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Replacing milk fat with pumpkin seed oil in yogurt

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ORIGINAL ARTICLE

Abstract

This study explored the potential of pumpkin seed oil (PSO) in enhancing the health benefits of yogurt by partially replacing milk fat. Yogurt was produced with varying amounts of PSO—0.0, 1, 2, 3, and 4%—to replace milk fat. Yogurt with 2% PSO showed higher levels of unsaturated fatty acids, conjugated linoleic acid, total phenols, and antioxidants, while also showing reduced amounts of free fatty acids and peroxide values. Although the texture remained unchanged, we observed significant differences in color, flavor, and overall acceptability between treated yogurt and control group. As the PSO content increased, color, flavor, and the overall acceptability of yogurt decreased.

Keywords: dairy; fatty acids; linoleic acid; phenol; plant oil; sensory

Introduction

Yogurt is commonly acknowledged as a nutritious food because of its significant content of protein, calcium, and riboflavin, as well as vitamins B6 and B12. The frequent consumption of yogurt has been linked to enhancements in immune system function, facilitation of lactose digestion, regulation of blood sugar levels, and reductions in the likelihood of developing allergies, constipation, diarrhea, colon cancer, and inflammatory bowel disease (Gómez-Gallego *et al*., 2018; Mckinley, 2005); however, it may be necessary to limit the intake of dairy products as its fats contain significant quantities of saturated fatty acids (SFA) and cholesterol (Givens, 2017; Gómez-Gallego *et al*., 2018; Markey and Kliem, 2020).

Milk fat has been classified as hypercholesterolemic due to its elevated long-chain SFAs and cholesterol (Astrup *et al.,* 2020). A correlation exists between dietary fat consumption and elevated susceptibility to obesity, atherosclerosis, cardiovascular disease, hypertension, and disorders associated with lipid oxidation (Badmus *et al*., 2023; Ganesan *et al*., 2018). Researchers observed several health advantages of decreasing SFAs by increasing the amounts of polyunsaturated and monounsaturated fatty acids (PUFAs and MUFAs) in different food products (Arias *et al.,* 2023; Barone *et al.,* 2019). Considerable efforts have been undertaken to develop food products that incorporate specific ingredients known to mitigate health risks. Vegetable oils have been utilized in food formulation to harness the potential health and dietary advantages associated with their content of MUFAs and PUFAs (Islam *et al*., 2023; Pattnaik and Mishra, 2022; Saini *et al*., 2021).

The conjugated linoleic acid (CLA) in milk fat has several advantageous health characteristics, including antioxidative, anticarcinogenic, antidiabetic, antihypertensive,

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immunomodulatory, and hypocholesterolemic benefits (Moslemi *et al*., 2023; Zahed *et al*., 2021). The focus on isomers of CLA has garnered considerable attention due to their noteworthy physiological attributes identified in human subjects (Paszczyk and Czarnowska-Kujawska, 2022; Wang *et al*., 2022). Microbial strains of lactic acid bacteria (LAB) have demonstrated the ability to produce CLA isomers, specifically C18:2 cis-9, trans-11, and C18:2 trans-10, cis-12, by the metabolism of linoleic acid (LA) present in milk or in controlled laboratory settings. Vegetable oils rich in LA can be used by LAB to synthesize CLA isomers in vitro (Ghosh *et al*., 2019; Kuhl and De Dea Lindner, 2016; Özer and Kılıç, 2021). Important PUFAs can be found in vegetable oils (Jurić *et al*., 2022). Animal cells cannot synthesize linoleic and alpha-linolenic acids; hence, they are recognized as a necessary human body component (Alessandri *et al*., 2008). According to research (Tindall *et al.,* 2019), eating foods high in oleic acid can help lower blood pressure and LDL cholesterol. In addition, vegetable oils provide the body with minute amounts of vitamin A, Vitamin D, tocopherols, lecithin, phytosterols, pigments, and various other constituents (Chen *et al*., 2011; Lee *et al*., 2022).

Pumpkin seed oil (PSO) is currently recognized as a nutraceutical and edible oil due to its inherent abundance of proteins, phytosterols, PUFAs, antioxidants, carotenoids, tocopherols, and trace elements like zinc (Topkafa *et al*., 2019; Siano *et al*., 2016). PSO is distinguished from other vegetable oils due to its unique nutritional profile. It includes arachidonic acid (0.27%), which is essential for the optimal functioning of the neurological system (Šamec *et al*., 2022). Oleic acid and linoleic acid predominate in PSO, which has more unsaturated fatty acids than saturated ones (USDA, 2019). Furthermore, PSO includes a range of vital constituents, including minerals, squalene, tocopherols, polyphenols, phytoestrogens, and carotenoids, all linked to diverse health advantages. PSO is employed in conventional medicine to treat intestinal parasite ailments related to the prostate and bladder (Delaš, 2010). PSO has garnered significant attention in the scientific community in recent years, leading to numerous studies exploring its phytochemical composition, mechanistic properties, and clinical effects.

Consequently, a growing body of assertions and claims suggests that PSO may possess functional food properties. Current literature has evaluated using pumpkin seeds as the primary raw material for oil extraction (Alasalvar *et al.*, 2021; Dotto and Chacha, 2020).

