

SENSORY ACCEPTANCE, QUALITY CHARACTERISTICS AND ANTIOXIDANT ACTIVITY OF YOGHURT FORTIFIED WITH HONEY AND POMEGRANATE PEEL

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Abstract

*Pomegranate (Punica granatum L.) peels and honey are rich in bioactive compounds and are promising as natural ingredients for functional product development. This study aimed to develop a novel yoghurt fortified with pomegranate peel and honey, and to investigate their effect on the sensory, physicochemical, textural, microbiological, and antioxidant properties throughout storage period (28 days). Fortification of yoghurt with 5 % of honey and 0.5 % of pomegranate peel powder showed a positive effect on several determinative properties such as syneresis, water-holding capacity, color, instrumental texture and sensory attributes. After the whole storage period, fortified yoghurt presented the same count of total lactic acid bacteria (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) as the control yoghurt that remained over 10^7 CFU/g. In addition, fortified yoghurt contained more polyphenols (nearly 7 fold higher) and displayed significantly ($p < 0.05$) higher antiradical activity (DPPH and ABTS radical scavenging activity) than control yoghurt. In conclusion, fortification of yoghurt with 5 % of honey and 0.5 % pomegranate peel powder offered a novel yoghurt with acceptable sensory characteristics, good physicochemical and textural properties, and interesting antioxidant activity without inhibiting the development of lactic acid bacteria.*

Key words: Yoghurt, fortification, pomegranate peel, honey, bioactive compounds, antioxidant activity

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1. INTRODUCTION

Yoghurts are fermented dairy products obtained from lactic acid fermentation by two species of lactic acid bacteria, that is, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. This fermentation leads to acidification and milk coagulation (Corrieu and Béal 2016). Yoghurt has become one of the prevalent choices and considered as a healthy food since it provides excellent sources of essential nutrients (Fazilah et al., 2018). Despite these promising values, yoghurt remains poor in some components such as natural antioxidants and dietary fiber. Food fortification is one of the most important processes for improvement of the nutrients quality and quantity in food. It can be a very cost effective public health intervention. Fortification of yoghurt will effectively reduce or prevent diseases associated with nutritional deficiencies (Gahruie et al., 2015).

In recent years, there have been a growing number of publications focusing on the

incorporation of natural ingredients to the yoghurt, such as dried nut (Ozturkoglu-Budak et al. 2016), green tea and green coffee powders (Dönmezet al., 2017), rice bran (Demirci et al., 2017) and *Spirulina* (Barkallah et al., 2017). In the new global economy, considerable interests in applying food and agricultural processing wastes as functional food ingredients have been evinced as the waste are rich in beneficial bioactive compounds such as antioxidants (Lai et al., 2017). Although studies have recognized, for instance, the chemical features and ethnomedical relevance of many agro-industrial by-products, but, surprisingly, their incorporations have not been closely studied. Besides, billion tons of agriculture by-products are produced each year along the agricultural and food industrialization (Lai et al., 2017), and most by-products, in particular those from fruit processing industries, are underutilized, which usually leads to economic and environmental issues (Sah et al., 2016).

Pomegranate (*Punica granatum* L.) peel presents worthwhile properties, which bestow it with high potential for valorization in food and nutraceutical applications as a source of dietary fiber, antioxidants, and biopolymers (Hasnaoui et al., 2014). Pomegranate peels are characterized by an interior network of membranes comprising almost 26-30 % of total fruit weight and are characterized by substantial amounts of phenolic compounds, including flavonoids (anthocyanins, catechins and other complex flavonoids) and hydrolyzable tannins (punicalin, pedunculagin, punicalagin, gallic and ellagic acid) (Ismail et al., 2012). Several studies have revealed the diverse activities of pomegranate peel such as anti-inflammatory (Larrosa et al., 2010), antimutagenic (Negi et al., 2003), antidiarrheal (Qnais et al., 2007), antibacterial (Malviya et al., 2014), and antioxidant properties (Negi et al., 2003). Indeed, pomegranate peel had markedly stronger antioxidant properties than the pulp, including scavenging or preventive capability against several reactive oxygen species and inhibiting low density lipoprotein (LDL) oxidation (Li et al. 2006). Although its ethnopharmacological potential and health beneficial properties, pomegranate peel is still underutilized in food systems: astringency is the key limiting factor in its utilization as food. Utilization of pomegranate peel as effective supplements and food additives in defined concentrations in various organoleptically acceptable food preparations would open new avenues for scientific research in the realm of food science and nutrition (Akhatar et al., 2015).

Addition of sugar and carbohydrate-rich products such as honey could mask the bitter taste of pomegranate peel (Kennas et al., 2018). Honey is the natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants. Honey consists essentially of different sugars, predominantly fructose and glucose as well as other substances such as organic acids, enzymes and solid particles derived from honey collection (Codex Alimentarius, 2001).

Thus, honey could be a suitable sweetener for manufacturing fermented dairy products such as yoghurt (Chikh et al., 2001; Dourado Gomes Machado et al., 2017). Enrichment of yoghurt with honey is recommended because it is a natural sweetener that possesses a wide range of beneficial nutritional properties. It highly improves the sensory quality of the finished product without having a detrimental effect on characteristic lactic acid bacteria (Varga, 2006).

