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ORIGINAL ARTICLE

Physicochemical properties of enriched yogurt with pistachio nut pellet

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Background: The improvement of structural characteristics of low-fat functional dairy products has always been of great importance. The purpose of the present study was to evaluate the physicochemical properties of low-fat probiotic yogurt fortified with pistachio-pellet.

Materials and Methods: Kalle-Ghuchi pistachio kernel residue (pellet) was prepared after extracting the oil through cold-pressing. Probiotic starter (ABT-10) was then added to low-fat milk and the sample was divided into three portions (T1-T3). T1 was the control. T2 and T3 were treated with 2% and 4% pistachio-pellet, respectively. After incubation at 42°C, the products were kept at 4°C for 14 days. During this period, the products' physicochemical properties were investigated. The results were analyzed using a one-way ANOVA ($P < 0.05$).

Results: A direct relationship was observed between the amount of pellet and the product acidity. Additionally, spontaneous syneresis reduction was detected during this period. Water holding capacity (WHC) of the fortified portions of yogurt was not affected by pellet treatment. Regarding yogurt color changes, lightness (L^*) reduction, greenness (a^*), and yellowness (b^*) enhancement were observed compared to the control portion.

Conclusions: Enriching yogurt with the proteins of pistachio pellet not only improves the physical structure of low-fat yogurt gel but may also enhance its functional properties, including bioactivity and being satisfactory for cardiovascular health.

Keywords: pellet; physicochemical properties; pistachio; yogurt

1. Introduction

Iran is one of the countries where pistachio (*Pistacia Vera* L.) is cultivated. In addition to having micro- and macro-nutrients, pistachio kernels have significant nutritional value and are used as food ingredients due to the presence of various antioxidant classes, such as anthocyanins, tocopherols, carotenoids, chlorophylls, flavonoids, isoflavones, proanthocyanidins, anacardic acids, cardanols, resveratrol, and vitamin C [1-3].

The amount of kernel oil varies in different types of pistachios, and, in some cases, it exceeds 75%. This oil is mainly used in food and cosmetics industry. Using the cold-press technique in pistachio kernel oil extraction reduces the amount of antioxidant destruction and also causes minimum organoleptic changes in the product [2]. It has been shown that the amount of total antioxidant activity (TAA) of pistachio hydrophilic extract, as a source of polyphenols, is higher than its lipophilic extract [4, 5]. After the extraction of pistachio oil, the remainder contains significant amounts of protein, fiber, and micronutrients, which can be used in many industries, including the food industry.

Nowadays, functional foods are widely used to improve people's well-being and health [6]. Products containing probiotic bacteria are examples of these functional foods

that have several health benefits [7]. Therefore, maintenance and improvement of physicochemical and functional properties of probiotic products are of great importance for the palatability, consumption, and marketability of these products.

The purpose of the present study was to investigate the physicochemical properties of protein-enriched probiotic yogurt by adding the nutritious pistachio residues, obtained after the extraction of kernel oil, in order to produce a nourishing functional product.

2. Materials and Methods

2.1. Pistachio preparation

The pistachios (Kalle-Ghuchi cultivar) were purchased from a local supermarket in the city of Kerman, Iran. In order to determine the physical properties of the nuts and their kernels, 50 individual nuts/kernels were randomly selected. The pistachio nut and the kernel were weighed using a scale and the three principal dimensions length, width, and height were measured using a Vernier caliper (with an accuracy of 0.02 mm). The length (L), width (W), and height (H) of pistachio nut and also the dimensions of pistachio kernel (i.e. length (l); width (w) and height (h)), were measured, as shown in Fig. 1 [8-10].

