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Tunisian persimmon (*Diospyros kaki* L.) jelly: Effect on physicochemical, microbiological, rheological, biological activities and sensory properties of bilayer yogurt

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ABSTRACT. The purpose of this study was to investigate the effects of the incorporation of the persimmon jelly on the quality of the bilayer yogurt. The samples were prepared using 10, 15and 20% layers of persimmon jelly. The products were stored at 4°C for four weeks. During this period, the physicochemical, rheological, microbiological and sensorial characteristics, phenolic contents and the antioxidant activity were investigated. The results showed that a* and b* color parameters have been increased with the amount of the jelly layer. Bilayer yogurts showed higher phenolic (2.245 ±0.11 mg AGE/g) and antioxidant capacities (58.91 ±0.51%) for Y20 than the control. The addition of persimmon jelly to yogurt did not affect the lactic bacteria growth. Regarding sensory evaluation, the scores for the different attributes increased as the amount of persimmon jelly added increased. Collectively, these findings revealed that persimmon fruit have the ability to improve the biological and the technological characteristics of yogurt as a functional food, which can be used as potential food ingredients.

Keywords: persimmon fruit; jelly; bilayer yogurt; phenol content; antioxidant activity; functional food.

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Introduction

Increasing consumer awareness of nutrition makes it more and more important to pay attention to health-enhancing foods. Therefore, consumers are increasingly willing to purchase food that, in addition to meeting their basic nutritional needs, the supply of portions of carbohydrates, proteins and fats, also has documented effects in terms of improving their well-being, health and reducing the risk of disease. Such foods are referred to as functional or enriched foods (Cory, Passarelli, Szeto, Tamez, & Mattei, 2018).

Dairy products are an important part of the human diet, and yogurt is one of the most popular fermented dairy products today. Yogurt is popular among consumers because of its flavor, as well as its nutritional value, and health-promoting properties. Furthermore, yogurt has highly desired functional food properties as an antioxidant, bioactive amino and fatty acids, and probiotics from starter culture (Hadjimbei, Botsaris, & Chrysostomou, 2022). Yogurt contains cultures of living bacteria that are good for gut health. Protein, calcium, and other vital elements are abundant in yogurt. It is also low in fat and calories, making it a healthy option for those attempting to maintain a healthy weight (Awad et al., 2023). Although the health benefits of yogurt have long been recognized, ongoing efforts to improve its nutritional value, sensory and functional properties, and to produce yogurts fortified with natural antioxidants from natural sources have piqued the public's interest and represent a novel approach to product development.

The persimmon fruit, *Diospyros kaki L.*, is a fleshy fibrous tropical deciduous fruit belonging to the *Ebenaceae* family. Native to East Asia, it is also grown in Brazil, Lebanon, Italy, Spain and North Africa (Food and Agriculture Organization Statistics [FAOSTAT], 2019). Over 400 species of persimmon are planted globally. *Diospyros kaki* L. (persimmon) is the most important specie (Murali, Hamid, Shams, & Hussain Dar, 2023).Persimmon is rich in vitamins, fiber, carbohydrates, minerals, phenolic compounds, proteins, and carotenoids. This indicates a variety of health benefits, including the treatment of cardiovascular disease, high cholesterol, and diabetes (Choudhary et al., 2022).

The delicate nature of persimmon, sensitive texture, poor handling applications and inadequate storage facilities have caused fruit processing and its addition to other foods to improve nutritional quality of the final products to be very important (Rashwan, Karim. Shishir, Bao, Lu, & Chen 2020).Thus, the aim of the current studyis to develop a new bilayer yogurt by adding persimmon jelly at different levels. The physicochemical, microbiological, rheological, antioxidant and sensory properties of the yogurt samples were investigated during the cold storage.

Materials and methods

Persimmon fruit

Persimmon fruits (*Diospyros kaki*) known in Tunisia as "Krima" were harvested in the north-west region of Tunisia at maturity stage. Persimmon fruits were transported immediately to the laboratory, where they were graded manually to remove all immature and damaged fruits, washed under running water, drained and peeled. The peeled fruits were then sealed in polyethylene bags and stored at 4°C until use.