In previous work, we have reported on the effect of pomegranate peel and honey fortification on physicochemical, physical, microbiological and antioxidant properties of yoghurt powder (Kennas et al., 2018). Presently, the aim of this paper was to develop a novel yoghurt fortified with honey and pomegranate peel, and to better investigate their effects on the physicochemical, sensorial, microbiological, firmness, and antioxidant properties of the yoghurt.

2. MATERIALS AND METHODS

Materials

Honey was collected from beekeeper in May in Beni Douala, Tizi Ouzou (Algeria). Pomegranate fruits were picked in October in Beni Douala. Fruits were washed then peeled and the peels were air dried under ambient conditions. The dried peels were ground to a fine powder. The pomegranate peel powder (PPP) was conserved in airtight packages. Commercial (YO-MIX[®] Real) mixed strain culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) was obtained from (DuPont/Danisco[®], Denmark).

Chemicals

Methanol, Folin-Ciocalteu phenol reagent, gallic acid monohydrate, 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH*), aluminum trichloride, and quercetin were procured from Sigma Chemical Company (St. Louis, MO, USA). Ethanol, sodium carbonate and sodium hydroxide were supplied by Pancreac (Spain). 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) was purchased from TCI (Tokyo

Chemical industry Company, LTD), and potassium persulfate ($K_2S_2O_8$) from VWR prolabo Chemicals (Pennsylvania, USA). All the chemicals used were of analytical grade.

Yoghurt preparation

Whole milk powder was reconstituted in distilled water to obtain 14 % solid fraction. The mixture was homogenized for 30 min at 65°C then subjected to heat treatment at 95°C for 5 min. After cooling to 42°C, the PPP and honey were added, respectively, in the proportions of 0.5 % and 1 % (Y1), 0.5 % and 2.5 % (Y2), 0.5 % and 5 % (Y3) whereas the control yoghurt (YC) was devoid of PPP and honey. After homogenization, each mix was inoculated with the starter culture. The mixtures were distributed in 100 mL plastic cups and incubated at 42°C until a pH of about 4.5. At the end of the fermentation, the yoghurt was stored at 4°C.

Physicochemical analysis

The total solids (TS) and titratable acidity (percentage of lactic acid produced) of yoghurt samples were determined by AOAC (1990) methods. The pH was measured using a pH meter (HI 2211, Hanna Instruments, Rumania).

Susceptibility to Syneresis and Water-Holding Capacity

The yoghurt susceptibility to syneresis (STS) was evaluated according to Michael et al. (2010). Yoghurt samples placed in a cup were weighed then maintained at an angle of 45° at 4°C overnight. After that, the separated whey was removed with a pipette, and the yoghurt cup was re-weighed. The Eq. 1 was used to calculate STS:

$$STS(\%) = \left(\frac{W_w}{W_y} \right) \times 100 \quad (1)$$

where W_w is the weight of whey and W_y is the initial weight of yoghurt sample.

The Water-Holding Capacity (WHC) of yoghurt was evaluated according to the method of Isanga and Zhang (2009) with slight modifications. Yoghurt sample was placed in test tube and centrifuged (HERMLE Z 300 K, HERMLE Labortechnik GmbH, Germany) at 4000 rpm for 30 min at 4°C.

The WHC was calculated using the Eq. 2:

$$WHC(\%) = \left(1 - \frac{W_1}{W_2} \right) \times 100 \quad (2)$$

where W_1 is the weight of whey after centrifugation and W_2 is the weight of yoghurt sample.

Textural analysis

The firmness of yoghurt was determined by means of a texture analyzer (Texture Analyser, TA Plus, LLOYD instruments, England). Firmness of yoghurt samples in plastic cups, at 4 °C, was measured with a 1.2 cm diameter probe. The probe was moved at a test speed of 1 mm/s from the surface until a penetration depth of 10 mm within the yoghurt sample.

Color measurements

Color was determined with a CR-10 CIELAB colorimeter (Minolta Co., Osaka, Japan) and results were noted as L^* , a^* and b^* coordinates. Color difference (ΔE) was calculated by taking the color values of the control yoghurt as the reference, by using Eq. 3:

$$\Delta E = \sqrt{(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2} \quad (3)$$

Microbiological analysis

The enumerations of lactic acid bacteria and the investigation of the total and fecal coliforms, molds and yeasts were carried out after 28 days of cold storage. Experiments were performed at last in duplicate. *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were analyzed according to the Official Journal of the Algerian Republic (N° 43, 2004). Coliforms, molds and yeasts measurement was done according to Guiraud (1998).

Sensory evaluation

Yoghurt samples, at 7 days of the storage (4 °C), were evaluated by a panel of 15 semi-trained people, aged 23-50 years being 07 men and 08 women, consisting of researchers and post graduate students of our laboratory. Each panelist received 04 samples of yoghurt; all samples were presented to the panelists in three digit codes; the sample presentation order was randomized for each panelist. Water was provided between samples to cleanse residual palate effect. Yoghurt samples were analyzed in terms of their appearance and color, body and texture, odor, taste, and overall

acceptability depending on a nine-point hedonic scale, ranging from 1 (dislike extremely) to 9 (like extremely) for each organoleptic characteristic.