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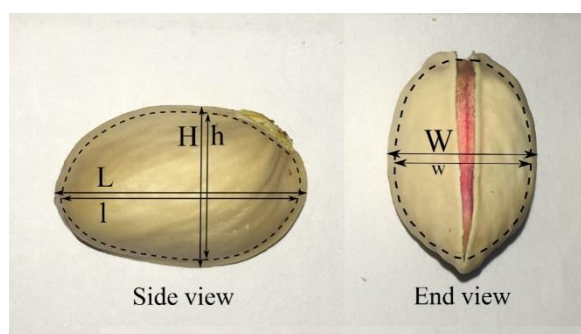


Fig.1. Three dimensions of pistachio nut and kernel while dash lines demonstrate the kernel position (adapted from [9])

2.2. Pistachio-pellet preparation

In order to prepare the pistachio-pellet, the oil was extracted using a screw press (Iran Cold Press®, Model 85 mm express, Iran). The pistachio kernels were introduced directly into the press while the tip of the press had previously been heated to ensure proper oil extraction. The oil temperature was controlled not to exceed 45°C. After oil extraction, the produced pistachio-pellets were stored at -18°C in dark glass bottles to avoid oxidation until the analysis and usage stages [11].

2.3. Chemical analysis of yogurt ingredients

To determine the moisture content and the dry matter, milled pistachio kernels and their pellets were heated using an oven at 103±2°C until a constant weight was reached [8, 9]. Dried samples were also analyzed for total ether-extractable fats using a Soxhlet extractor, and the total proteins were determined through the standard micro-Kjeldahl method in at least triplicate. The proteins were calculated by the protein factor of 6.25. Milk protein (micro-Kjeldahl), fat (Gerber method), and moisture content (oven method) were also determined [12-14].

2.4. Yogurt preparation

The whole yogurt preparation procedure was performed in a hygienic condition. The homogenized and pasteurized milk was heated up to 85°C for 30 min, followed by cooling down to 42°C. The probiotic yogurt starter culture ABT-10 (Chr. Hansen®, Denmark) containing *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis subsp. lactis* BB-12® was then added at a concentration of 0.05% w/v to the milk. The cultured milk was divided into three equal portions (T1, T2, and T3). The dry matter of T2 and T3 were increased by 2% and 4% with pistachio-pellet fine powder, respectively, while T1 received nothing (the control group). All the 3 groups were separately poured into 250-mL plastic cups and incubated at 42°C until their pH decreased to 4.6. The cups were then stored at 4±1°C for 14 days and the physicochemical tests were performed at 1, 7, and 14 days of storage. The experiments were performed triplicate.

2.5. pH determination

A Selecta pH meter 2001 (J.P. Selecta, Spain) was used to determine the pH of the yogurt samples on the above-mentioned days at room temperature [15].

2.6. Titratable acidity (TA)

Ten grams of each yogurt samples were diluted with distilled water (2-fold) and titrated with NaOH (0.1 M) in the presence of phenolphthalein. The TA of a single cup of yogurt per replication was expressed as the percent of lactic acid (v/w) [15].

2.7. Color parameters

Color parameters L^* (lightness: 100 representing white and 0 representing black), a^* (redness-greenness: positive representing red and negative representing green), and b^* (yellowness-blueness: positive representing yellow and negative representing blue) were measured using a digital imaging modified method described by Yam and Papadakis (2004) with a slight modification. Photos of the samples were captured using a Canon digital camera (PowerShot SX20 IS, 12.1 megapixels, Japan) installed in a situation described by Abbasvali *et al.* (2012). The images were analyzed 4 times per sample in the lab mode to obtain L^* , a^* , and b^* color values using Photoshop version 11.0. Color values were then averaged to obtain a mean value for each yogurt cup per replication [16, 17].

