

Impact of *Prangos Ferulacea* on Some Microbial, Physicochemical and Sensory Properties of Probiotic Low Fat Yogurt Containing *Lactobacillus Casei*

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Abstract

Background: The *Prangos ferulacea* (PF)–yogurt is a traditional food in Iran. This study investigated the effects of PF on the microbial, physicochemical and sensory properties of probiotic yoghurt.

Methods: Pasteurized low fat milk was heated up to 85°C, cooled to 40°C, and then mixed with conventional and *Lactobacillus casei* starter cultures incubated at 37°C until pH decreased to 4.6. Then, the cooked PF was added to yogurt and stored at 5°C for 21 days. Acidity, Syneresis, probiotic colony count and sensory evaluation of yoghurt was determined during the storage time. The experiments were replicated for three times. Probiotic yogurt (PY) was examined as the control and probiotic yoghurt containing 10, 20 and 30% *Prangos ferulacea* (PFY) as the samples.

Results: Total titratable acidity of PFYs compared to PY was not significant during 21 days. The syneresis rate of PFY yogurt showed significant differences compared to PY during storage time (P<0.001). Comparison of the mean scores of sensory attributes (taste, odor, syneresis, mouth feel and color) of PFY yogurt showed that there were no significant differences with PY. Enumeration of *lactobacillus casei* (logCFU/mL) revealed significant differences in PFYs compared to PY in each experimental day (P=0.040). At the end of the storage time (day 21), the highest number of *L.casei* was observed in PY and PFY 20% and the lowest in PFY30%.

Conclusion: Adding *Prangos ferulacea* (20%) to probiotic yogurt prevented an increase in acidity, a decrease in syneresis rate and an increase in the number of probiotic bacteria during 21 days. Results showed that the highest number of probiotic bacteria was seen in probiotic yogurt containing 20 percent *Prangos ferulacea*; probably, the existing fiber and some nutrients in *Prangos ferulacea* promoted the viability of probiotic bacteria.

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Introduction

Prangos ferulacea (*Jashir* in Persian) is a plant which grows in mountains and regions with cold weather. It is

found in Iran, Turkey and other regions of Middle-East and Mediterranean regions. *P. ferulacea* which belongs to the *Umbelliferae* family “consists of about 30 species”,¹ “Fifteen species”² of *Prangos ferulacea* are found in

Iran; of them five species are endemic. The *Prangos ferulacea* is a medicinal plant in Iranian traditional medicine administered as an anti-fever and anti-ache. Some species of *Prangos ferulacea* are used as emollient, carminative,³ tonic, anti-flatulent, anthelmintic, anti-fungal, and antibacterial agents.^{4,5} Chemical analysis of the genus *Prangos ferulacea* determined some components including various coumarins, alkaloids, flavonoids, terpenoids and γ -pyrone derivatives.⁶⁻⁹ Antioxidant activity of *prangos ferulacea* has been reported by some researchers.^{10,11} Yogurt is one of the traditional fermented dairy products and one of the most popular milk products produced by two lactic acid bacteria as fermentation starter (*Lactobacillus delbrueckii subsp. Bulgaricus* and *Streptococcus thermophilus*). Dairy products, especially yogurt, are the best well-known carrier for transmission of probiotic organisms to the consumers.¹² Probiotics have been defined as "live micro-organisms confer a health benefit on the host" when administered in adequate amounts.¹³ Some micro-organisms in the human gut system are lactic acid producer, such as *Lactobacillus* and *Bifidobacterium* which are probiotics. Probiotics are beneficial micro-organisms with functional properties including treatment of lactose intolerance, diarrhea, constipation, allergies, inflammatory bowel disease, irritable bowel syndrome and peptic ulcer; stimulation of the immune system and prevention of autoimmune diseases. They also lower cholesterol and have anti-cancer and cholesterol lowering effect.¹⁴⁻¹⁷ *Lactobacillus casei* belongs to *Lactobacillaceae* family. The morphology and properties of *L. casei* are rod-shaped colonies about 1mm diameter (white, shiny and smooth), non spore-former, gram-positive, negative-catalase, mesophyl, microaerophilic, and that is the normal inhabitant of the oral cavity and the digestive tract in humans. The addition of *L. casei* into yogurt as starter can promote extra nutritional and physiological values and improve the technological and nutritional properties of the product as a probiotic functional food.^{18,19} *L. casei* is defined as a highly viable and stable bacteria in fermented milk products such as yogurt during storage.²⁰ With respect to the fact that the *Prangos ferulacea*-yogurt is traditionally produced in Iran and the effect of this plant on the viability of probiotic and characteristics of probiotic yogurt is not yet known, the aim of this study was to investigate the effect of adding different percentages of *Prangos ferulacea* (10, 20 and 30%) on themicrobial, physiochemical properties and sensory characteristics of probiotic yogurt containing *Lactobacillus casei*.