Extraction of phenolics from yoghurts

5 g of each sample was mixed with 25 mL of methanol using a magnetic stirrer. The mixture was filtered by Whatman No.1 paper. Then, the solvent was removed from the filtrate at 40°C under vacuum by using rotary evaporator. The extract was redissolved in 5 mL of methanol and stored at 4°C until use.

Total phenolic content

Total phenolic content (TPC) was measured according to Singleton et al. (1999). 500 µL of 10 fold-diluted Folin-Ciocalteu reagent was added to 100 µL of methanolic solution of each extract. After shaking, the mix was left to stand for 8 min before adding 400 µL of sodium carbonate solution (7.5 %). The mix was left to stand in dark at room temperature for 1 h. Absorbance was measured by using an ultraviolet spectrophotometer (Optizen Pop, Mecasys Co. Ltd., Daejeon, Korea) at 765 nm. The TPC was expressed as gallic acid equivalents in mg per 100 g (GAE/100 g).

Total flavonoid content

The total flavonoid content (TFC) of yoghurt extracts was determined according to the method of Djeridane et al. (2006), with some modifications. 1000 µL of methanolic solution of extract was mixed with 1000 µL of AlCl₃ methanolic solution (2 %). After incubation for 15 min in dark at room temperature, the absorbance was read at 430 nm. The TFC was expressed in mg quercetin equivalents per 100 g (QE/100 g).

Antioxidant properties

DPPH radical scavenging activity

The DPPH radical scavenging activity (DPPH RSA %) of the phenolic extracts was determined by following the method of Brand-Williams et al. (1995). 100 µL of the sample solution (2.5 mg/mL) was mixed with 3.9 mL of DPPH* methanolic solution (75 µM). Absorbance of the mixture at 517 nm was recorded after 30 min of incubation. Antioxidant activity was expressed as

percentage inhibition of DPPH* and was calculated using the Eq. 4:

$$\text{DPPH RSA (\%)} = \left[\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right] \times 100 \quad (4)$$

where A_{blank} is the absorption of the blank (containing all reagents except the extract), A_{sample} is the absorbance values of the DPPH* solution after the addition of the extract.

ABTS radical scavenging assay

The 2, 2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS^{•+}) radical scavenging assay was done according to Re et al. (1999). ABTS was dissolved in water to a 7 mM concentration. The ABTS^{•+} radical was generated by mixing the ABTS solution with 2.45 mM potassium persulfate in the dark at room temperature (20 °C) for 16 h. The working solution was prepared by diluting the previous solution with ethanol until the absorbance at 734 nm was 0.70±0.02. An aliquot (100 µL) of each extract (2.5 mg/mL) was mixed with 2.9 mL of the ABTS^{•+} working solution, and the absorbance was recorded after 10 min at 734 nm. The ABTS^{•+} scavenging ability of the sample was calculated using Eq.5:

$$\text{ABTS RSA (\%)} = \left[\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right] \times 100 \quad (5)$$

where A_{blank} is the absorption of the blank containing only ABTS^{•+} solution, A_{sample} is the absorbance of the ABTS^{•+} solution after the addition of the sample.

Statistical analysis

All tests were done in triplicate and the data are expressed as mean ± standard deviations. One-way analysis of variance (ANOVA) and Duncan's tests for comparisons was used to determine significant differences at the 95 % confidence level ($\alpha = 0.05$) between results using the software STATISTICA 7.1 (Statsoft Inc, France).

3. RESULTS AND DISCUSSION

Sensory evaluation

Table 1 presents the scores recorded for the sensory evaluation of yoghurts. As can be seen from the table, Y1 and Y2 have similar ratings for the different hedonic attributes. Y3 has

higher scores than other yoghurts in terms of taste and overall acceptability ($p < 0.05$). It can be deduced that, honey, at supplementation rate of 5%, could not only mask the bitter taste of pomegranate peel when it was added to yoghurt at rate of 0.5%, but it could also enhance its acceptability. This could be associated, according to Dourado Gomes Machado et al. (2017), with the dominating honey sweetness that may provide an enjoyable and desirable flavor to yoghurts. There was no significant difference ($p > 0.05$) among the yoghurt samples in terms of body and texture, and odor scores. Regarding the color and appearance, all fortified yoghurt samples had significantly higher ratings than control yoghurt which states that the fortification changed positively the color of yoghurt. It should be noted that increasing honey addition level did not improve the color of yoghurt, indicating that the color is in fact mainly due to PPP. In this study, increasing honey enrichment level improved taste perception compared with control yoghurt. Similarly, Varga (2006) stated that increasing the fortification level of honey increased the sweetness of yoghurt.

Values are Mean \pm SD ($n = 15$); YC: Control yoghurt; Y1: Yoghurt fortified with 0.5 % of pomegranate peel powder and 1 % of honey; Y2: Yoghurt fortified with 0.5 % of pomegranate peel powder and 2.5 % of honey; Y3: Yoghurt fortified with 0.5 % of pomegranate peel powder and 5 % of honey. Values followed by different letters in the same line differ significantly ($P < 0.05$). The results were given for 7 days.

Yoghurt fortified with 5 % of honey and 0.5 % of pomegranate peel powder (Y3) was chosen as the best final product and selected for further analyzes. Thus, control yoghurt was taken as reference.

