0.01^{Ba}	0.249±0.02^{Eb}	0.103±0.01^{Ea}	0.184±0.02^{Db}	0.103±0.00^{Fa}	0.169±0.02^{Eb}
Control	0.404±0.03^{Aa}	0.540±0.02^{Ab}	0.436±0.03^{Aa}	0.447±0.02^{Ab}	0.238±0.04^{Aa}	0.356±0.04^{Ab}	0.430±0.00^{aa}	1.171±0.00^{Ab}

Values are given as mean standard deviation. A-F: different letters indicate a significant difference ($p < 0.05$) in the same column. a-b: different letters indicate a significant difference ($p < 0.05$) on the same line. Samples with the same letters indicate no significant difference ($p < 0.05$).

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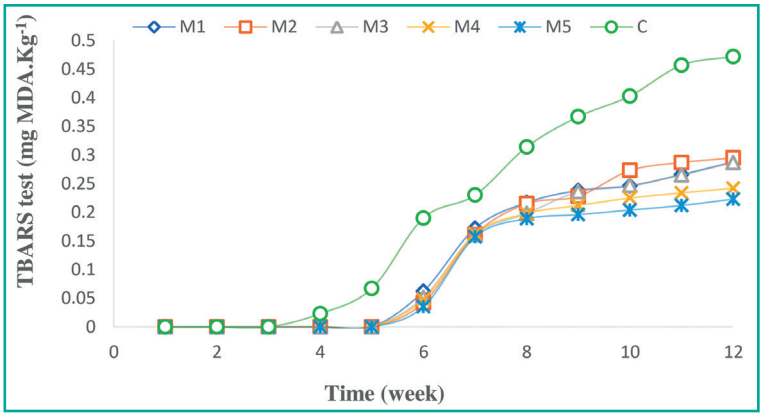

FIGURE 2: *Monitoring the TBARS test of formulated margarines. Vertical bars represent the TBARS test (mg MDA.Kg^{-1}) and the horizontal arm represent the time (week).*

dienes (Table 4). In fact, conjugated triene values are generally in line with those of conjugated dienes. Statistically, time has a significant effect from week 4 onwards.

TBARS test

The detection of MDA in formulated margarines (Figure 2) is quantified from week 6. It varies from 0.050 (M5) to 0.089 mg MDA.Kg^{-1} (M1) and from 0.204 (M5) to 0.267 (M2) mg MDA.Kg^{-1} at week 12. Statistically, time has a significant effect from week 6 onwards. whereas the commercial margarine recorded MDA levels from week 4 onwards, ranging from 0.152 to 0.469 mg MDA.Kg^{-1} at week 12. The results indicate that formulated margarines, especially margarines M4 and M5, accumulate less substances (MDA) than the control margarine, which confirms the values obtained above, and ensures the good stability of margarines formulated with honey. The results obtained fall within the ranges reported by Panpinat et al. (2018) and Shin et al. (2021) on the oxidative stability of margarine enriched with different β-sitosterol ester structures and based on duck fat, respectively. However, they are lower to those reported by Nor Adilah et al. (2020), which indicates that in addition to storage temperature, the nature of the packaging also affects lipid oxidation.

Rancimat test

The Rancimat method determines oxidative stability by detecting the volatile products (by-products of lipid oxidation) formed after heating the samples. Margarine M5 shows higher oxidative stability with an oxidation induction time of 19.11 h than margarines M2, M4, M1 and M3, which had induction times of 16.78 h, 16.20 h, 15.68 h and 15.65 h, respectively (Figure 3). The oxidative stability of margarine M5 can be explained by the nature and quality of antioxidants contained in honey H5. The results also show that the addition of natural antioxidants at low concentrations can retard lipid oxidation, thus extending the margarine's shelf life. However, the low induction time of margarines can be explained by the prooxidant properties of certain antioxidants at high concentrations (Liu et al., 2020). Furthermore, Chikhoune et al. (2017) reported that in lipid systems such as margarine, the addition of antioxidant at low concentration is highly essential for their oxidative stability and antioxidant structure and doses revealed a paradoxical effect in the lipid oxidation phenomenon, confirming the results obtained, and asserting that honey is an effective singlet oxygen scavenger, as it can also neutralize molecular oxygen free radicals through a combination of physical, and chemical reactions.