2.8. Spontaneous syneresis

The siphon method was used to determine the amount of spontaneous whey separation in the set-yogurt [18]. After being taken from the cold room (4°C), a cup of yogurt was immediately weighed and tilted at an angle of 45° for whey collection using a syringe. The cup of yogurt was weighed again. To prevent further outflow of whey from the set yogurt, the siphoning was repeated within 10 s. The spontaneous syneresis (%) was expressed as Equation 1.

$$\text{Spontaneous syneresis (\%)} = \frac{\text{whey (ml)}}{\text{initial yogurt sample (g)}} \times 100 \quad (1)$$

2.9. Susceptibility to syneresis (STS)

Determination of STS was evaluated using the drainage method with some modifications [15, 18, 19]. Approximately 50 g of the set yogurt was cut in a single action by a ladle. The gel was then weighed and drained on a 38-µm aperture sieve for 15 min at room temperature. Susceptibility to syneresis (%) was calculated using Equation 2.

$$\text{STS(\%)} = \frac{\text{whey (ml)}}{\text{initial yogurt sample (g)}} \times 100 \quad (2)$$

2.10. Water-holding capacity (WHC)

The yogurt samples were stirred 25 times clockwise and anticlockwise with a glass rod to measure their WHC utilizing the centrifugation method (Universal 320 R, Hettich Zentrifugen, Germany) with several modifications [18]. Thirty grams of yogurt were centrifuged at 3313×g for 15 min at 10°C, and the WHC was calculated as Equation 3.

$$WHC(\%) = \frac{1 - \text{Whey (ml)}}{\text{initial yogurt sample (g)}} \times 100 \quad (3)$$

2.11. Statistical analysis

The obtained data were represented as mean±standard deviation (SD) and were then analyzed using IBM SPSS Statistics version 19.0. Differences in between-subjects variables were considered significant at p<0.05 using a one-way ANOVA, followed by the Duncan's post-hoc test.

3. Results

The appropriate kernel: nut weight ratio in Kalle-Ghuchi cultivar and the size of its dimensions are among the important physical factors for marketing of this product (Table 1).

The extraction of pistachio oil by means of screw cold-pressing reduced the amount of sample oil by 62.9%. This oil reduction was accompanied by an increase in the pellet

protein percentage from 9.35% to 27.70%. Furthermore, this oil extraction decreased the indices of pellet lightness (*L**), greenness (*a**), and yellowness (*b**) values in comparison to the kernel (Table 2).

Enrichment of the yogurt with the pistachio-pellet decreased the pH value compared to the control group during the first 7 days. Notably, the increase in the acidity of the fortified yogurt was observed not only regarding the plain yogurt but also with an increment in the amount of added pellet (from T3 to T2) over a period of 14 days (Table 3).

Table 1. Weight and dimensions (length, width and height) of Kalle-Ghuchi pistachio used (mean±SD; n=50)

	Weight (g)	Length (mm)	Width (mm)	Height (mm)
Nut	1.18±0.12	19.29±1.78	12.94±1.15	12.53±1.11
Kernel	0.60±0.09	16.46±0.99	10.25±0.66	10.02±0.83

Table 2. Physicochemical characteristics of the yogurt ingredients (mean±SD)

	Pistachio kernel	Pistachio pellet	Milk
Dry matter (%)	97.00±0.18	94.88±0.01	9.8±0.61
Moisture (%)	3.00±0.18	5.12±0.01	89±0.72
Protein (%)	9.35±1.62	27.71±3.71	3.3±0.84
Fat (%)	49.63±0.18	18.43±1.8	1.5±0.11
L* color parameter	52±8.96	30.3±6.72	93±1.03
a* color parameter	-9.4±4.85	-3.95±1.67	0±2.12
b* color parameter	43.95±4.85	23.4±2.91	3±1.27

Table 3. Physicochemical characteristics of yogurts samples during 14 days of storage (mean±SD; n=3)