Persimmon Jelly formulation

The persimmon jelly was formulated as described by Curi et al. (2017) with some modifications. Peeled fruits were mixed in a blender and filtered to obtain a clarified juice.

The proportions of the ingredients used to make the jelly recipe, expressed in relation to the total weight (sugar and pulp) were as follows: 60% clarified juice, 40% sugar. Initially, the clarified juice was added to sugar and then processed in an open stainless-steel pan heated at a medium temperature (100°C). The cooking process continues with a hand refractometer (Euromex Holland) reading of 65°Brix soluble solids. The jelly was poured hot into sterilized glass bottles, allowed to cool, and stored at +4°C.

Bilayer-yogurt manufacture

Yogurt samples were prepared following a pilot process. The fresh cow milk (15 L) was obtained from the Central Dairy of the North (Tunisia) and then heated at 75°C for 30s.Milk powder (30g L^{-1}) and sugar (60g L^{-1}) were added and mixed. After that, the mixture was pasteurized at 95°C 5sec^{-1} and then rapidly cooled with chilled water to 44°C. The mixture was inoculated with the freeze-dried starter culture yogurt at 2% (v/v) (Chr. Hansen, Denmark). The samples were fermented in bottles (90 mL) then agitated uniformly, already containing the jelly layer, for 3-4h at 44°C then cooled until 4°C. Three percentages of *Diospyros kaki* L. jelly layer were tested (10,15 and 20%) (w/v) as follows: Control, Y10(yogurt with a 10% of persimmon jelly), Y15 (yogurt with a 15% of persimmon jelly) and Y20 (yogurt with a 20% of persimmon jelly).

Physicochemical analysis

The pH was assessed with a pHmeter probe calibrated at 20°C. The titratable acidity and the total solids were determined according to Agil et al. (2013).

The color parameters L* (100 white-0 black), a* (green (− 60)- red (+ 60)) and b*(blue (−60)-yellow (+ 60)) were measured using a colorimeter(Minolta Chroma Meter CR-300, Tokyo, Japan).

Determination of phenolic content and antioxidant activity

Yogurt extraction

The bilayer yogurt samples (2 g) were homogenized with methanol in water (70:30, v:v). The mixtures were left under stirring for 4h in the dark and at room temperature. Then, the mixture was centrifuged at 4000 g for 10 min.at 4°C (Ersan, Ozcan,Akpinar-Bayizit, & Sahin, 2018). The supernatant was collected and used for analysis.

The antioxidant activity of extracted samples was determined by the DPPH (2,2-Diphenyl-1-picrylhydrazyl) scavenging method (Oliveira et al., 2011). For this, 1mL of the extracts at different concentrations was mixed with 1mL of the DPPH. After stirring, the tubes were placed in the dark for 30 min.; the absorbance was then measured at 517 nm using a spectrophotometer (Jenway, 6705 UV/VIS).

DPPH radical-scavenging activity was calculated as follow:

$$\text{Scavenging rate (\%)} = (1 - \frac{\text{Abs sample}}{\text{Abs blank}}) \times 100$$

and the total phenolic content of experimental samples were analyzed according to the method of Oliveira et al. (2011).The extract (100 µL) was dissolved in 0.5 mL of the Folin reagent (1/10 dilution) and 1 mL of distilled water. Then, the solution was well stirred and kept at room temperature for 3 min. After that, 2 mL of 20% (w/v) sodium carbonate (Na_2CO_3) was added and the mixture was incubated for 2h in the dark at room temperature. Then, the absorbance of the solution was measured by a spectrophotometer at 760 nm.

Total phenol content was estimated from a linear regression equation deduced from the calibration curve (Y = 3.865X-0.054, R2= 0.998) and expressed as mg GAE/g of extract.

Microbiological analysis

Bacterial enumerations were carried out at 1, 7, 14, 21 and 28 days in duplicate of each experimental lots. Serial dilutions were carried out in sterile test tubes using peptone water. For the enumeration of *L.delbrueckii* subsp.*bulgaricus*, the MRS Agar (Sigma-Aldrich, France) was used, and plates were incubated anaerobic conditions at 37°C for 48. The M17 Agar (Sigma-Aldrich, France) was used for selective enumeration of *S.thermophilus* by incubating anaerobically at 37°C for 48h. Bacterial enumerations were carried out in 1, 7, 14, 21 and 28 days using spread plate technique and colony counts were pointed out as $logCFUg^{-1}$.