Materials and Methods

Preparation of Cooked *Prangos Ferulacea*

Prangos ferulacea was bought from Sepidan city, northwest of Shiraz, Fars Province, Iran. Five kilogram of *Prangos ferulacea* was soaked in a 10

liter tap water and then left for one hour. Then, the water in the dish was removed and water was added to it. Then *Prangos ferulacea* was cooked with water for 15 minutes. Cooking stage was repeated anew. Then, the plant was placed on the screen for one hour. Prepared *prangos ferolacea* was packaged and stored at refrigerator.

Preparation of Starter Culture

Low fat milk (1.5% fat) was heated up to 85°C and cooled to 40°C. Probiotic *Lactobacillus casei* (DVS) and common (contains *Lactobacillus bulgaricus* and *Streptococcus thermophilus*) starter cultures (Chris-Hansen Denmark) were mixed thoroughly with the preheated milk followed by incubation at 40°C for 6 hours; then, the yoghurt was stored at 4°C.

Preparation of *Prangos Ferulacea*-Yoghurt (PFY)

Yoghurt was produced by adding 2-3 percent of common and probiotic starter cultures with 1L of preheated milk followed by incubation at 37°C until pH decreased to 4.6. PFY with varying compositions (10%, 20%, 30% w/v) were made by mixing mashed *Prangos ferulacea* into produced yoghurts, respectively. All the samples were stored at 4°C.

L. Casei Counts

The enumeration of *L. casei* was performed in MRS agar media (Merck, Germany) containing Vancomycin that was prepared according to the manufacturer's instruction (Merck, Germany). About 2 mL of Vancomycin solution (Sigma Aldrich Co.) was added to 1000 mL MRS agar. Then, 10-fold serial dilutions were prepared in sailing-peptone (8.9% NaCl+0.1% peptone). One ml of each dilution was inoculated into plates followed by adding MRS Vancomycin agar. The plates were incubated anaerobically, using gas generating pack A (Merck, Germany) at 37°C for 72 h. White, shiny, smooth colonies of 1.0mm diameter were counted.²¹

Total Titratable Acidity

Total Titratable Acidity on a basis of lactic acid percentage was measured in milk and yoghurt samples according to the methods described in the Iranian National Standards number 2852.²²

Syneresis Measurement

The released whey in the yoghurt samples was measured according to Mazloomi and colleagues by inverting a 50g sample at 5°C on a wathman paper

number 40 placed on the top of a funnel. The quantity of whey collected after 1 h of drainage at refrigerator (5°C) was used as an index of syneresis.²³

Sensory Evaluation

Sensory evaluation was conducted by 7 trained panelists using self-made questionnaire based on regulation of Iranian National Standards number 695 (2002) for sensory evaluation of dairy products. The samples were provided to panelists on days 1 and 21, in a random order using identical containers coded with random numbers. The taste, odor, mouth-feel, appearance and color of the samples were evaluated according to a 0-5 point scale, “very weak=0, weak=1, moderately=2, good=3, very good=4”. The total score was calculated as the sum score of all the attributes.²²

Statistic and Analysis

Data analyses were performed using SPSS statistical software, version 16 (SPSS). One way ANOVA was used to analyze the data on microbial and physicochemical properties of yogurts. In all cases, the Duncan post hoc test was performed for comparison. Non-parametric methods (*Kruskal-Wallis*) were performed to determine the statistical differences of the sensory data, and where appropriate, T-tests were performed for comparison of two means. A P value <0.05 was considered statistically significant for all analyses. All parameters per replication on days 1, 7, 14, and 21 were determined in three cups of samples. The experiments were performed in triplicate.

The Study Date and Place

The current research was conducted in the first half of 2014.

Results

Effect of P.F on Acidity in Probiotic Yoghurts during Days of Storage at 4°C

Data of Lactic Acid percentage as Total Titratable Acidity (TTA) in all types of yogurts during storage up to 21 are shown in Table 1. As the amount of *Prangos ferulacea* and storage time increased, the percentage of lactic acid in samples increased in such a way that the highest percentage of lactic acid was related to the yogurt containing 30 percent of *Prangos ferulacea* on the last day; however, such differences were not significant compared to control yogurt.