Characteristic/sample	Storage time		
	Day 1	Day 7	Day 14
pH			
T1	4.57±0.00 ^a	4.44±0.01 ^a	4.43±0.02
T2	4.35±0.01 ^b	4.29±0.01 ^b	4.36±0.04
T3	4.40±0.04 ^b	4.36±0.04 ^b	4.44±0.00
Titrateable acidity (lactic acid %)			
T1	0.88±0.01 ^a	0.97±0.01 ^a	1.03±0.01 ^a
T2	1.22±0.10 ^b	1.28±0.00 ^b	1.41±0.07 ^b
T3	1.54±0.11 ^c	1.50±0.02 ^c	1.59±0.01 ^c
Susceptibility to syneresis (STS) (%)			
T1	21.94±1.37 ^a	13.03±0.18	7.80±1.62
T2	15.50±0.70 ^b	9.52±1.28	10.29±0.45
T3	17.51±0.70 ^b	11.70±0.99	9.08±1.14
Spontaneous syneresis (%)			
T1	14.18±3.12	3.93±0.39 ^a	6.27±1.48 ^a
T2	10.44±1.66	1.93±0.25 ^b	2.25±0.08 ^b
T3	8.86±2.00	2.88±0.31 ^b	1.37±0.21 ^b
Water-holding capacity (WHC) (%)			
T1	46.24±1.55	39.39±1.09	44.96±2.97
T2	39.56±1.10	37.07±0.25	38.17±1.54
T3	43.32±3.84	39.79±0.91	40.34±0.14

T1: control (yogurt); T2: yogurt + 2% rise in dry matter by pistachio pellet; T3: yogurt + 4% rise in dry matter by pistachio pellet.
^{a-c}: Different letters in the same columns of each character, indicate a statistically significant difference (p<0.05).

On day 1, the spontaneous syneresis rate measured immediately after taking the yogurt from the cold room (4°C), did not differ among the different groups, while susceptibility to syneresis of the control group at room temperature after 15 min of draining was higher than the

enriched yogurt samples. Spontaneous syneresis of the enriched samples was significantly lower than the control group after 7 and 14 days. On day 14, the reduction of spontaneous syneresis was even more evident when comparing fortified yogurt with the control group (plain

yogurt), and even the T3 samples showed a lower amount of syneresis than the T2 samples (1.37% versus 2.25%) (Table 3).

Plain yogurt samples (the control group) had less lightness than the milk (Tables 2 and 4). Although there was not any difference in the lightness of fortified yogurt samples on the first day, after 7 and 14 days, enriched yogurts started to show less lightness (Table 4). The color parameter “*a**” indicated an increase in the greenness of the yogurt samples containing pistachio pellet compared to the control yogurt within 14 days. The increase in the yogurt yellowness in comparison with the initial milk is clear in the control group (Tables 2 and 4). The higher amount of *b** value in the enriched yogurt samples in comparison with the control group indicates higher yellowness of these fortified products. Notably, after 7 and 14 days, the differences in yellowness were observed not only in comparison with the control group but also in T2 and T3 (Table 4).

4. Discussion

The presence of significant amounts of protein and calcium in pistachio and its pellets makes it an appropriate candidate for the enrichment of food, especially dairy

products. In addition, the presence of substantial amounts of fiber in pistachio-pellet is likely to increase its use in products containing beneficial bacteria, including probiotics [2].

So far, pistachio protein, which mainly contains globulin (66%), albumin (25%), glutelin (7.3%), and prolamin (2%), has been used in the preparation of edible films [20, 21]. In this study, the protein content of pistachio kernel was measured at 9.35%, which was similar to the findings of Hayoglu and Gamli [3].

The pistachio-pellet application as a source of protein and carbohydrate in yogurt production increased the titratable acidity of dairy products compared to the control group, such that after 14 days the produced lactic acid in T2 and T3 samples reached 1.41% and 1.59% levels, respectively (Table 3). This could be due to the role of pistachio-pellet nutrients as a prebiotic and to the increase in the milk solid content to improve the activity of lactic acid bacteria of the yogurt samples [22]. In addition, pistachio nut paste has been shown to be slightly acidic, and at 20°C its pH declines to 5.61 after 7 months of storage [23].