However, the potential of using PSO to make yogurt, a largely unexplored area, is a pressing need in the field of functional food research. Further exploration in this direction could lead to significant advancements in the field. This study explored the effects of different fat levels and ratios of PSO on the production of yogurt. A thorough analysis was conducted on various aspects of the resulting product, including its chemical composition, texture, microbiological profile, and sensory characteristics. The aim was to assess how these factors influenced the overall quality and acceptance of the yogurt.

Materials and Methods

Materials and reagents

Pumpkin (*Cucurbita pepo*) seeds were collected from the authorized markets in Riyadh governorate, Saudi Arabia. Cold pressing was performed in a laboratory prototype apparatus. The extracted oil was later separated from the sediment by centrifugation at 5000 rpm (Hettich Zentrifugen, model Universal 32R) for 10 min at 24°C and stored in a freezer at −20°C. The cow milk sample utilized in this study had a fat content of 4%. The conjugated linoleic acid standard was procured from Sigma Chemical Co., based in St. Louis, USA. Each solvent, including hexane, methanol, and chloroform, was of GC quality; unless otherwise noted, all compounds were of analytical grade. Commercial yogurt cultures, comprising *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. bulgaricus and supplemental *Lactobacillus acidophilus* cultures were supplied by the Microbial unit in Riyadh, Saudi Arabia. These cultures were stored in a −18°C freezer. This procedure was carried out close to the flame and in a clean environment.

According to Ivanova *et al*., (2017) the emulsion was created using commercially available PSO in the amounts listed in Table 1 and glycerol monostearate as an emulsifier in a concentration of 1 $g.dm^{-3}$.

Preparation of emulsion form of pumpkin seed oil

To generate emulsions, the oil phase and dispersion medium were combined using an IKAR T18 digital

Table 1. The fat content of various yogurt prepared with PSO.

*Sample	Fat content of product %	Pumpkin seed oil%	Milk fat%
CY	4	0	4
T1	4	1	3
T2	4	2	2
T3	4	3	1
T4	4	4	0

C, Control yogurt; T1, yogurt made from 1% PSO; T2, yogurt made from 2% PSO; T3, yogurt made from 3% PSO; T4, yogurt made from 4% PSO.

Ul-tra-Turrax laboratory homogenizer at 55–60°C for 5 min. The mixture was continuously swirled at a speed of 15,000 rpm. This process continued until the emulsifier was entirely dissolved (Ivanova *et al*., 2017).

Preparation of yogurt

The traditional procedure for making fermented milk with a set coagulum was used to acquire the experimental samples (Tamime and Robinson, 2007). The necessary vegetable oil emulsifier was then combined with the milk, which was then homogenized in a GEA homogenizer (Niro Soavi, Germany) at 250 bar and 60°C. Finally, batch pasteurization of the treated milk took place for 5 min at 92°C (Corrieu and Béal, 2016). According to the supplier's recommendations, milk was chilled to 45°C before adding the yogurt starter culture (2% v/v) and adjunct culture of *Lactobacillus acidophilus* (0.8% v/v). The mixture was thoroughly mixed before dividing into 250 mL portions and incubating at 45°C. pH = 4.6 marked the end of the fermentation process, and the produced yogurt was then refrigerated at 4°C. The control sample contained 4% (w/v) milk fat, while in the treated samples, the fat of milk was replaced with PSO in the proportion of 25% (1%), 50% (2%), 75% (3%), and 100% (4%). The yogurt samples were stored at a temperature of 4 ± 1°C and subjected to 14 days of analysis. The yogurt treatments can be summarized as follows: The yielded yogurt was stored at 4 ± 1°C and subjected to 14 days of analysis. The yogurt treatments (T) can be summarized as follows:

C: Control yogurt: yogurt made with 4% milk fat;
T1: Yogurt made with 1% PSO and 3% milk fat;
T2: Yogurt made with 2% PSO and 2% milk fat;
T3: Yogurt made with 3% PSO and 1% milk fat;
T4: Yogurt made with 4% PSO.

Methods of analysis

The antioxidant activity percentage (AO) of the produced yogurt was assessed using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) inhibition method. The total phenolic content (TPC mg GAE/g) was determined according to the instructions provided by Singleton *et al.* (1999) and Van Hung and Morita (2008). The determination of the total carotenoids (TCC) in yielded yogurt was conducted by Hagenimana *et al.* (1998) and the results were represented as micrograms per kilogram ($\mu g.kg^{-1}$) using the formula. The investigation adhered to the accepted protocols described in Horwitz (1997) to quantify total solids, protein, milk fat, titratable acidity, and ash content in raw milk and yogurt. The pH levels of the milk and yogurt samples were determined using a Hanna Instrument 8021 pH meter.

Sensory evaluation

Fifty instructors and graduate students with experience in food-related courses who were acquainted with the assessed features of the PSOY were given samples of the produced yogurt. 100 mL single-use plastic cups were used to analyze each sample in this investigation at ten °C. According to Kim *et al.* (2022), the yogurt samples were evaluated for texture, color, flavor, and overall acceptability using a 5-point hedonic scale, with ratings ranging from 5 (excellent) to 1 (very terrible).