Physicochemical characteristics

Total solids content of yoghurt samples are presented in Table 2. TS of the control and fortified (Y3) yoghurts were 13.71 ± 0.09 and 15.56 ± 0.23 %, respectively. The fortified yoghurt had significantly higher ($p < 0.05$) value of TS than control yoghurt. These results were consistent with Ozturkoglu-Budak et al. (2016)

whom reported that total solids content increased after the addition of dried nut to the yoghurt.

Figure 1 shows the evolution of pH values during the fermentation process. The initial pH value of the control yoghurt blend (6.12 ± 0.02) was slightly higher ($p < 0.05$) than that fortified yoghurt (Y3) blend (5.94 ± 0.02). This result may be attributed to a low pH value in honey and PPP. Dourado Gomes Machado et al. (2017) reported that addition of honey to goat yoghurt blend leads to reduction of the initial pH due to the natural acidity of honey. Following 4 hours of incubation, no statistical differences ($p > 0.05$) in pH values were observed between yoghurt samples. The similar acidification curves show that honey and PPP had no influence on the fermentation process.

During storage time, the pH values (Table 2) decreased from 4.54 ± 0.02 to 4.41 ± 0.01 and from 4.52 ± 0.02 to 4.36 ± 0.01 for YC and Y3, respectively. Our findings are in agreement with Varga (2006) who found similar pH values between yoghurt samples when testing the effect of honey on the characteristic microflora of yoghurt. The pH values were similar ($p > 0.05$) on day 1, whereas at the end of storing time, the results showed significant difference ($p < 0.05$) between control and fortified yoghurts. Meanwhile, titratable acidity of the yoghurt samples (Table 2) increased after the storage time. However, the acidity increase was significantly higher in the fortified yoghurt (1.13 ± 0.07 to 1.38 ± 0.03). These results may be associated with a possible positive effect of the presence of honey on the post acidification of yoghurt. Our findings were compatible with the results of Dourado Gomes Machado et al. (2017) whom observed that acidity increased over the course of the storage period in yoghurt fortified with honey. Furthermore, these authors reported that addition of honey seemed to stimulate the lactic acid metabolism and consequently the post acidification of yoghurt. Similarly, Demirci et al. (2017) reported a decrease in the pH and an increase in the titratable acidity during the storing time of yoghurt supplemented with rice bran.

Table 1: Sensory scores of yoghurt samples

	YC	Y1	Y2	Y3
Color and appearance	4.20±1.52 ^b	5.43±1.70 ^a	5.87±1.60 ^a	5.37±2.18 ^a
Odor	5.30±1.77 ^a	5.13±1.77 ^a	5.10±1.98 ^a	4.73±1.79 ^a
Body and Texture	5.73±1.22 ^a	6.40±0.83 ^a	6.60±1.45 ^a	6.53±0.99 ^a
Taste	3.90±0.85 ^c	4.60±1.40 ^{bc}	5.53±1.67 ^b	6.67±0.38 ^a
Overall acceptability	4.35±0.72 ^b	4.13±1.08 ^b	4.53±1.44 ^b	6.10±1.39 ^a

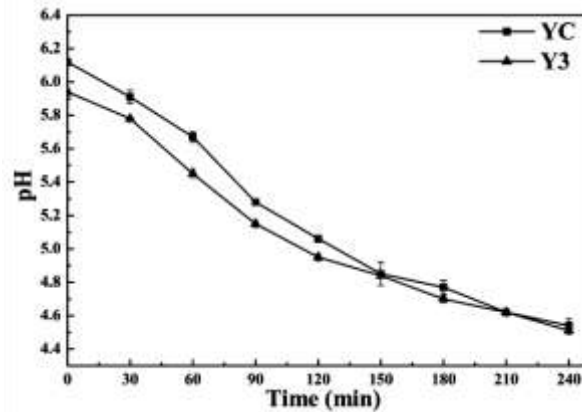


Fig. 1: pH curves of fortified (Y3) (0.5 % of pomegranate peel powder and 5 % of honey) and control (YC) yoghurts during fermentation period

Table 2: Physicochemical characteristics and firmness of fortified and control yoghurts

Characteristic		d 1	d 28
TS (%)	YC	13.71±0.09 ^{bA}	13.89±0.55 ^{bA}
	Y3	15.56±0.24 ^{aA}	15.96±0.61 ^{aA}
pH	YC	4.54±0.02 ^{aA}	4.41±0.01 ^{aB}
	Y3	4.52±0.02 ^{aA}	4.36±0.01 ^{bB}
Acidity (%)	YC	1.07±0.02 ^{aB}	1.32±0.05 ^{bA}
	Y3	1.13±0.07 ^{aB}	1.38±0.03 ^{aA}
STS (%)	YC	5.18±0.66 ^{aB}	9.56±0.50 ^{aA}
	Y3	3.28±0.87 ^{bB}	7.61±0.80 ^{aA}
WHC (%)	YC	44.56±0.49 ^{bB}	47.63±1.05 ^{bA}
	Y3	48.32±0.14 ^{aB}	51.08±1.41 ^{aA}
Firmness (g)	YC	34.89±0.17 ^{bB}	38.97±0.23 ^{bA}
	Y3	45.47±1.35 ^{aB}	50.74±0.78 ^{aA}

Values are Mean±SD of three trials. YC: Control yoghurt; Y3: Yoghurt fortified with 0.5 % of pomegranate peel powder and 5 % of honey; TS: Total solids; STS: Susceptibility To Syneresis; WHC: Water-Holding Capacity.