Effect of P.F on Syneresis in Probiotic Yoghurts during Days of Storage at 4°C

The data in Table 2 shows that the syneresis rate of the yogurt containing different percentages of *Prangos ferulacea* was significantly lower than the control on days 1, 7, 14 and (P<0.001). The lowest amount of syneresis was observed in PFY20 and PFY30 at the end of the experiment (P<0.001).

Effect of P.F on Sensory Properties on Probiotic Yoghurt during the Storage Days at 4°C

The mean scores of sensory attributes of yogurts obtained from the questionnaires (Table 3) determined that the scores of mouth feel related to yogurt samples

Table 1: Comparison of the Mean±SE of titratable acidity between yogurt samples and control and in repeated samples

| Treatments | 1 (day) | 7 (Day) | 14 (Day) | 21 (Day) | P value |
|------------|-----------|------------------------|-----------|-----------|---------|
| PY | 1.13±0.35 | 1.07±0.12 ^a | 1.1±1.00 | 1.15±0.05 | 0.816 |
| PFY10 | 0.97±0.15 | 1.03±0.06 ^a | 1.17±0.15 | 1.2±0.27 | 0.432 |
| PFY20 | 1.07±0.21 | 1.03±0.06 ^a | 1.23±0.15 | 1.4±0.35 | 0.333 |
| PFY30 | 1.20±0.27 | 1.33±0.15 ^b | 1.37±0.15 | 1.4±0.21 | 0.665 |
| P value | 0.720 | 0.021 | 0.201 | 0.433 | |

PFY=Probiotic Yoghurt containing L. Casei; PFY10=Probiotic+10%Prangos ferulacea; PFY20=Probiotic+20%Prangos ferulacea; PFY30=Probiotic+30%Prangos ferulacea; ^{a, b, c} Different superscripts letters in the same column indicate a statistically significant difference or (P<0.05)

Table 2: Compare of the Mean±SE of syneresis is between yogurt samples and control and in repeated samples

| Treatments | 1 (day) | 7 (Day) | 14 (Day) | 21 (Day) |
|------------|--------------------------|---------------------------|-----------------------------|--------------------------|
| PY | 14.27±1.136 ^a | 14.375±0.946 ^a | 14.938±0.625 ^a | 14.7±0.819 ^a |
| PFY10 | 10±1.541 ^b | 8.75±1.936 ^b | 10.013±0.44791 ^b | 12±1.803 ^b |
| PFY20 | 7.04±2.002 ^c | 5.9±1.917 ^c | 7.238±0.37722 ^c | 7.033±0.404 ^c |
| PFY30 | 5.352±2.097 ^c | 3±1.671 ^d | 5.7±0.245 ^d | 5.467±0.577 ^c |
| P value | <0.001 | <0.001 | <0.001 | <0.001 |

PFY=Probiotic Yoghurt containing L. Casei; PFY10=Probiotic+10%Prangos ferulacea; PFY20=Probiotic+20%Prangos ferulacea; PFY30=Probiotic+30%Prangos ferulacea; ^{a, b, c} Different superscripts letters in the same column indicate a statistically significant difference or (P<0.05)

Table 3: Mean±SE scores of sensory evaluation (7 panelists) of various yogurts on days 1 and 21 of storage at 4°C

| Sensory values | PY | PFY10 | PFY20 | PFY30 | P value |
|------------------|--------------------------|------------------------|-------------------------|-------------------------|---------|
| Taste Day 1 | 2.57±0.79 | 2.86±0.7 | 2/00±1/00 | 2/00±1/00 | 0.202 |
| Odor Day 1 | 3.00±1.00 | 3±0.58 | 2.71±0.95 | 2.42±0.79 | 0.542 |
| Appearance Day 1 | 2.29±1.25 ^{a,b} | 3±0.58 ^b | 2.14±0.9 ^{a,b} | 1.57±0.976 ^a | 0.073 |
| Color Day 1 | 2.57±0.976 | 3.29±0.488 | 2.86±0.69 | 2.57±0.787 | 0.073 |
| mouth-feel Day 1 | 2.57±0.79 ^b | 2.57±0.79 ^b | 1.86±0.9 ^{a,b} | 1.29±0.756 ^a | 0.017 |
| Taste Day 21 | 2.86±1.07 | 1.86±0.9 | 1.86±1.21 | 1.43±0.976 | 0.098 |
| Odor Day 21 | 3/00±0.82 | 1.86±0.9 | 2.29±1.25 | 1.71±1.11 | 0.117 |
| Appearance Day21 | 2.43±1.134 | 1.86±0.9 | 2.29±1.11 | 1.14±1.07 | 0.129 |
| Color Day21 | 3.14±0.69 | 2.29±0.95 | 2.29±1.25 | 1.71±1.11 | 0.129 |
| mouth-feel Day21 | 3.14±0.69 ^b | 1.57±0.79 ^b | 1.71±1.25 ^b | 0.29±0.49 ^a | <0.001 |