Microbiological analyses

Antibacterial activity of honey

The evaluation of the inhibitory effect of the different honeys tested on the three bacterial strains is expressed by the diameter of the zone of inhibition and the results are shown in table 5.

H2 honey shows antibacterial activity against *S. aureus,* and *Ps. aeruginosa* at all the concentrations used, on the other hand its activity against *E. coli* is detectable from the 50% concentration. Also, H1 honey shows antibacterial activity against *S. aureus* (all concentrations used), *E. coli* (50, 75 and 100%), and *Ps. aeruginosa* (75 and 100%). However, the H3,

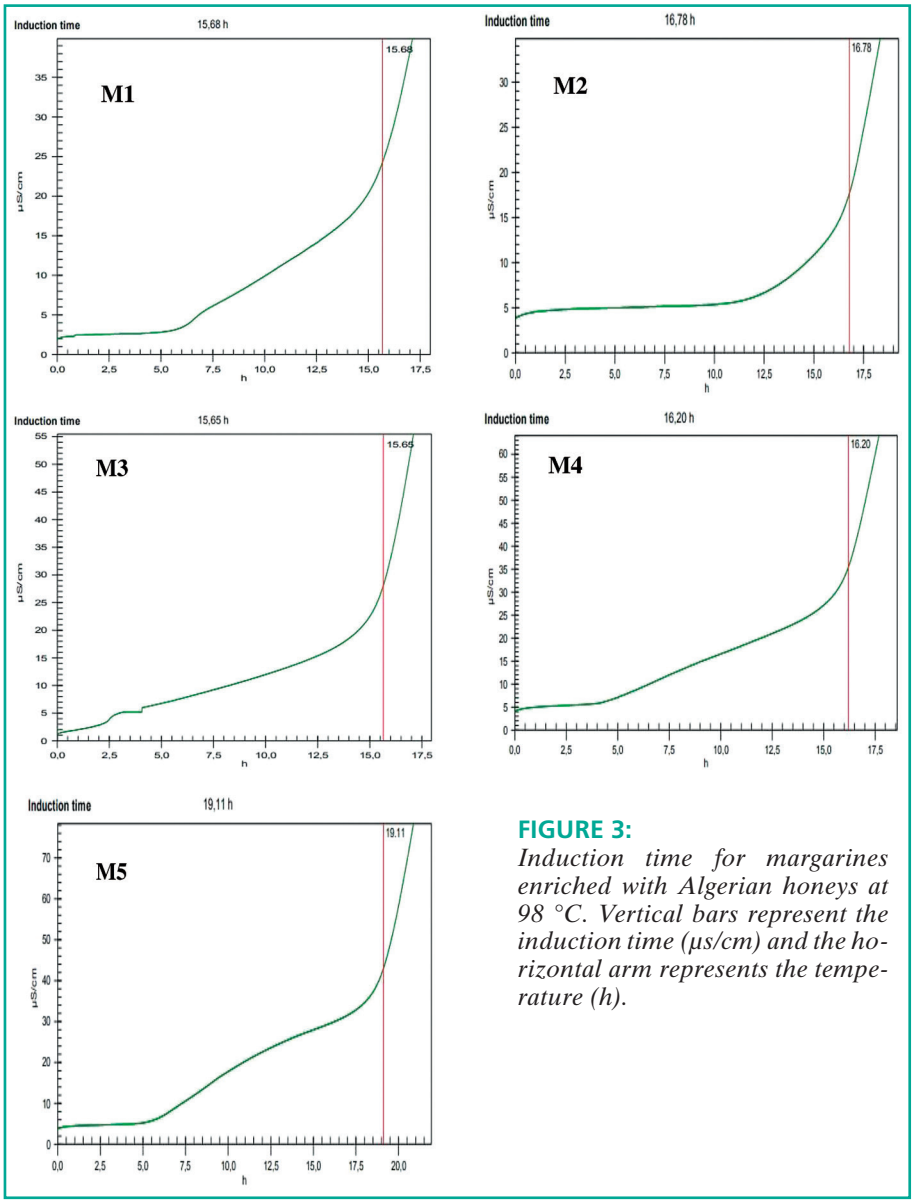

FIGURE 3:
Induction time for margarines enriched with Algerian honeys at 98 °C. Vertical bars represent the induction time (µs/cm) and the horizontal arm represents the temperature (h).