^{a-b} Means in the same column with different lowercase letters show significant differences between yoghurt samples ($p < 0.05$).

^{A-B} Means in the same raw with different uppercase letters show significant differences for same type of yoghurt sample between refrigerated storage periods ($p < 0.05$).

Susceptibility to Syneresis and Water-Holding Capacity

Syneresis is a common defect in yoghurt products results from excessive disarray of curd stability. On the other hand, Water-Holding Capacity is one of the desirable features for yoghurt quality which is related to

the water keeping ability of proteins within the yoghurt curd Demirci et al. (2017). According to Corrieu and Béal (2016) syneresis is, generally, affected by the protein content and physical treatments of the mix.

As shown in Table 2, significant reduction in the tendency to syneresis was observed in Y3

compared to YC at the first day of storage. This may be attributed to the higher total solids content of Y3 compared to YC (Table 3). A significant ($p < 0.05$) increase in serum separation, varying from 5.18 ± 0.66 to 9.56 ± 0.50 % in YC and from 3.28 ± 0.87 to 7.61 ± 0.80 % in Y3, was noted after cold storage. This observation may be explained by a higher casein aggregation which is attributed to the post acidification of yoghurt during storage. Ozturkoglu-Budak et al. (2016) reported that increase in syneresis during the storage period is usually associated with casein rearrangement. At the end of the storage period, syneresis of control and fortified yoghurts showed similar values ($p > 0.05$); nevertheless that of control yoghurt was slightly higher. On the other hand, the water-holding capacity (WHC) of fortified yoghurt (48.32 ± 0.14 %) was higher ($p < 0.05$) compared to that of control yoghurt (44.56 ± 0.49 %) at the first day of storage. After 28 days of storage, a similar significant increase in WHC was detected in yoghurt samples; however, WHC of fortified yoghurt was significantly ($p < 0.05$) greater than that of control yoghurt. This may be due to the dietary fibers present in the fortified yoghurt. According to Hasnaoui et al. (2014), pomegranate peels could be considered as potential sources of pectin with excellent WHC values.

Instrumental Texture Analysis

Yoghurt gel structure is the result of casein aggregation by pH reduction and disulphide bonding between κ -caseins and denatured whey proteins. Indeed, texture is an important attribute of yoghurt quality Sah et al. (2016). The values of yoghurt firmness are shown in Table 2. Results show that firmness value increase significantly ($p < 0.05$) after addition of 0.5 % of PPP and 5 % of honey. The increased firmness of the fortified yoghurt may be due to the increased TS content of fortified yoghurt. The gel texture could be also modified by the interaction between phenolic compounds and proteins. Indeed, polyphenols are known to interact with proteins such as caseins (O'Connell and Fox, 2001; Papadopoulou and Frazier, 2004). Dönmez et al. (2017) suggested

that protein-polyphenol interaction promoted the strength of the casein network and stabilized yoghurt structure by increasing the consistency and reducing the syneresis rate. On the other hand, for both yoghurts (control and fortified), the firmness increased significantly ($p < 0.05$) after 28 days of cold storage. The post acidification of yoghurt during storage may be the cause of the increase in firmness values; beyond, pH reduction may improve gel strengthening as the isoelectric point of caseins is reached. This same firmness rise was reported by Ozturkoglu-Budak et al. (2016) and Sah et al. (2016) in their works on the addition of dried nut and pineapple peel powder, respectively, on yoghurt.

Color measurements

Sample color was measured using a colorimeter after 1 and 28 days of storage and the data is shown in Table 3. The addition of honey and PPP had an effect ($p < 0.05$) on L^* and b^* parameters. However, the red/green coordinate (a^*) was not affected by the fortification ($P > 0.05$). Y3 had higher yellowish color (b^*) and lower lightness value than YC. The increasing b^* value (more yellowness) may be attributed to the yellowish color of honey and PPP. The statistical analysis indicated that storage had tendency to change L^* and b^* coordinates of the fortified yoghurt. However, the change could be considered as minor (3% for L^* and 10 % for b^*). Additionally, the total ΔE is important, so that all differences encountered between L^* , a^* , b^* color values of the samples and control are taken into account (Dönmez et al., 2017). After 1 day of storage, the ΔE value (3.13 ± 0.6) indicated minor difference between fortified and control yoghurts. The ΔE value unregistered in first day was significantly lower than that unregistered in 28th day of storage (4.81 ± 0.5), which could be attributed to the L^* and a^* values reduction. Color is an important attribute in food; it is the first characteristic perceived by the consumers and thus often influences the consumer's preference (Sah et al., 2016), thus, it's judicious to note that pomegranate peel powder could be useful as natural colorant in yoghurt formulations.