PFY=Probiotic Yoghurt containing *L. Casei*; PFY10=Probiotic+10%*Prangos ferulacea*; PFY20=Probiotic+20%*Prangos ferulacea*; PFY30=Probiotic+30%*Prangos ferulacea*; ^{a, b, c} Different superscripts letters in the same column indicate a statistically significant difference or (P<0.05)

(containing PF) compared with the control yogurt on the first day had a significant difference (P=0.017). Also, there was a significant difference between the control yogurt and samples on day 21 (P<0.001). The scores of other sensory attributes belonging to the yogurt samples had no significant differences compared to the control yogurt on days 1 and 21. The mean of sensory scores of all types of yogurts on day one (P=0.021) showed significant differences between yogurts also on day 21 (0.027).

Effect of P.F on Viability of Probiotic Bacteria in Probiotic Yoghurt during the Storage Days at 4°C

Figure 1 shows that highest number of *L. casei* on day 21 was observed in PFY20. The number of *Lactobacillus casei* (log CFU/ml) in yogurt samples (containing PF) had significant differences compared to the control yogurt on day 1 (P=0.009), day 7 (P=0.040), day 14 (P<0.001), and day 21 (P<0.001). The number of probiotic in PFY30 showed significant differences between days 1 and 21 (P=0.047) and also

between days 7 and 21 (P=0.020). Enumeration of probiotic in PFY10 indicated a significant difference between days 1 and 21 (P=0.047). The number of probiotic in other samples and control yogurt on the first day was not significant compared to other days during the storage.

Discussion

We found that there were no significant differences between the amount of lactic acid in the treatments (PFY10, 20, 30%) compared to the control yogurt (PY) (except on day 7) at the end of the experiment period. This finding is similar to the results of the research conducted by Mazloomi and colleagues,²³ Aghajani and colleagues,²⁵ Yeganehzad and colleagues,²⁴ and in contrast to Zainoldin and Baba.²⁷

The syneresis rate of yogurt samples (PFY10, 20, 30%) was lower during storage time and compared to the control yogurt (PY) it showed significant differences. Probably, the reason for the reduction in syneresis is an increase in *Prangos ferulacea* due to