Fat extraction from yogurt

The fat extraction technique used in this work adhered to the method by Kinsella *et al.* (1990). A 100 g yogurt sample was immersed in a combined solution of 50 mL of chloroform and 100 mL of methanol for 3 min. The mixing procedure employed a vortex mixer Model L5M manufactured by Silverson in the United Kingdom. Subsequently, 50 mL of chloroform was added to the solution, and the mixture was agitated for 30 s. Subsequently, 50 mL of water was added to the mixture and stirred for 30 s. The organic phase, comprising the fat obtained from the yogurt, was separated using a centrifugal funnel. Heat treatment removed the solvent in a rotating evaporator set at 75°C.

Fatty acid composition

The fatty acids (FAs) in isolated fat were subjected to re-esterification using a methanolic potassium hydroxide solution, forming their respective methyl esters. The identification of the methyl esters of FAs was conducted using a Varian 3800 apparatus manufactured by Varian Techtron in the United States. This analysis employed a gas chromatographic technique. The identification of fatty acids in milk fat was performed using analytical standards provided by Supelco, a company based in the United States. Of the 57 fatty acids (FAs) detected in the chromatograms, 49 FAs were identified. Pecová *et al.* (2019) utilized the ratio between the peak area of each fatty acid (FA) and the total peak area of all detected acids to determine the proportion of each FA.

Physicochemical properties

The method of AOCS (Brühl, 1997) was employed to determine the acid value, iodine value, free fatty acid content, peroxide value, saponification value, and unsaponifiable matter.

Syneresis and viscosity

The identification of syneresis in different yogurt samples was reported by Wu *et al.* (2000). A yogurt sample weighing 25 g was subjected to a draining process for 2 h at a temperature of 7°C. This process involved the use of filter paper (no. 589/2, S&S, Dassel, Germany). The syneresis was determined by dividing the weight of the collected permeate by the yogurt weight and expressing the result as grams of permeate per 100 g of yogurt. The viscosity of yogurt samples was determined by employing a Rotational viscometer-type Lab-Line Model 5437. The findings are presented in Centipoise (c.P) units.

Evaluation of the viability of starter culture

After fermentation and cold storage, the viability of the starter culture in various yogurt milk treatments was assessed. The growth of *S. thermophilus* was inhibited by using the MRS agar medium utilized at a reduced pH level of 5.5 to quantify the viable cells of *Lactobacillus delbrueckii* subsp. *L. bulgaricus* and *L. acidophilus* (Süle *et al*., 2014). The viable count of *S. thermophilus* was counted using M17 agar. *S. thermophilus* and *Lactobacillus* spp. plates were incubated aerobically at 43°C for 48 h (Jordano *et al*., 1992). Gram staining was used to confirm the development of starting culture on the media. Under identical conditions, the experiments were replicated thrice, and each viable cell count was done in triplicate.

Statistical analysis

A two-way analysis of variance (ANOVA) was calculated to compare the data. The mean ± SD was used to express all results. At $P < 0.05$, differences were deemed significant. Every treatment was examined in four repetitions at predetermined intervals.

Results and Discussion

Some chemical and phytochemical properties of pumpkin seed oil

Table 2 provides a detailed overview of the chemical and photochemical properties of PSO. These properties include the acid value (AV), free fatty acids (FFAs), peroxide value (PV), iodine value (IV), saponification value (SV), and unsaponifiable matter (UM). The specific values for these properties in PSO are as follows: 1.52 mg KOH/g, 0.76%, 1.22 mEqO_2/kg, 120.20 g/100 g, 176.12 mKOH/g, and 0.64%. These findings are consistent with those of Samuel *et al*. (2017) who reported the following values for PSO: 1.41 mg KOH/g, 0.71%, 1.17 mEqO_2/kg, 119.67 g/100 g, 179.04 mKOH/g, and 0.47% for AV, FFA, PV, IV, SV, and UM, respectively. Furthermore, the total phenolic (TF) and total carotenoid (TC) contents, as well as the percentage of DPPH inhibition of PSO, were measured at 24.80 mg GAE/g, 124.18 µg/kg, and 72.60%, respectively. These results are in line with those reported by Siano *et al*. (2016) who found that the total flavonoid content (TF), total phenolic content (TC), and DPPH inhibition percentage of PSO to be 52.40 mg GAE/g, 107.50 µg/kg, and 25.87%, respectively.

Table 2. Some chemical and phytochemical properties of PSO.

Components	Pumpkin seed oil
Acid value (mgKOH/g)	1.52 ± 0.06
Free fatty acid (%)	0.76 ± 0.02
Peroxide value mEqO_2/kg)	1.22 ± 0.04
Iodine Value (g/100 g)	120.20 ± 3.4
Saponification value (mKOH/g)	176.12 ± 2.6
Unsaponifiable matter (%)	0.64 ± 0.01
Total Phenol (mg GAE/g)	24.80 ± 0.45
Carotenoids (µg.kg^{-1})	124.18 ± 4.8
% DPPH Inhibition	72.60 ± 1.2

Titratable acidity, pH, free fatty acids, and peroxide values of yogurt made from PSO