Microbiological analysis

The results of microbiological analysis of yoghurt samples are presented in Table 4. In the present study, after the whole storage period (28 days), the viable counts of lactic acid bacteria remained over 10^7 CFU/g. Besides, no significant difference ($p > 0.05$) was observed between control and fortified yoghurts. Our results show that yoghurt samples are in accordance with the Algerian legislation (Official Journal of the Algerian Republic, N° 35, 1998) that requires a minimum of viable lactic acid bacteria of at least equal to 10^7 CFU/g at the time of consumption. Chick et al. (2001) reported that

honey was not inhibitory to *Streptococcus thermophilus* and *Lactobacillus bulgaricus* when added to nonfat dry milk at a level of 5 %. Varga et al. (2006) also reported that the addition of honey at 1.0 % to 5.0 % did not influence the survival of characteristic microorganisms in yoghurt during storage period (06 weeks) at 4 °C. Yeasts, molds, and coliforms were not detected in the yoghurt samples after the whole storage period (Table 4). Therefore, the produced yoghurt formulations were safe and suitable for human consumption. Consequently, it is clear that the yoghurt preparation and storage was performed under excellent hygienic conditions.

Table 3: Color characteristics of yoghurt samples.

days	<i>L</i> *		<i>a</i> *		<i>b</i> *		ΔE
	YC	Y3	YC	Y3	YC	Y3	
d 1	49.30±1.41 ^{aA}	46.47±0.71 ^{bA}	-4.17±0.32 ^{aB}	-3.57±0.23 ^{aA}	12.80±0.36 ^{bA}	13.90±0.10 ^{aA}	3.13±0.6 ^A
d 28	49.57±1.01 ^{aA}	44.93±0.59 ^{bB}	-3.47±0.29 ^{aA}	-3.27±0.40 ^{aA}	13.30±0.10 ^{aA}	12.53±0.32 ^{aB}	4.81±0.5 ^B

Values are mean±standard deviation of triplicates. YC: Control yoghurt; Y3: Yoghurt fortified with 0.5 % of pomegranate peel powder and 5 % of honey; ΔE : color differences.

^{a-b} Means in the same raw with different lowercase letters show significant differences between yoghurt samples ($p < 0.05$).

^{A-B} Means in the same column with different uppercase letters show significant differences for same type of yoghurt sample between refrigerated storage periods ($p < 0.05$).

Table 4: Results of microbiological analysis of fortified and control yoghurts after 28 days of storage.

	Sample	Viable count (CFU/g of yoghurt)	Standards*
Total coliforms	YC	00	10/g
	Y3	00	
Fecal coliforms	YC	00	01/g
	Y3	00	
Yeasts	YC	00	00
	Y3	00	
Molds	YC	00	00
	Y3	00	
Lactic acid bacteria	YC	$15.6 \pm 0.85 \times 10^7$ ^a	$\geq 10^7$
	Y3	$17.6 \pm 1.34 \times 10^7$ ^a	

Values are mean±standard deviation of duplicates. YC: Control yoghurt; Y3: Yoghurt fortified with 0.5 % of pomegranate peel powder and 5 % of honey; CFU: Colony Forming Unit.

*Official Journal of the Algerian Republic (N° 35, 1998)

^{a-b} Means in the same column with different lowercase letters show significant differences between yoghurt samples ($p < 0.05$).

Table 5: Total phenolic and flavonoid contents and antioxidant activity of fortified and control yoghurts after 1 and 28 days of storage

		Period of storage (days)	
		d 1	d 28
TPC (mg GAE/100 g)	YC	9.58±2.49 ^{bA}	8.83±0.79 ^{bA}
	Y3	64.10±0.80 ^{aA}	55.81±3.46 ^{aB}
TFC (mg QE/100 g)	YC	/	/
	Y3	4.66±0.01 ^A	2.89±1.10 ^B
DPPH RSA (%)	YC	3.02±1.56 ^{bA}	0.93±0.50 ^{bA}
	Y3	18.03±2.45 ^{aA}	6.52±0.55 ^{aB}
ABTS RSA (%)	YC	8.04±0.94 ^{bA}	7.11±1.78 ^{bA}
	Y3	18.02±4.69 ^{aA}	12.10±2.03 ^{aB}

Values are mean±standard deviation of triplicates. YC: Control yoghurt; Y3: Yoghurt fortified with 0.5 % of pomegranate peel powder and 5 % of honey; TPC: Total Phenolic Content as gallic acid equivalents in mg per 100 g of yoghurt (GAE/100 g); TFC: Total Flavonoid Content in mg quercetin equivalents per 100 g of yoghurt; DPPH RSA: DPPH Radical Scavenging Activity; ABTS RSA: ABTS Radical Scavenging Activity.

^{a-b} Means in the same column with different lowercase letters show significant differences between yoghurt samples ($p < 0.05$).

^{A-B} Means in the same row with different uppercase letters show significant differences for same type of yoghurt sample between refrigerated storage periods ($p < 0.05$).

Total phenolic content, total flavonoid content and antioxidant activities

The TPC, TFC and antioxidant power of yoghurt samples are displayed in Table 5. As expected, the yoghurt containing pomegranate peel powder and honey had higher TPC (64.10±0.80 mg GAE/100 g) than control yoghurt (9.58±2.49 mg GAE/100 g). The TPC of fortified yoghurt (Y3) was nearly 7 fold higher than that of control sample, indicating evidence of fortification effect. After 28 days of storage, the TPC of fortified yoghurt was significantly lower ($p < 0.05$) than the initial TPC value. Nevertheless, the reduction was not very extensive (nearly 13%). This observation is supported by the reduction in TFC between 1 and 28 days where values varied from 4.66±0.01 and 2.89±1.10 mg QE/100 g, respectively.