Table 4. Color values of yogurt samples during 14 days of storage (mean±SD; n=3)

	Color parameters on day								
	L*			a*			b*		
	1	7	14	1	7	14	1	7	14
T1	82.25±2.38	79.63±2.20 ^a	77.88±1.81 ^a	-1.38±1.60 ^a	-0.50±0.93 ^a	-0.88±1.25 ^a	12.00±1.93 ^a	12.13±2.17 ^a	13.00±1.85 ^a
T2	80.13±4.05	74.50±2.39 ^b	73.25±1.91 ^b	-3.38±1.51 ^b	-2.13±1.46 ^b	-2.63±1.30 ^b	26.50±3.96 ^b	15.88±2.53 ^b	17.63±3.29 ^b
T3	80.88±2.23	73.88±1.96 ^b	71.63±2.20 ^b	-3.00±1.60 ^{ab}	-3.13±1.55 ^b	-3.25±0.46 ^b	28.63±4.69 ^b	20.38±3.58 ^c	24.13±2.17 ^c

T1: control (yogurt); T2: yogurt + 2% rise in dry matter by pistachio pellet; T3: yogurt + 4% rise in dry matter by pistachio pellet.

^{a-c}: Different letters in the same columns indicate a statistically significant difference ($p < 0.05$).

The syneresis is defined as the breaking down of protein gel network and the separation of its serum phase [24]. The amount of whey separation from yogurt is evaluated in a variety of methods. Spontaneous syneresis measures the whey separation on the surface of set yogurt gel, while the drainage method (STS) brings about the rate of whey leakage from its cutting surface [18].

In this study, the 2% increase in the dry matter of milk by pistachio-pellet reduced the spontaneous whey separation of yogurt by 26.38%, 50.89%, and 64.11%, during the days 1, 7, and 14, respectively. The decrease in spontaneous syneresis of T3 groups, in which the amount of dry matter increased by 4%, was 37.52%, 26.72%, and 78.15%, respectively, on the same days (Table 3). It has been shown that increasing the total solid content can play an important role in reducing the whey isolation [25-27]. Although some use high levels of milk fat in order to reduce yogurt syneresis, adding pistachio-pellet not only enriches yogurt in terms of protein but also enhances its antioxidant content [28]. In this study, increased acidity did not have a stimulating effect on the spontaneous syneresis of the enriched samples (Table 3).

Since susceptibility to syneresis for gels like cottage cheese is more informative than yogurt, there was no significant difference in drainage rate between the control group and

the enriched yogurt samples except for the first day [18]. The amount of whey isolated in the centrifuge method (WHC) is also influenced by other factors, such as the rheological properties and the product rigidity, and, in this study, no significant difference was found among the groups (Table 3).

The decrease in the enriched yogurts lightness on days 7 and 14 is due to the addition of pistachio-pellet, when its lightness was 22 times less than the pistachio kernel. Pistachio and its pellet are green in color due to their chlorophyll content. Finally, the increase in the yellowness (*b**) and also the greenness (*a**) indices is due to the presence of pistachio pigments in the fortified yogurts.

5. Conclusions

Low-fat yogurts have an important role in cardiovascular-friendly diets due to a reduced fat content. Since the presence of fat has an important effect on a product's texture and the improvement of its physical and technological properties, the marketability and palatability of the products are affected by fat reduction. There are several strategies, including using gum, to improve this condition. After extracting pistachio kernel oil, the residual pellet has a high protein and fiber content. The above properties, as well as

the presence of antioxidant compounds in the pellet, make it an ideal candidate for the preparation of yogurt as a dairy product with a protein matrix. In this study, pistachio-enriched yogurt, which has a high nutritional value, has shown favorable physicochemical characteristics, especially in terms of spontaneous syneresis reduction and also syneresis susceptibility which, accordingly, could provide a suitable place in people's diet.

Conflicts of interest

The authors declare no conflicts of interest.

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