Table 3 presents the impact of substituting milk fat with PSO on the acidity, pH, FFAs, and PV values of the yogurt produced after 14 days of refrigeration. The results displayed in Table 3 indicate that the pH values rose in the treatments after replacing the milk fat with PSO. Unlike the pH results, the treatments involving substituting milk fat with PSO showed a distinct reduction in acidity levels. The control yogurt had the highest acidity value of 0.84 on the first day of manufacture. The acidity values decreased in the treatments that replaced the milk fat with PSO, where the values were 0.82, 0.83, 0.82, and 0.80 in the samples that replaced the milk fat with 25, 50, 75, and 100% pumpkin seeds oil, respectively. Following a 14-day storage period, the levels of acidity of the control yogurt and the yogurt treatments featuring the substitution of milk fat with 25, 50, 75, and 100% PSO were recorded as 1.2, 1.02, 0.96, 0.92, and 0.90%, respectively. All yogurt treatments exhibited an increase in titratable acidity values. The pH levels declined over the storage time and reached their lowest point, possibly due to the lower acidity of PSO compared to milk fat (Amin *et al.,* 2020; Gedi, 2022). The present results align with the conclusions of Zheng *et al*., (2022), who found that yogurt containing perilla seed oil displayed higher pH than the control throughout storage for 0–28 days. Fennel essential oil may not have affected the pH of yogurt, according to Ben Abdesslem *et al*. (2020) who theorized that this was because it differs from PSO. According to Perina *et al*., (2015) adding vegetable oil emulsion to yogurt ($P \le 0.05$) changed its pH considerably. As microbial metabolism converted lactose to lactic acid, the pH of all samples dropped as storage time was extended (de Campo *et al.,* 2019).

All yogurt samples, both fresh and kept in storage, fell within the acceptable pH range for set-type yogurt.

Table 3 also presents the data on the proportion of free fatty acids (FFA) found in yogurt samples. The control yogurt exhibited the highest free fatty acids (FFA) level. At the same time, a statistically significant difference in FFA content was observed in yogurt treatments where milk fat was replaced with PSO ($P \leq 0.05$). The study by Barrantes *et al.* (1996) proposes that the varying fatty acid composition of milk fats may affect the rate of lipolysis and the possible production of free fatty acids (FFA) in yogurt, facilitated by the lipolytic activity of microbial lipase. The study conducted by Gunstone and Harwood (2007) revealed that milk fat primarily comprises short-chain fatty acids, while PSO mostly comprises fatty acids with carbon chain lengths of 16 and 18. The higher FFA content in control yogurt may be attributed to the faster hydrolysis of short-chain FFAs during digestion (Deeth and Fitz-Gerald, 2006).

In Table 3, the PVs of several yogurts are shown on Day 0 of incubation and Day 14 of storage. During the storage period, all yogurt treatments in which milk fat was replaced with PSO exhibited lower PV than the control yogurt. The observed difference exhibited statistical significance ($P \leq 0.05$), although the higher unsaturation levels in the vegetable oils were employed as replacements. Table 3 demonstrates that the control yogurt displayed heightened vulnerability to oxidation due to its elevated free fatty acids (FFA) levels. Additionally, the lower natural antioxidant content of milk fat than vegetable oils contributed to this heightened susceptibility. The yogurt treatments with the highest PV contained 1% (w/v) PSO, and a decline in PV was seen as the ratio of PSO rise. In these yogurt treatments, milk fat was substituted with PSO. This might be due to the less linolenic acid and more natural antioxidants content in PSO (Pușcaș *et al.*, 2020; Siano *et al.*, 2016). As observed in Table 3, storage duration had a substantial impact on the PV of yogurts ($P \leq 0.05$). These findings align with those published by Farmani *et al.* (2016). Moreover, Ivanova *et al.* (2017) found that the FFA content and PV of yogurt were considerably reduced when milk fat was replaced with canola, sesame, or olive oil.

Total phenolic content, antioxidant activity, whey syneresis, and viscosity of yogurt made from PSO

The study examined the impact of incorporating pumpkin seed oil (PSO) on the properties of yogurt during a 14-day refrigerated storage period (Table 4). According

Table 3. Titratable acidity, pH, free fatty acids and peroxide values of yogurt made from PSO during storage at refrigerator temperature for 14 days.