Viscosity behavior determination

The rheological characteristics of the bilayer yogurts were determined at 25°C with a programmable strain/ stress controlled rheometer (Rheomat RM180, Germany), at 1st, and 28 days of storage. A cone-plate geometry was applied in this study. The flow curves of all samples are described by shear stress (σ) as a function of shear rate (γ) (Essaidi et al., 2023).

Sensory analysis

Hedonic test was carried out on day 14 of storage to evaluate the acceptance of bilayer yogurts flavored with persimmon jelly at different levels. The untrained consumer panel members (62 panelists) were the users of bilayer yogurt. They were served the three samples of bilayer yogurt in a plastic transparent cup coded with an arbitrary three digit number and asked to rate their preferences for color, amount of jelly, odor, texture, sweet taste, acidic taste, fruity taste and overall acceptability using a 9-point hedonic scale (1=dislike extremely to 9= like extremely) (de Campo et al., 2019).

Statistical analysis

The data were analyzed by using SPSS statistics 20.0 to perform statistical analysis of the results (ANOVA). Tukey test ($p<0.05$) significance level was performed to determine significant differences between the means. Data are presented as mean ± standard deviation. The experiments were carried out in triplicate.

Results and discussion

Physico-chemical characteristics

The physicochemical characteristics of different jelly bilayer yogurts at 1,7, 14,during storage are shown in Table 1. Bilayer yogurts (Y10, Y15 an Y20) have lower pH values compared to the control sample ($p<0.05$) with a significant decrease throughout storage at 4°C for 28 days. Similar pH changes were found by Khubber, Quijal, Tomasevic, Remize, and Barba(2022) ofgoat milk yogurt supplemented with Isabel grape preparation.These findings should be associated with the degradation of the lactose by the bacteria's metabolic activity during yogurt fermentation and storage, involving the development of lactic acid and other organic acids.

Table 1. Physicochemical properties of bilayer yogurts during storage.

Parameter	Storage time (days)				
pH	1	7	14	21	28
Control	$4.51{\pm}0.01^{cE}$	$4.48{\pm}0.01^{cD}$	$4.46{\pm}0.01^{bC}$	$4.34{\pm}0.01^{cB}$	$4.30{\pm}0.01^{aA}$
Y10	$4.44{\pm}0.01^{bE}$	$4.40{\pm}0.01^{bD}$	$4.36{\pm}0.02^{aC}$	$4.32{\pm}0.02^{bB}$	$4.29{\pm}0.02^{aA}$
Y15	$4.42{\pm}0.01^{bE}$	$4.39{\pm}0.02^{abD}$	$4.36{\pm}0.02^{aC}$	$4.31{\pm}0.01^{bB}$	$4.28{\pm}0.02^{aA}$
Y20	$4.39{\pm}0.02^{aC}$	$4.36{\pm}0.01^{aC}$	$4.32{\pm}0.01^{aB}$	$4.29{\pm}0.02^{aA}$	$4.27{\pm}0.02^{abA}$
Titrable Acidity (%)					
Control	$86.33{\pm}0.58^{aA}$	$97.33{\pm}1.15^{bB}$	$101{\pm}1^{cC}$	$102.67{\pm}0.58^{aC}$	$104.67{\pm}1.53^{aD}$
Y10	$88{\pm}1^{bA}$	$92{\pm}1^{aB}$	$93{\pm}1^{aB}$	$101{\pm}1^{aC}$	$103{\pm}1^{aD}$