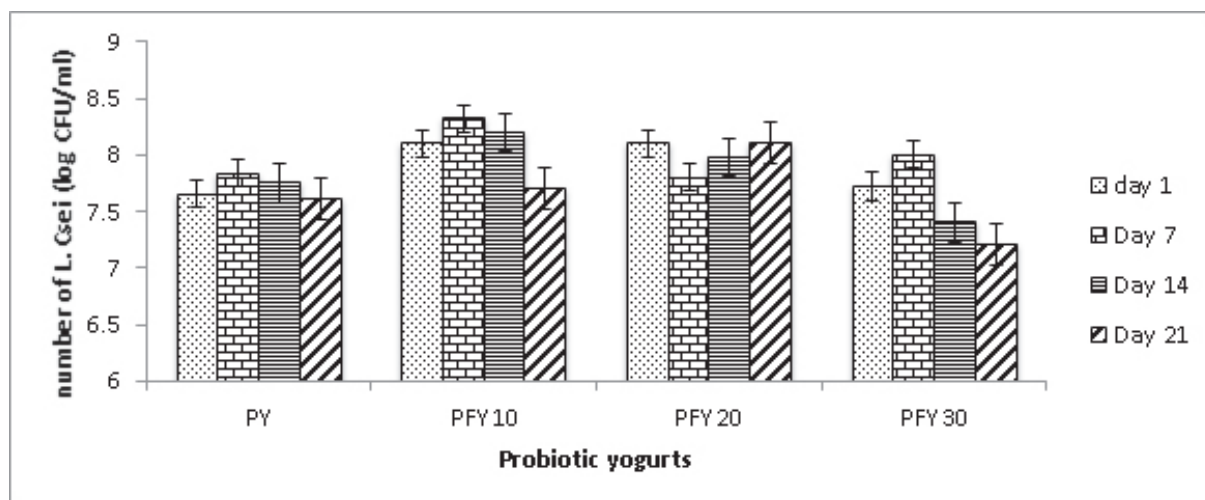


Figure 1: This figure shows the mean±SE lactobacillus casei count (log CFU/ml) of various types of yogurt during storage up to 21 days. PFY=Probiotic Yoghurt containing *L. Casei*; PFY10=Probiotic+10%*Prangos ferulacea*; PFY20=Probiotic+20%*Prangos ferulacea*; PFY30=Probiotic+30%*Prangos ferulacea*

the presence of fiber in it and its role in absorbing the water molecules. Other researchers such as Aghajani and colleagues,²⁵ Yeganehzad and colleagues,²⁴ Aryana and McGrew,²⁶ and Dehghan and colleagues²⁸ confirmed our findings about syneresis. A study by Dehghan and colleagues in the area of the effect of inoculating microbial cultures and incubating temperature on the yogurt containing *Lactobacillus casei* showed that the lowest rate of syneresis was observed in incubated yogurts at a temperature of 37°C.²⁸ Mazloomi and colleagues showed that adding inulin up to two percent to probiotic yogurt containing *Lactobacillus acidophilus* would cause a reduction in syneresis.²³ But a research by Zainoldin and Baba showed that all the fruit enriched yogurts had higher percentages of syneresis compared to the control yogurt.²⁷

The scores of sensory attributes of yogurt samples (containing PF) had not significant differences compared to the control yogurt (PY). A study on the effect of the prebiotic on probiotic yogurt containing *Lactobacillus casei* conducted by Aghajani and colleagues showed that yogurt samples containing prebiotic (inulin, lactulose, and oligofructose) had the best sensory properties compared to the control yogurt.²⁵ The study of Aryana and McGrew on the qualitative properties of probiotic yogurt with *Lactobacillus casei* and different quantities of prebiotics (short, medium and long inulin) showed that the taste of the yogurt containing long chain inulin were significantly different with the control yogurt during the storage.²⁶

In this study, enumeration of *Lactobacillus casei* bacteria during storage revealed that the highest number of *Lactobacillus casei* was observed in probiotic yogurt containing 20 percent *Prangos ferulacea* on day 21. Probably, PF has some nutrients and fiber which improved the viability of this probiotic. Some researchers reported that the number of *L. casei* in yogurts containing inulin during storage was higher compared to the control yogurt; this is similar to our results.^{23, 25, 26, 29} Another study by Sendra and colleagues showed that addition of lemon fiber to fermented milk with *L. casei* enhanced the growth and survival of this probiotic during storage.²⁹ Mazloomi and colleagues concluded that the highest number of *Lactobacillus acidophilus* bacteria was observed in probiotic yogurt containing 2 percent inulin on day 14 compared to the controls.²³ Aryana and McGrew reported that the number of *Lactobacillus* in yogurts containing inulin was higher than other yogurts.²⁶ According to Donkor and colleagues, the yogurt fermented by common and probiotic bacteria (*Lactobacillus acidophilus* and *L. casei*, respectively) were mixed with different percentages of maize starch powder (having high amylose). The mixture was kept in the refrigerator for 28 days. The number

of probiotics in different types of yogurt containing maize starch powder (having high amylose) led to an extended life span compared to the control yogurt during storage.³⁰ Yeganehzad and colleagues showed that substituting soy milk with cow milk would lead to an increase in the total number of the probiotic bacteria in yogurt samples and samples containing 10 and 20-percent soy had enough number of probiotic on day 21.²⁴

Conclusion

Results of this study showed that the lowest percentage of lactic acid was in PY on day 21. The highest sensory score and the number of probiotic bacteria in yogurt samples were in probiotic yogurt containing 20 percent *Prangos ferulacea* on day 21. *Prangos ferulacea* can enhance the probiotic yogurt containing *Lactobacillus Casei* because of having fiber and some nutrients and promoted the viability of probiotic bacteria *Lactobacillus casei* in yogurt while the remained numbers of probiotic were higher than the minimum therapeutic dosage of probiotics (log10⁶CFU/ml) during storage. The best consumption time is the first week after production. Therefore, the produced yogurt including probiotic yogurt *L. casei* containing different percentages of *Prangos ferulacea* can be introduced as a probiotic product.

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Conflict of Interest: None declared.

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