Parameters	Storage period (Day)	Treatments				
		C	T1	T2	T3	T4
Acidity%	0	0.84 ± 0.02^{FGH}	0.82 ± 0.04^{GH}	0.83 ± 0.03^{FGH}	0.82 ± 0.03^{GH}	0.80 ± 0.04^{H}
	7	0.95 ± 0.04^{BCD}	0.92 ± 0.02^{CDE}	0.88 ± 0.04^{DEFG}	0.86 ± 0.05^{EFGH}	0.84 ± 0.02^{FGH}
	14	1.12 ± 0.01^{A}	1.02 ± 0.03^{B}	0.96 ± 0.02^{BC}	0.92 ± 0.03^{CDE}	0.90 ± 0.04^{CDEF}
	LSD 0.0789					
pH values	0	4.62 ± 0.07^{AB}	4.64 ± 0.04^{A}	4.62 ± 0.04^{AB}	4.63 ± 0.04^{A}	4.64 ± 0.05^{A}
	7	4.40 ± 0.06^{E}	4.50 ± 0.05^{D}	4.54 ± 0.08^{CD}	4.60 ± 0.09^{AB}	4.58 ± 0.07^{BC}
	14	4.14 ± 0.08^{G}	4.30 ± 0.05^{F}	4.42 ± 0.06	4.50 ± 0.04^{D}	4.52 ± 0.06^{D}
	LSD 0.0522					
FFA (%w/w)	0	1.02 ± 0.02^{C}	0.46 ± 0.01^{HI}	0.40 ± 0.01^{I}	0.36 ± 0.02^{I}	0.34 ± 0.01^{I}
	7	2.33 ± 0.01^{B}	0.78 ± 0.02^{DEF}	0.66 ± 0.03^{FG}	0.62 ± 0.01^{G}	0.58 ± 0.02^{GH}
	14	2.80 ± 0.04^{A}	0.92 ± 0.02^{CD}	0.85 ± 0.03^{DE}	0.78 ± 0.02^{EF}	0.70 ± 0.03^{FG}
	LSD 0.1249					
PV (meqO_2/kg fat)	0	1.70 ± 0.02^{D}	1.50 ± 0.01^{FG}	1.42 ± 0.02^{CH}	1.26 ± 0.01^{I}	1.12 ± 0.01^{J}
	7	1.84 ± 0.03^{C}	1.65 ± 0.02^{DE}	1.58 ± 0.01^{EF}	1.40 ± 0.03^{GH}	1.32 ± 0.02^{HI}
	17	2.14 ± 0.02^{A}	1.96 ± 0.04^{B}	1.70 ± 0.03^{D}	1.58 ± 0.02^{EF}	1.46 ± 0.03^{G}
	LSD 0.1160					

Means in the same row that are denoted by several little letters differ significantly ($P \leq 0.05$). Each yogurt sample underwent a triplicate analysis after each experiment was carried out in triplicate. LSD, The smallest difference; C, control yogurt; T1, yogurt made from 1% PSO; T2, yoghurt made from 2% PSO; T3, yoghurt made from 3% PSO; T4, yoghurt made from 4% PSO.

to Sah *et al.* (2014), the antioxidant activity in yogurt is primarily attributed to physiologically active peptides. However, in this study, we found that the antioxidant activity of the yogurt increased significantly with the addition of PSO, and this effect was proportional to the amount of PSO added. The PSO-enriched yogurt consistently exhibited higher antioxidant activity throughout the storage period than the control group. This suggests that PSO has the potential to enhance the antioxidant capacity of yogurt due to its high levels of polyunsaturated fatty acids, bioactive compounds, and polyphenols (Amin *et al*., 2020; Gedi, 2022). Specifically, the yogurt containing 4% PSO showed significant antioxidant activity throughout the 14-day storage period, indicating the potential industrial benefits of using PSO to boost the antioxidant capacity of yogurt. These findings are in line with previous studies by Farmani *et al.* (2016), Ivanova *et al.* (2017), and Zheng *et al.* (2022), which also reported increased total phenolic content (TPC) and radical scavenging activity (RSA) in yogurt when various vegetable oils were added or substituted for milk fat.

Whey separation in yogurt is due to the rearrangement of gel network particles (Tamime and Robinson, 2007). Table 4 presents the quantitative data on whey separation in yogurt samples containing PSO compared to the control yogurt. The study found that the syneresis (whey separation) increased significantly toward the end of the shelf life, with the yogurt sample containing 4% PSO exhibiting the highest syneresis. As the ratio of PSO decreased, a statistically significant reduction in syneresis was observed, with the control yogurt showing the lowest syneresis. This pattern is consistent with the findings of Abdullah *et al.* (2012) who reported higher syneresis in yogurt samples containing enzymatically interesterified palm olein compared to the control. Similarly, Barrantes *et al.* (1996) found that substituting milk fat with various plant oils led to increased syneresis during storage, likely due to the lower melting point and viscosity of plant oils compared to animal fats, which can result in a looser texture when added to yogurt. Additionally, the study suggests that the viscosity of the yogurt may be influenced by the ability of the protein to immobilize water and the strength of the gel network, which is enhanced as the viscosity of the oil increases, leading to reduced whey loss (Luna *et al.,* 2004; Tamime and Robinson, 2007).

Table 4 displays the viscosity of yogurts containing PSO after 14 days of storage. The study found that all PSO-enriched yogurts had lower viscosity than the control sample. Specifically, the yogurt containing 4% PSO exhibited the lowest viscosity, while the control yogurt had the highest viscosity. As the ratio of PSO increased

Table 4. The total phenolic content, antioxidant activity, whey syneresis, and viscosity of yogurt produced using PSO, observed over 14 days of storage at refrigeration temperature.