Y15	91.33±0.58^{cA}	92.67±0.58^{aA}	93±1^{aA}	101.33 ±2.08^{aB}	103±1.00^{aB}
Y20	91±1^{cA}	93.67 ±0.58^{aB}	96 ±1^{bB}	100.33±2.08^{aC}	102.67 ±1.53^{aC}
Dry matter (%)					
Control	11.71±0.02^{aD}	10.41±0.03^{aA}	11.98±0.18^{dA}	11.21±0.07^{aC}	10.81±0.09^{aB}
Y10	19.33±0.1^{bE}	15.71±0.16^{bA}	18.29±0.19^{bD}	16.69±0.12^{bB}	17.16±0.04^{bC}
Y15	21.33±0.08^{cD}	18.98±0.11^{cB}	17.94±0.1^{cA}	19.75±0.11^{cC}	19.56±0.28^{cC}
Y20	22.55±0.23^{dB}	21.9±0.18^{dA}	25.32±0.15^{dE}	24.27±0.05^{dD}	23.78±0.1^{dC}

a.b Values with different letters in the same column indicate significant difference at p <0.05.A.b values with different letters in the same row indicate significant difference at p<0.05.Control: control yogurt; Y10; Y15; Y20 yogurts with 10, 15 and 20% of persimmon jelly layer

Titratable acidity values were higher (p <0.05) in the bilayer yogurts than the control sample and slightly increased for all yogurts during storage. The acidity of the bilayer yogurts was affected both by the storage time and by the amount of persimmon jelly added. Similar results were obtained by Arslan and Bayrakci (2016) for yogurts incorporated with dried persimmon marmalade and fresh puree, respectively.

Table 2. Color parameters evolution of bilayer yogurts during storage.

Parameter	Sample	Storage time (days)				
		1	7	14	21	28
L*	Control	83.07±0.12 ^{dD}	82.89±0.1 ^{dD}	82.40±0.2 ^{dC}	74.00±0.4 ^{dB}	72.4±0.2 ^{dA}
	Y10	80.23±0.31 ^{cD}	77.45±0.1 ^{cC}	77.16±0.01 ^{cC}	71.9±0.06 ^{cB}	68.44±0.3 ^{cA}
	Y15	76.75±0.21 ^{bD}	75±0.12 ^{bC}	74.70±0.2 ^{bC}	70.41±0.2 ^{bB}	64.96±0.01^{bA}
	Y20	72.05±0.65 ^{aC}	71.89±0.09 ^{aC}	71.45±0.02 ^{aC}	64.18±0.01 ^{aB}	58.88±0.4 ^{aA}
a*	Control	-1.76±0.67 ^{aC}	-2.34±0.52 ^{aBC}	-2.59±0.2 ^{aB}	-5.94±0.1 ^{aA}	-6.53±0.4 ^{aA}
	Y10	+1.12±0.74 ^{bB}	+0.65±0.12 ^{bB}	+0.47±0.1 ^{bB}	-2.63±0.1 ^{bA}	-2.77±0.08 ^{bA}
	Y15	+1.99±0.44 ^{bC}	+1.65±0.35 ^{bC}	+1.52±0.3 ^{cC}	-1.24±0.05 ^{cB}	-1.83±0.2 ^{cA}
	Y20	+3.26±0.11 ^{cC}	+2.79±0.88 ^{cC}	+2.73±0.2 ^{dC}	+1.43±0.1 ^{dC}	+0.11±0.2 ^{dA}
b*	Control	+4.86 ±0.9 ^{aA}	+5.09±0.13 ^{aA}	+5.65±0.3 ^{aA}	+7.39±0.3 ^{aB}	+8.69±0.1 ^{aC}
	Y10	+5.22±0.22 ^{abA}	+6.12±0.2 ^{bB}	+6.36±0.01 ^{bB}	+11.62±0.03 ^{bD}	+11.17±0.4 ^{bC}
	Y15	+5.36±0.31 ^{abA}	+6.22±0.11 ^{bB}	+6.55±0.6 ^{bB}	+13.31±0.3 ^{cC}	+13.40±0.01 ^{cC}
	Y20	+6.04±0.61 ^{bA}	+6.43±0.57 ^{bA}	+7.31±0.04 ^{cB}	+14.55±0.02 ^{dC}	+14.94±0.02 ^{dC}

a.b Values with different letters in the same column indicate significant difference at p < 0.05.A.B Values with different letters in the same row indicate significant difference at p< 0.05.Control: control yogurt; Y10; Y15; Y20 yogurts with 10, 15 and 20% of persimmon jelly layer.

The treated yogurt samples initially measured significantly different total solids content. The addition of persimmon jelly increases the total solids content in all yogurts. However, these values showed a decrease which can be related to the increase of syneresis during the storage time.