TABLE 5: *Results of antibacterial activities of Algerian honeys.*

	H1	H2	H3	H4	H5
IZD S 25%	10.33±0.58[a]	9.00±1.00 [b]	0.00±0.00[c]	0.00±0.00[c]	0.00±0.00[c]
IZD S50%	15.33±0.58[a]	9.67±1.53[a]	0.00±0.00[c]	0.00±0.00[c]	0.00±0.00[c]
IZD S 75%	16.67±1.53 [a]	16.33±3.51[b]	6.97±0.00[d]	9.33±1.16[c]	0.00±0.00[e]
IZD S 100%	20.67±1.5 [c]	17.33±4.04 [c]	11.67±2.08[d]	10.67±0.58 e	25.33±5.03 [a]
IZD E 25%	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]
IZD E 50%	9.67±0.58 [b]	15.33±5. [a]	0.00±.00[c]	0.00±0.00 [c]	0.00±0.00 [c]
IZD E 75%	15.33±0.58 [b]	16.00±3.52 [a]	0.00±0.00 [e]	10.67±1.16 [d]	12.00±0.00 [c]
IZD E 100%	19.67±0.58 [b]	18.33±3.79 [c]	8.67±0.58 [e]	10.67±1.16 [d]	20.00±0.00 [a]
IZD Ps 25%	0.00±0.00 [b]	16.33±3.22 [a]	0.00±0.00 [b]	0.00±0.00 [b]	0.00±0.00 [b]
IZD Ps 50%	0.00±0.00 [d]	23.33±4.16 [a]	10.33±0.58 [b]	5.23±3.96 [c]	0.00±0.00 [d]
IZD Ps 75%	14.67±0.58 [d]	36.00±4.00 [a]	15.33±1.53 [c]	10.67±0.58 [e]	23.67±1.53 [b]
IZD PS 100%	17.33±3.06 [d]	42.67±3.06 [a]	19.33±1.16 [c]	15.00±1.00 [e]	29.00±1.00 [b]

IZD S: inhibition zone diameter S. aureus, IZD E: inhibition zone diameter E. coli, IZD Ps: inhibition zone diameter Ps. aeruginosa, 25; 50; 75; 100 %: different concentrations of honey. Samples with the same letters indicate no significant difference a>b>c>d>e, (p < <0.05).

H4 and H5 honeys indicate no inhibition against *S. aureus* and *E. coli* (25 and 50%), and *Ps. aeruginosa* (25%). The importance of the inhibition can be enlightened by the sensitivity of each strain tested facing each other the different concentrations of honey used. At lower concentrations, *E. coli* and *Ps. aeruginosa* appear resistance to honey which could be attributed to its gram-negative structure (Breijyeh et al., 2020). The results are consistent with those reported by and Hunter et al. (2021) in honeys from Australia.

Microbiological analyses of prepared margarines

All formulated margarines show a total absence of micro-organisms over three months of analysis, despite the presence of factors that cause a microbiological risk in aqueous phase such as water and milk compared with fat phase, indicating a healthy quality suitable for human consumption. This is due to the good microbiological quality of the raw materials used in the manufacture of these margarines, the control of the hygiene of the place as well as the antibacterial power of honey exerted in margarines following its addition as a natural preservative.

Correlations

The Spearman correlation analysis shown in Table 6a reveals a strong, statistically significant positive correlation between honey color, and polyphenol content ($r = 0.91$, $p < 0.001$). Similarly, color reveals a positive correlation with the IC_{50} DPPH test ($r = 0.91$, $p < 0.001$). A highly significant correlation is recorded between phenolic compounds ($r = 0.66$, $p < 0.01$), and they also recorded a positive correlation with antioxidant activities. The results obtained are in agreement with Hunter et al. (2021) and Amessis-Ouchemoukh et al. (2021), testing to the correlations obtained, and confirming that Algerian honeys are a good source of antioxidants.

Furthermore, the results, illustrated in the Table 6b, show that flavonoids contained in the formulated margarines present statistically significant negative correlations with schaal test, conjugated dienes at 2 and 30 °C and the rancimat test, as well as a highly significant negative correlation with TBA, indicating that the richer the margarines are in flavonoids, the better their oxidative stability. Negative correlations were observed between FRAP and CUPRAC antioxidant activities with conjugated dienes at 2 °C, TBP, and peroxide value, indicating that the better the antioxidant activity, the more stable the margarine. Indeed, margarines M4 and M5 confirm the correlations obtained.