Parameters	Storage period (Day)	Treatments				
		C	T1	T2	T3	T4
Total phenolic content (mg/00 g)	0	50.20 ± 1.1^{J}	62.50 ± 1.3^{G}	70.90 ± 1.6^{D}	78.20 ± 1. 2^{B}	82.50 ± 1.5^{A}
	7	35.80 ± 1.4^{L}	46.80 ± 1.4^{K}	61.50 ± 1.3^{GH}	67.40 ± 1.5^{E}	73.90 ± 1.1^{C}
	14	24.50 ± 1.2^{M}	35.60 ± 1.5^{L}	55.70 ± 1.2^{I}	59.60 ± 1.4^{H}	65.20 ± 1.2^{F}
	LSD 2.1955					
Radical scavenging activity RSA %	0	20.30 ± 1.3^{HI}	23.20 ± 1.12^{G}	26.40 ± 1.18^{CD}	28.80 ± 1.3^{B}	32.50 ± 1.3^{A}
	7	17.50 ± 1.14^{J}	20.80 ± 1.20^{H}	24.60 ± 1.26^{EF}	25.70 ± 1.10^{DE}	27.60 ± 1.6^{BC}
	14	12.80 ± 1.18^{L}	14.50 ± 1.22^{K}	19.20 ± 1.28^{I}	23.40 ± 1.20^{FG}	25.50 ± 1.4^{DE}
	LSD 1.1687					
Whey syneresis (mL/100 g)	0	25.00 ± 1.12^{G}	27.00 ± 1.08^{FG}	29.00 ± 1.14^{EF}	31.00 ± 1.14^{DE}	33.00 ± 1.12^{CD}
	7	27.00 ± 1.14^{FG}	29.00 ± 1.09^{EF}	32.00 ± 1.16^{CD}	33.00 ± 1.12^{CD}	36.00 ± 1.14^{AB}
	14	31.00 ± 1.09 ^{DE}	34.00 ± 1.16^{BC}	36.00 ± 1.12^{AB}	37.00 ± 1.09 ^{A}	38.00 ± 1.08^{A}
	LSD 2.4517					
Viscosity (c. P)	0	3580 ± 106^{C}	3260 ± 77.0^{F}	3190 ± 85.0^{G}	3080 ± 95.0^{I}	3020 ± 102^{J}
	7	3630 ± 124^{B}	3350 ± 72.0^{E}	3270 ± 88.0^{F}	3120 ± 100^{H}	3080 ± 95.0^{I}
	14	3760 ± 114^{A}	3410 ± 85.0^{D}	3340 ± 94.0^{E}	3250 ± 90.0^{F}	3140 ± 114^{H}
	LSD 24.420					

Means in the same row that are denoted by several little letters differ significantly (P ≤ 0.05). Each yogurt sample underwent a triplicate analysis after each experiment was carried out in triplicate. LSD, The smallest difference; C, control yoghurt; T1, yogurt made from 1% PSO; T2, yogurt made from 2% PSO; T3, yogurt made from 3% PSO; T4, yogurt made from 4% PSO.

in the yogurt composition, the viscosity of the samples decreased. This suggests that the higher level of crystallinity and saturated fatty acids (SFA) in milk fat-based yogurt may contribute to its increased viscosity. The study also indicates that an increase in SFA content with an increase in PSO may lead to higher viscosity in PSO-enriched yogurt. These findings align with the results of Farmani *et al*. (2016) who reported that yogurt containing vegetable oils had lower viscosity than the control yogurt.

Viable bacterial count of yogurt with different concentrations of PSO during 14 days of storage at 4°C

The study examined the bacterial count in yogurt with different concentrations of pumpkin seed oil (PSO) during 14 days of storage at 4°C. Over the 14-day period, the microbial populations in the yogurts made with various concentrations of PSO as milk fat replacements did not show significant deviations ($P \leq 0.05$) (Table 5). This suggests that the type of fat used does not affect microbial growth. A similar trend was observed when canola and sesame oil were added to yogurt analogs (Farmani *et al.,* 2106).

Sensory properties of yogurt containing PSO

Table 6 presents the influence of fat composition on the sensory attributes of yogurt containing PSO. The control yogurt and the products with 1 and 2% (w/v) PSO acquired the highest flavor ratings, while those with 3 and 4% (w/v) PSO received the lowest ratings. The distinct flavor of yogurt is attributed to lactic acid and carbonyl molecules, particularly acetaldehyde. Color is a critical quality of yogurt, and as indicated in Table 6, all yogurts scored above 4, except for those containing 3 and 4% PSO. Yogurts made with 1 and 2% (w/v) PSO, as well as the control yogurt, did not differ significantly in color scores ($P \leq 0.05$). The yogurt containing 4% (w/v) PSO received the lowest color ratings, possibly due to the superior color of PSO.

There were no statistically significant differences in the texture of yogurts containing PSO compared to the control group. The study revealed statistically significant color, flavor, and overall acceptability variations between the control and treatment groups, as determined by statistical analysis of the yogurts' sensory characteristics.

Table 5. Viable *Streptococcus thermophiles*, *Lactobacillus acidophilus*, and *Lactobacillus bulgaricus* count of yogurt with different concentrations of PSO insert as fat replacer during 14 days of storage at 4°C.