The color is a very important parameter of dairy products for consumer preferences. The initial values of the lightness parameters L* are higher in the control sample (83.07±0.12) than in the persimmon jelly bilayer yogurts (Y10, Y15, and Y20) (Table2). This may be related to the presence of persimmon jelly layer yogurts that gives them color. The values of the color parameters a* and b* increased with the amount of persimmon jelly added where the highest values are observed in the Y20 sample of jelly (a*= +3.26±0.11; b*= +6.04±0.61) which has an orange-red color, characteristic of the color of the persimmon jelly.

Phenolic content and antioxidant capacity

The phenolic contents and antioxidant capacity values of all persimmon jelly bilayer yogurts are summarized in Table 3. The amount of persimmon jelly added has significantly influenced the total phenol contents of the yogurts and enhanced their antioxidant capacities compared to the control (p <0.05).

Table 3. Phenolic content and antioxidant activity of bilayer yogurts during storage.

Parameter	Storage time (days)	Control	Y10	Y15	Y20
TPC (mgAGEg^{-1})	1	0.65±0.09 ^{aA}	1.03±0.03 ^{bA}	1.33 ±0.12 ^{cA}	3.29±0.15 ^{dA}
	28	0.61±0.08 ^{aA}	0.9±0.10 ^{aB}	1.1±0.09 ^{aB}	2.25±0.11^{bB}
DPPH (%)	1	32.71±0.31 ^{aA}	54.97±0.41 ^{bA}	60.16±0.23 ^{bcA}	65.65±0.42 ^{cA}
	28	17.09±0.23 ^{aB}	54.09±0.32 ^{bA}	57.01±0.26 ^{bB}	58.91±0.51 ^{bB}

a.b Values with different letters in the same column indicate significant difference at p<0.05.A.B Values with different letters in the same row indicate significant difference at p<0.05.Control: control yogurt; Y10; Y15; Y20 yogurts with 10, 15 and 20% of persimmon jelly layer.

During storage, there was a decrease in total phenols in all samples, which proved to be non significant (p >0.05). The results obtained for antioxidant activity show that the quantity of persimmon jelly added to the yogurts significantly influenced their antioxidant activity (p <0.05). Piechowiak, Balawejder, Grzelak-

Błaszczy, Oracz, and Matłok,(2023) reported that the addition of jelly fruit in food improves his antioxidant activity which has already demonstrated by Allouache, Mahmoudi, Ben Haj Koubaier, Bouzouita, and Snoussi, (2024). Also, Khatoon, Ali, Liu, and Muzammil, (2021) investigated that persimmon enhanced antioxidant activity of yogurt. Our findings forboth total phenol content and antioxidant activity decreased during storage. At the same, Arslan and Bayrakci, (2016) reported that the antioxidant activity of yogurts fortified with phenolic extracts decreased during storage. Also, Durmus, Capanoglu, and Kilic-Akyilmaz (2021) reported that this decrease is related to the binding effect between polyphenol and dairy protein by lowering the number of free hydroxyls.

Rheological behavior

The flow curves variation of the apparent viscosity (η) as a function of the shear rate of bilayer yogurts at the 1st and the 28th days of storage is presented in Figure1. The apparent viscosity of the all samples drops as the shear rate increases. These results confirm that the samples are a non-Newtonian fluid with shear thinning behavior. Also, the results showed that all the bilayer yogurts have a shear thinning character with a flow indexes (n<1). A significant difference between all the samples was recorded during the storage period of 28 days ($p<0.05$) and the yogurt consistency (k) increased with persimmon jelly addition. Our results are in agreement with those of Essaidi et al. (2023) who indicated that the apparent viscosity increase in syrup fortifying yogurt may be due to the interaction between phenolic compounds and the proteins in the persimmon bilayer yogurts.