Moreover, the presence of antioxidants in formulated margarines is due to the added honey. The antioxidant activity carried by the phenolic compounds in honey acts as

TABLE 6a: *Correlation between color, phenolic compounds and antioxidant activities of honey.*

	L*	a*	b*	TPC	TF	IC50 DPPH	FRAP	CURPAC
L*	1.00							
a*	-0.91***	1.00						
b*	1.00	-0.91***	1.00					
TPC	0.91***	-0.83***	0.91***	1.00				
Flavonoids	0.47	-0.36	0.47	0.66**	1.00			
IC50 DPPH	0.91***	-0.81***	0.91***	0.98***	0.66**	1.00		
FRAP	0.34	-0.20	0.34	0.57*	0.87***	0.56*	1.00	
CUPRAC	0.20	-0.07	0.20	0.37	0.20	0.36	0.17	1.00

*TPC: total phenolic content, TF: total flavonoids. *= p < 0.05, ** = p < 0.01, *** = p < 0.001.*

TABLE 6b: *Correlation between phenolic compounds, antioxidant activities and oxidative stability tests of margarines enriched with honey.*

	TPC	TF	FRAP	CUPRAC	IP	Schal test	DC 2°C	DC 30°C	TC 2°C	TC 30°C	TBA	Rancimat
TPC	1.00											
TF	0.54*	1.00										
FRAP	0.57*	0.93***	1.00									
CUPRAC	0.76**	0.43	0.47	1.00								
IP	-0.15	0.28	0.20	-0.61*	1.00							
Schaal test	-0.28	-0.53*	0.47	-0.32	0.50	1.00						
DC 2°C	-0.33	-0.60*	-0.52*	0.19	-0.83***	-0.49	1.00					
DC 30°C	0.03	-0.53*	-0.41	0.34	-0.87***	-0.62*	0.87***	1.00				
TC 2°C	-0.15	-0.24	-0.23	-0.22	-0.15	0.10	0.14	0.30	1.00			
TC 30°C	0.23	0.15	0.20	-0.10	0.14	0.22	-0.28	0.04	0.77**	1.00		
TBA	0.10	-0.72**	-0.68**	0.16	-0.61*	-0.90***	0.62*	0.79***	0.21	-0.01	1.00	
Rancimat	-0.20	0.61*	0.57*	-0.04	0.38	0.73**	-0.34	-0.64*	-0.46	-0.38	-0.90***	1.00

*TPC: total phenolic content, TF: total flavonoids, PI: peroxid index, DC: dienes conjugated, TC: trienes conjugated. * = p < 0.05, ** = p < 0.01,*** = p < 0.001.*

electron or hydrogen donors, slowing down the production of by-products and conjugated dienes, and reducing the formation of hydro-peroxides (El Shourbagy and El-Zahar, 2014).

Principal component analysis of margarines enriched with honey

PCA is applied to analyze and identify formulated margarines that share similar characteristics from a number of five margarines enriched with honey. PCA explains 63.81% of the variance. The principal component (CP-1; 34.24%) represents in the positive part physico-chemical characteristics (acidity, color, pH and salt content), and oxidative stability tests (PI, schaal, conjugated dienes and trienes at 2 °C, and rancimat tests). The negative section shows melting point, water content, conjugated dienes, and trienes at 30 °C, phenolic compound and antioxidant activity. CP-2 (29.57%), on the other hand, shows the rancimat test, color (a*b*), acidity, antioxidants, and FRAP test in the positive section, while the other tests for oxidative stability, physico-chemical parameters, and CUPRAC test are in the negative section (Figure 4a).

The CP-1 discrimination illustrated in Figure 4b shows four groups of margarines. The positive part is characterized by the presence of two groups. The first group, formed by margarine M5, is located on the left-hand side and represents the best oxidative stability according to the Rancimat test, along with acceptable values in color and salt content. The second homogeneous group, formed by margarines M1 and M3, is also located on the left and recorded values close to the oxidative stability tests, as well as pH and color (L*). The negative side also features two groups, the first group, located on the right, represents margarine M2, which recorded the best antioxidant activity measured by CUPRAC. The last group, located on the left-hand side, represents margarine M4, which recorded the highest antioxidant content, and the best antioxidant activity measured by FRAP.

The PCA results illustrate a significant difference between formulated margarines, with the exception of margarines M1 and M3. These two margarines form a homogeneous group in terms of physicochemical parameters and oxidative stability tests. In fact, the correlation circle shows similarities between the TBA, Schaal, and PI tests, as well as between conjugated dienes, and trienes at 2 °C, and between antioxidants, and antioxidant activity, which means, and indicates very strong correlations between them, and confirms the results already obtained.