The phenolic content of control yoghurt may be a consequence of several factors including: consumption of particular fodder crops by cattle, catabolism of proteins by bacteria and contamination with sanitising agents (O'Connell and Fox, 2001). However, some substances contained in yoghurt like aromatic

amines could react with Folin-Ciocalteu reagent (Singleton et al., 1999).

Similar behavior was found in the same table with antioxidant activities (DPPH and ABTS RSA). Thus, it is obvious that antiradical capacities of yoghurt sample increased significantly for both methods after addition of pomegranate peel. However, the antiradical capacities of fortified yoghurt decreased significantly after 28 days of storage. Interestingly, fortified yoghurt which had the highest phenolic content still exhibited higher antiradical capacity compared to the control yoghurt. Chouchouli et al. (2013) showed that storage time can affect antioxidant activity of supplemented yoghurts, since the antioxidant compounds are unstable during storage because they are sensitive to factors like temperature, light, pH, oxygen and water activity (Ventura et al. 2013). Our results show that addition of honey and pomegranate peel powder seems to enhance the antioxidant power of yoghurt which could be associated, but not exclusively, with the increase in the content of phenolics since these compounds are widely recognized as antioxidants.

4. CONCLUSION

Honey and pomegranate peel powder were successfully incorporated as ingredients for the production of novel yoghurt with bioactive compounds and beneficial health effects. The sensory attributes have shown that fortifying yoghurt with 5 % honey and 0.5 % PPP offered the most acceptable product. This level of supplementation had no effect on fermentation process of yoghurt. The fortified yoghurt showed a higher color rating that remains relatively stable during the overall storage, indicating that PPP may be useful as natural colorant in yoghurt formulations. In comparison to the control, a significant reduction in the tendency to syneresis and a significant increase in the water-holding capacity were observed in fortified yoghurt. Besides that, fortified yoghurt with 5 % of honey and 0.5 % of PPP had firmer texture compared with control. Fortified yoghurt displays higher phenolic and flavonoid contents, and exhibits higher antioxidant activity than control yoghurt. In addition, the fortification level did not affect the viability of starter bacteria. Finally, this present study demonstrated that honey, at rate of 5 %, could be a convenient way to mask the bitter taste of pomegranate peel when added to yoghurt at rate of 0.5 % which may attract consumers that are impressively attired by the health benefits of functional products.

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5. REFERENCES

[1]. Corrieu G. and Béal C. Yoghurt: The Product and its Manufacture. In: Caballero B., Finglas P. and Toldrá F, editors. The Encyclopedia of Food and Health. Oxford, UK: Academic Press; 2016. pp. 617-624.

- [2]. Fazilah N. F., Ariff A. B., Khayat M. E., Rios-Solis L. and Halim M. Influence of probiotics, prebiotics, synbiotics and bioactive phytochemicals on the formulation of functional yogurt. *Journal of Functional Foods*, 48: 2018; 387-399.
- [3]. Gahruie H. H., Eskandari M. H., Mesbahi G. and Hanifpour M. A. Scientific and technical aspects of yogurt fortification: A review. *Food Science and Human Wellness*, 4: 2015;1-8.
- [4]. Ozturkoglu-Budak S., Akal C. and Yetisemiyen A. Effect of dried nut fortification on functional, physicochemical, textural, and microbiological properties of yogurt. *Journal Dairy Science*, 99: 2016; 8511-8523.
- [5]. Dönmez Ö., Mogol B. A. and Gökmen V. Syneresis and rheological behaviors of set yogurt containing green tea and green coffee powders. *Journal Dairy Science*, 100: 2017; 1-7.
- [6]. Demirci T., Aktaş K., Sözeri D., Öztürk H. İ. and Akın N. Rice bran improve probiotic viability in yoghurt and provide added antioxidative benefits. *Journal of Functional Foods*, 36:2017; 396-403.
- [7]. Barkallah M., Dammak M., Louati I., Hentati F., Hadrich B., Mechichi T., Ayadi M. A., Fendri I., Attia H. and Abdelkafi S. Effect of *Spirulina platensis* fortification on physicochemical, textural, antioxidant and sensory properties of yogurt during fermentation and storage. *LWT -Food Science and Technology*, 84: 2017; 323-330.
- [8]. Lai W. T., Khong N. M. H., Lim S. S., Hee Y. Y., Sim B. I., Lau K. Y. and Lai O. M. A review: Modified agricultural by-products for the development and fortification of food products and nutraceuticals. *Food Science and Technology*, 59:2017; 148-160.
- [9]. Sah B. N. P., Vasiljevic T., McKechnie S. and Donkor O. N. Physicochemical, textural and rheological properties of probiotic yogurt fortified with fibre-rich pineapple peel powder during refrigerated storage. *LWT-Food Science and Technology*, 65: 2016; 978-986.
- [10]. Hasnaoui N., Wathelet B. and Jiménez-Araujo A. Valorization of pomegranate peel from 12 cultivars: Dietary fibre composition, antioxidant capacity and functional properties. *Food Chemistry*, 160:2014; 196-203.
- [11]. Ismail T., Sestili P. and Akhtar S. Pomegranate peel and fruit extracts: A review of potential anti-inflammatory and anti-infective effects. *Journal Ethnopharmacology*, 143: 2012; 397-405.
- [12]. Larrosa M., González-Sarriás A., Yáñez-Gascón M. J., Selma M. V., Azorín-Ortuño M., Toti S., Tomás-Barberán F., Dolara P. and Espín J. C. Anti-inflammatory properties of a pomegranate extract and its metabolite urolithin-A in a colitis rat model and the effect of colon inflammation on phenolic metabolism. *Journal Nutritional Biochemistry*, 21:2010; 717-725.