Properties	Treatments	Storage period (days)		
		0	7	14
Streptococcus thermophiles (log cfu/mL)	C	6.84 ± 0.42^{C}	7.22 ± 0.63^{B}	6.66 ± 0.55^{G}
	T1	6.86 ± 0.60^{C}	7.24 ± 0.75^{AB}	6.70 ± 0.64^{EF}
	T2	6.84 ± 0.92^{C}	7.26 ± 0.63^{A}	6.72 ± 0.83^{DF}
	T3	6.85 ± 0.54^{C}	7.25 ± 0.42^{AB}	6.74 ± 0.92^{D}
	T4	6.86 ± 0.70^{C}	7.26 ± 0.65^{A}	6.68 ± 0.76^{FG}
	LSD 0.0336			
Lactobacillus acidophilus (log cfu/mL)	C	6.38 ± 0.73^{C}	6.62 ± 0.33^{B}	6.20 ± 0.54^{F}
	T1	6.40 ± 0.32^{C}	6.66 ± 0.41^{AB}	6.22 ± 0.72^{F}
	T2	6.42 ± 0.25^{C}	6.68 ± 0.66^{AB}	6.24 ± 0.43^{EF}
	T3	6.46 ± 0.63^{C}	6.70 ± 0.44^{A}	6.26 ± 0.35^{DF}
	T4	6.44 ± 0.34^{C}	6.66 ± 0.52^{AB}	6.28 ± 0.63^{D}
	LSD 0.0284			
Lactobacillus bulgaricus (log cfu/mL)	C	6.60 ± 0.65^{E}	6.80 ± 0.66^{C}	6.56 ± 0.42^{F}
	T1	6.64 ± 0.56^{D}	6.82 ± 0.45^{BC}	6.63 ± 0.35^{E}
	T2	6.66 ± 0.44^{D}	6.84 ± 0.74^{B}	6.68 ± 0.56^{D}
	T3	6.65 ± 0.62^{D}	6.86 ± 0.53^{A}	6.64 ± 0.44^{D}
	T4	6.66 ± 0.45^{D}	6.82 ± 0.62^{BC}	6.64 ± 0.66^{D}
	LSD 0.0298			

Means in the same row that are denoted by several little letters differ significantly ($P \leq 0.05$). Each yogurt sample underwent a triplicate analysis after each experiment was carried out in triplicate. LSD, The smallest difference; C, control yogurt; T1, yogurt made from 1% PSO; T2, yogurt made from 2% PSO; T3, yogurt made from 3% PSO; T4, yogurt made from 4% PSO.

However, there was no significant difference in texture ($P < 0.05$). Adding PSO generally led to decreased organoleptic measures of color, flavor, and overall acceptability, except for texture. Specifically, compared to the control group, the yogurt with 2% PSO yielded the most favorable results in this experiment. Abdullah *et al*. (2012) found that substituting up to 43% (w/w) of milk fat with enzymatically interesterified palm olein in a fermented dairy product with a yogurt-like consistency did not adversely affect sensory scores, including taste, flavor, texture, and overall acceptability.

However, sensory ratings were significantly reduced when milk fat was completely replaced. Kim *et al*. (2022) in their study observed that substituting milk fat with oils rich in PUFAs negatively affected the flavor of fermented products. However, it did not significantly impact the traditional yogurt texture.

Fatty acid composition of yogurt containing PSO

The comparison of the fatty acid composition of the yogurt samples containing PSO and the control yogurt is presented in Table 7. Yogurt enriched with PSO exhibited higher levels of PUFAs than the control yogurt, indicating the potential to adjust the fatty acid composition for specific target demographics. The yogurt containing 2% PSO showed the highest percentage of PUFAs ($P < 0.05$). Among all yogurt treatments, the control yogurt had the highest concentration of SFAs ($P < 0.05$). Compared to the control, the yogurt with 2% (w/v) PSO displayed the highest concentration of linoleic acid. The presence of short- and medium-chain fatty acids in yogurt containing PSO was significantly reduced, reaching very low levels ($P < 0.05$). These findings align with previous research by Farmani *et al*. (2016) who replaced milk fat with vegetable oils such as canola and sesame. Previous studies have demonstrated that substituting SFAs with MUFAs and PUFAs, particularly oleic acid and linoleic acid, leads to increased levels of high-density lipoprotein (HDL) cholesterol and decreased levels of low-density lipoprotein (LDL) cholesterol (Yu-Poth *et al.,* 2000). Adding PSO, which has a high linoleic acid content, to yogurt increased the CLA content of the final product. The CLA content rose from 0.60% in the control yogurt to 1.80% in yogurt containing 2% (w/v) PSO. Certain starter cultures, particularly those used in yogurt production, can convert linoleic acid into conjugated linoleic acid. This conversion occurs when vegetable oils containing significant linoleic acid are present (Al-Hindi and Abd El Ghani, 2016; Kuhl and De Dea Lindner, 2016).

Conclusion

An innovative approach to enhancing the nutritional value of dairy products involves the incorporation of

Table 6. The sensory properties of yogurt containing PSO during storage at refrigerator temperature for 14 days.