Conclusions

The incorporation of honey in aqueous phase reveals that all formulated margarines have recorded an improved physico-chemical quality that meets standards. The addition of honey confers on all margarine formulations a richness in phenolic compounds, and good antioxidant activity. The results of fatty acid profile and oxidative stability tests during 3 months‘ storage showed that margarines with added honey were more resistant to oxidation than the control. Margarines M4 and M5 were the best formulations in terms of oxidative stability. In addition, the antimicrobial power of honey attributed microbiological stability to all margarines enriched with honey. The Spearmane correlation attests that these Algerian honeys are an excellent source of antioxidants. Specifically, honey flavonoids prevent margarine oxidation, and the PCA confirms the correlations obtained. The substitution of honey for synthetic additives (α-tocopherol and potassium sorbate) has given rise to an innovative and conclusive approach. The future industrial producer of this new product can conquer this new offer while creating, and proposing a new strategic space in line with consumer needs.

Author statement

Nawel Guenaoui: Conceptualization, Methodology, Software, Writing – Original Draft, Writing-Review & Editing. Salim Ouchemoukh: Supervision, Writing – review

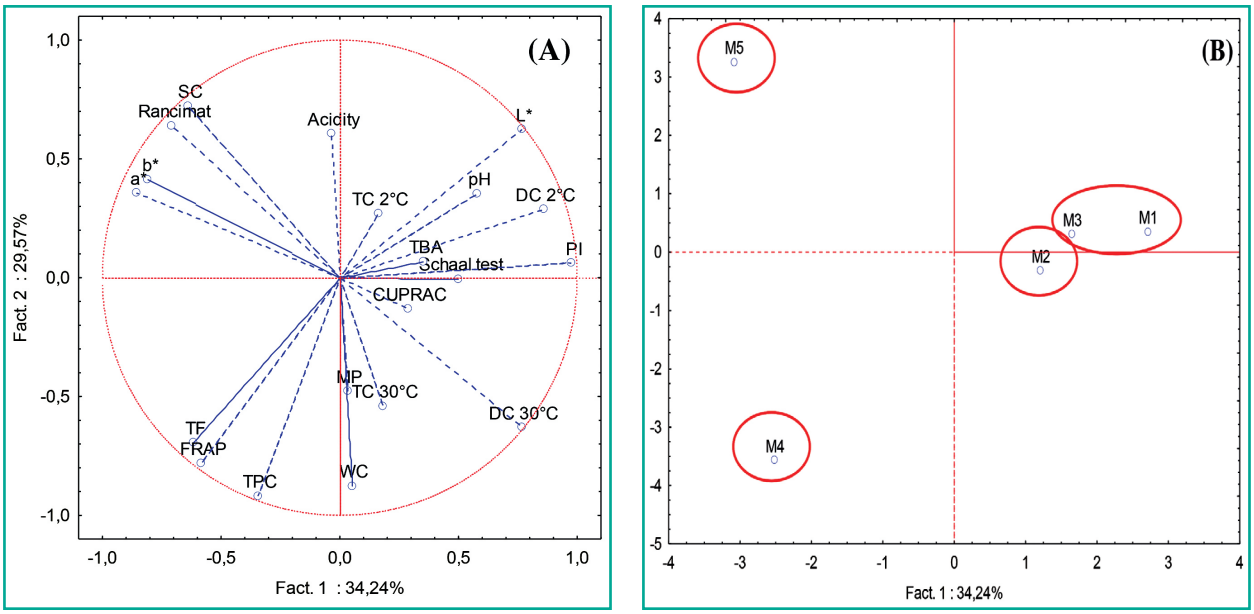

FIGURE 4: *Principal component analysis (PCA) biplot of physicochemical parameters (pH, acidity, melting point, salt and water content), oxidative stability (Schaal test, diene and triene tests (2 and 30 °C), TBA). (A): projection of the individuals 725 on the factorial plane (1 × 2) and (B): projection of the variables on the factorial plane (1 × 2). TPC: total phenolic content, TF: total flavonoids, PI: peroxid index, DC: dienes conjugated, TC: trienes conjugated, MP: meleting point, WC: water content, SC: salt content.*

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Conflicts of interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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