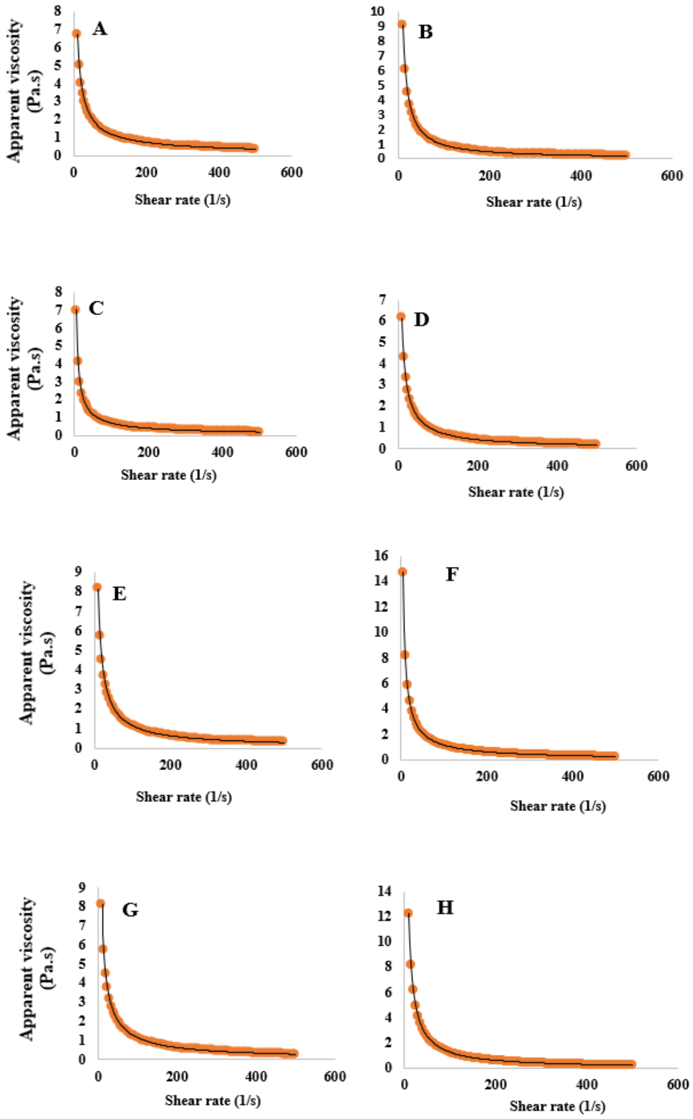

Figure 1. Apparent viscosity for (A; E) control, (B;F) bilayer yogurts with 10%, (C; G) bilayer yogurts with 15%, (D ; H) bilayer yogurts with 20 % at the First and the 28thdays of storage respectively.

Microbiological quality

Persimmon jelly incorporation to yogurt did not negatively affect the lactic bacteria growth. Figure 2 showed that the number of *S. thermophilus* and *L. bulgaricus* reached approximately ⩾10^7 CFU g^{-1} during 28 days of storage,which obviously indicates their conformity to the standards (Khubber, Quijal, Tomasevic, Remize, & Barba, 2022).

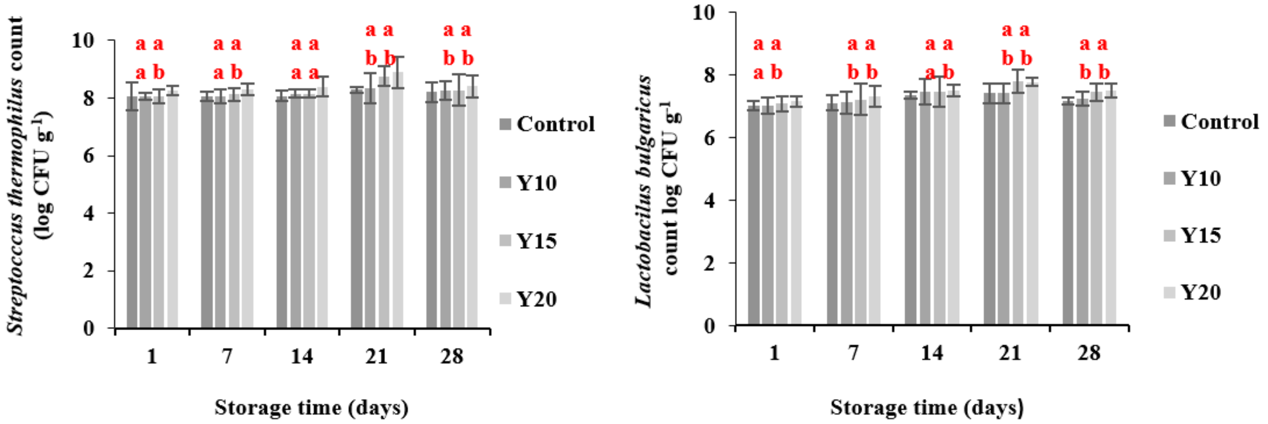

Figure 2. Lactic bacteria changes of bilayer yogurts during storage.Control: control yogurt; Y10; Y15; Y20 yogurts with 10, 15 and 20% of persimmon jelly layer