- [13]. Negi P. S., Jayaprakasha G. K. and Jena B. S. Antioxidant and antimutagenic activities of pomegranate peel extracts. *Food Chemistry*, 80: 2003; 393-397.
- [14]. Qnais E. Y., Elokda A. S., Abu Ghalyun Y. Y. and Abdulla F. A. Antidiarrheal Activity of the Aqueous Extract of *Punica granatum* (Pomegranate) Peels. *Pharmaceutical Biology*, 45:2007; 715-720.
- [15]. Malviya S., Jha A. A. and Hettiarachchy N. Antioxidant and antibacterial potential of pomegranate peel extracts. *Journal of Food Science Technology*, 51: 2014; 4132-4137.
- [16]. Li Y., Guo C., Yang J., Wei J., Xu J. and Cheng S. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food Chemistry*, 96: 2006; 254-260.
- [17]. Akhtar S., Ismail T., Fraternal D. and Sestili P. Pomegranate peel and peel extracts: Chemistry and food features. *Food Chemistry*, 174:2015; 417-425.
- [18]. Kennas A., Amellal-Chibane H., Kessal F. and Halladj F. Effect of pomegranate peel and honey fortification on physicochemical, physical, microbiological and antioxidant properties of yoghurt powder. *Journal of the Saudi Society of Agricultural Sciences*, in press:2018. <https://doi.org/10.1016/j.jssas.2018.07.001>
- [19]. Codex Alimentarius (2001). Revised codex standard for honey, Codex standard, 12: 1981; 1-7.
- [20]. Chick H., Shin H. S. and Ustunol Z. Growth and acid production by lactic acid bacteria and bifidobacteria grown in skim milk containing honey. *Journal of Food Science*, 66:2001; 478-481.
- [21]. Dourado Gomes Machado T. A., Gomes de Oliveira M. E., Ferreira Campos M. I., Antonino de Assis P. O., Leite de Souza E., Madruga M. S., Bertoldo Pacheco M. T., Estevez Pintado M. M. and De Cássia Ramos Do Egypto Queiroga R. Impact of honey on quality characteristics of goat yogurt containing probiotic *Lactobacillus acidophilus*. *LWT - Food Science and Technology*, 80:2017; 221-229.
- [22]. Varga L. Effect of acacia (*Robinia pseudo-acacia* L.) honey on the characteristic microflora of yogurt during refrigerated storage. *International Journal of Food Microbiology*, 108: 2006; 272-275.
- [23]. AOAC. Official Methods of Analysis of the Association of Official Analytical Chemists. AOAC, Washington D.C. 1990.
- [24]. Michael M., Phebus R. K. and Schmidt K. A. Impact of a plant extract on the viability of *Lactobacillus delbrueckii* ssp. *Bulgaricus* and *Streptococcus thermophilus* in nonfat yogurt. *International Dairy Journal*, 20: 2010; 665-672.
- [25]. Isanga J. and Zhang G. Production and evaluation of some physicochemical parameters of peanut milk yoghurt. *LWT Food Science and Technology*, 42:2009; 1132-1138.
- [26]. Guiraud J. P. *Microbiologie alimentaire*. Paris. France: Dunod; 1998.
- [27]. Singleton V. L., Orthofer R. and Lamuela-Raventós R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymology*, 299:1999; 152-178.
- [28]. Djeridane A., Yousfi M., Nadjemi B., Boutassouna D., Stocker P. and Vidal N. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry*, 97:2006; 654-660.
- [29]. Brand-Williams W., Cuvelier M. E. and Berset C. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28: 1995; 25-30.
- [30]. Re R., Pellegrini N., Proteggente A., Pannala A., Yang M. and Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine*, 26: 1999; 1231-1237.
- [31]. O'Connell J. E. and Fox P. F. Significance and applications of phenolic compounds in the production and quality of milk and dairy products: a review. *International Dairy Journal*, 11:2001; 103-120.
- [32]. Papadopoulou A. and Frazier R. A. Characterization of protein-polyphenol interactions. *Trends in Food Science Technology*, 15:2004; 186-190.
- [33]. Chouchouli V., Kalogeropoulos N., Konteles S. J., Karvela E., Makris D. P. and Karathanos V. T. Fortification of yoghurts with grape (*Vitis vinifera*) seed extracts. *LWT - Food Science and Technology*, 53: 2013; 522-529.
- [34]. Ventura J., Alarcón-Aguilar F., Roman-Ramos R., Campos-Sepulveda E., Reyes-Vega M. L., Boone-Villa D., Jasso-Villagómez E. I., Aguilar C. N. Quality and antioxidant properties of a reduced-sugar pomegranate juice jelly with an aqueous extract of pomegranate peels. *Food Chemistry*, 136:2013; 109-115.