Property	Storage period (Day)	Treatments				
		C	T1	T2	T3	T4
Flavor (5)	0	4.9 ± 0.3^{A}	4.8 ± 0.2^{B}	4.7 ± 0.3^{C}	4.2 ± 0.2^{G}	4.0 ± 0.1^{H}
	7	4.8 ± 0.2^{B}	4.6 ± 0.3^{D}	4.6 ± 0.2^{D}	3.7 ± 0.1^{I}	3.5 ± 0.2^{J}
	14	4.6 ± 0.4^{D}	4.4 ± 0.2^{E}	4.3 ± 0.3^{F}	3.4 ± 0.2^{K}	3.1 ± 0.1^{L}
	LSD 0.0688					
Texture (5)	0	5.0 ± 0.1^{A}	5.0 ± 0.1^{A}	5.0 ± 0.2^{A}	4.9 ± 0.2^{B}	4.9 ± 0.3^{B}
	7	5.0 ± 0.2^{A}	5.0 ± 0.3^{A}	5.0 ± 0.1^{A}	4.8 ± 0.2^{C}	4.8 ± 0.2^{C}
	14	4.8 ± 0.3^{C}	4.7 ± 0.2^{D}	4.7 ± 0.2^{D}	4.6 ± 0.1^{E}	4.6 ± 0.1^{E}
	LSD 0.0727					
Color (5)	0	5.0 ± 0.2^{A}	4.8 ± 0.3^{B}	4.7 ± 0.2^{C}	4.2 ± 0.1^{H}	3.6 ± 0.2^{K}
	7	5.0 ± 0.4^{A}	4.6 ± 0.3^{D}	4.5 ± 0.2^{E}	4.0 ± 0.3^{I}	3.3 ± 0.1^{L}
	14	5.0 ± 0.2^{A}	4.4 ± 0.3^{F}	4.4 ± 0.3^{G}	3.7 ± 0.1^{G}	3.0 ± 0.2^{M}
	LSD 0.0589					
Overall Acceptability (5)	0	4.9 ± 0.2^{A}	4.8 ± 0.3^{B}	4.8 ± 0.2^{B}	4.0 ± 0.1^{H}	3.7 ± 0.2^{I}
	7	4.8 ± 0.3^{B}	4.6 ± 0.2^{D}	4.5 ± 0.3^{E}	3.6 ± 0.2^{I}	3.2 ± 0.1^{J}
	14	4.7 ± 0.2^{C}	4.4 ± 0.1^{F}	4.3 ± 0.2^{G}	3.1 ± 0.1^{J}	3.0 ± 0.2^{K}
	LSD 0.0846					

Means in the same row that are denoted by several little letters differ significantly ($P \leq 0.05$). Each yogurt sample underwent a triplicate analysis after each experiment was carried out in triplicate. LSD, The smallest difference; C, control yogurt; T1, yogurt made from 1% PSO; T2, yogurt made from 2% PSO; T3, yogurt made from 3% PSO; T4, yogurt made from 4% PSO.

Table 7. Fatty acids percentages (FA/total FAs) in yogurt manufactured by replacing milk fat with PSO (50%) as compared to the control sample.

Fatty acids		CY	PSOY2
Saturated fatty acid	Butyric (C4)	2.22 ± 0.04	1.04 ± 0.2
	Caproic (C6)	1.76 ± 0.01	0.86 ± 0.01
	Caprylic (C8)	2.34 ± 0.12	1.28 ± 0.08
	Capric (C10)	8.64 ± 0.15	5.04 ± 0.018
	Lauric (C12)	6.45 ± 0.05	4.15 ± 0.03
	Myristic (C14)	5.10 ± 0.2	3.30 ± 0.1
	Myristoleic acid (C14:1)	0.44 ± 0.01	0.26 ± 0.01
	Pentadecanoic acid (C15)	1.02 ± 0.02	0.60 ± 0.02
	Palmitic (C16)	27.00 ± 0.5	22.30 ± 0.3
	Palmitoleic acid (C16:1)	0.66 ± 0.01	0.42 ± 0.01
	Heptadecanoic acid (C17:0)	0.40 ± 0.01	0.30 ± 0.01
	Heptadecenoic acid (C17:1)	0.30 ± 0.002	0.28 ± 0.001
	Stearic (C18)	9.20 ± 0.3	8.30 ± 0.1
Mono-unsaturated fatty acid	Oleic (C18:1)	30.10 ± 0.6	23.50 ± 0.4
	Vaccenic acid (C18:1)	0.50 ± 0.01	0.30 ± 0.02
Polyunsaturated fatty acid	Linoleic (C18:2)	2.10 ± 0.04	25.72 ± 0.2
	Linolenic (C18:3)	1.30 ± 0.02	0.68 ± 0.01
	Arachidonic (C20)	1.05 ± 0.03	0.27 ± 0.02
Total saturated fatty acid		64.35 ± 1.1	47.73 ± 0.95
Total unsaturated fatty acid		35.65 ± 0.8	52.27 ± 0.5
Total mono-unsaturated fatty acid		30.60 ± 0.3	23.80 ± 0.25
Total polyunsaturated fatty acid		5.05 ± 0.1	28.47 ± 0.2
Conjugated linoleic acid		0.60 ± 0.1	1.80 ± 0.3

CY, control yogurt; PSOY2, yogurt made from 2% PSO.

unsaturated fatty acids. The addition of pumpkin seed oil (PSO) to yogurt, followed by storage at 4°C, resulted in a significant improvement in the overall quality of the yogurt. This enhancement was evidenced by an increase in both the fatty acid content and antioxidant capacity, as well as an improvement in sensory evaluation, with the sample containing 2% PSO receiving the highest rating. Research indicates that substituting 50% of the milk fat with PSO may yield a new, healthier fat source for fortified yogurt. Overall, the findings of this study underscore the potential industrial applications of PSO within the dairy sector.

Institutional Review Board Statement

The research approval was given by the Institutional Review Board (IRB) of Princess Nourah bint Abdulrahman University, Riyadh, KSA. Before participating in the study, each participant provided written consent (IRB Log Number: 23-0678).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

Research data will be provided by the researcher when needed.

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Conflicts of Interest

The authors declare no conflicts of interest.

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