As shown (Figure 2), the *S. thermophilus* and *L. bulgaricus* are the most numerated bacteria in the persimmon jelly bilayer yogurts and their numbers progressed with the persimmon jelly amount, than the control. For *S.thermophilus* numbers varied from 8.06 to8.89 log CFUg^{-1} and from 8.04 to 8.28 log CFUg^{-1}, in the bilayer yogurts and in the control, respectively. For *L. bulgaricus*, the counts varied from 7.02 to 7.80 log CFUg^{-1} and from 7.01 to 7.42 log CFUg^{-1} in the bilayer yogurts and in the control, respectively. Our findings were in agreement with those found by Arioui, Ait Saada, and Cheriguene,(2016) for yogurt added with different concentrations of pectin.

Sensory profiles

Nowadays, the consumption of yogurt and other dairy products is very high, which is why the sensory aspect plays such an important role in this consumption. The use of fruit in these products not only offers consumers greater choice, but also enhances their nutritional quality and therapeutic value, thanks to the incorporation of bioactive compounds whose beneficial effects on health and the prevention of diet-related illnesses are well known Silvaet al. (2017).

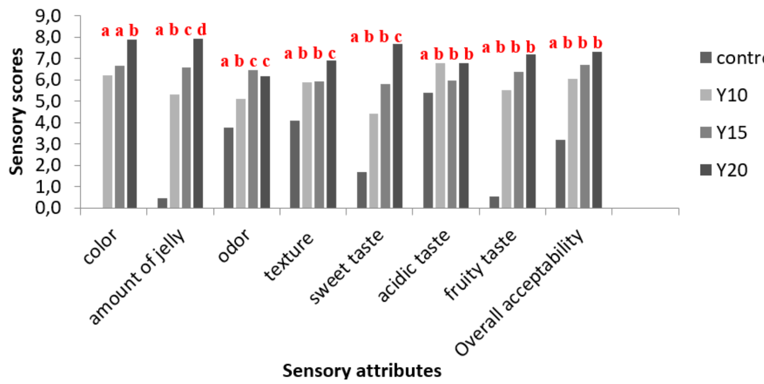

Figure 3. Sensory quality of bilayer yogurts. Control: control yogurt; Y10; Y15; Y20 yogurts with 10, 15 and 20% of persimmon jelly layer

The results of sensory evaluation of yogurts on the basis of color, amount of jelly, odor, texture, sweet taste, acidic taste, fruity taste and overall acceptability are summarized in Figure 3.

The Y20 bilayer yogurt sample had significantly higher color, amount of jelly, texture, sweet taste, fruity taste scores and overall acceptability, than those containing 15 and 10% of the persimmon jelly, respectively. For odor and acidic taste, there was no significant difference ($p < 0.05$) in yogurts with 10 and 20% of the persimmon jelly (5.1-6.2) and (6.8- 6.8). Therefore, both yogurts were well appreciated by the panelists, on the whole, the scores for the various sensory attributes increased as the concentration of persimmon jelly added increased.

Conclusion

In conclusion, the results show that persimmon jelly addition to bilayer yogurt has significantly improved its quality in terms of physicochemical, biological, and microbiological characteristics and sensory attributes. Furthermore, our study shows that including persimmon jelly in yogurt is beneficial because the selected food matrix has successfully maintained high levels of phenolic compounds and antioxidant activity throughout storage. This contribution is a key step toward the industrial-scale valorization of underutilized fruits. Persimmon jelly has been successfully included in yogurt and this study could lead to new prospects for the food industry by encouraging the use of these underutilized fruits in various industrial applications, in addition to diversifying a range of food products.

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