Pomegranate Peel Extract Fortified Yogurt: Effect on Physicochemical, Microbiological and Sensory Quality of Functional Dairy Product

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ABSTRACT

Background: The study aimed to evaluate the effect of incorporating *Punica granatum* (pomegranate) hydroethanolic extract as a functional ingredient in yogurt on the physicochemical, sensory quality and microbiological count of freshly prepared and refrigerated stored (4°C) yogurts.

Methods: Pomegranate peels were dried, ground and extracted with 70% ethanol, then concentrated using a rotary evaporator and finally lyophilized to obtain pomegranate peel powder (PPP). Yogurts were prepared using pasteurized semi-skimmed cow's milk, with phenolic extracts of pomegranate peels added at 10.0 mg, 20.0 mg and 30.0 mg per 100 mL of milk; a control product was simultaneously prepared without such pomegranate peel extract. The physicochemical analysis included pH, acidity, syneresis and viscosity measurements. Microbiological analysis was performed to determine the viability of *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus.* Sensory scoring of the yogurt was carried out by 25 panelists using a 5-point scale.

Result: Initial pH values of freshly prepared yogurt ranged between 4.48 and 4.64, decreasing to pH values of 4.3 and 4.15 at 21st day of refrigerated (4°C) storage. The titratable acidity of yogurt increased from initial value of 75°D for freshly prepared control product to 99°D as noted on 21st day of storage. Syneresis at the 21st of storage were 35.98%, 37.88%, 39.34%, 39.22% for yogurt samples control, experimental yogurt containing PPE of 10.0 mg, 20.0 mg and 30.0 mg respectively. The viscosity of yogurt utilizing 10.0 mg PPE peaked to value of 769.63 cP on 21st day of storage. *Streptococcus thermophilus* count peaked to 8.39 log CFU/g for experimental sample containing 10.0 mg of PPE as noted on 7th day of storage. Yogurt samples containing 20.0 mg and 30.0 mg of PPE received the superior overall acceptability scores over others. The extract improved yogurt's physicochemical and sensory properties, enhancing its nutritional value and consumer acceptance.

Key words: Microbial quality, Physicochemical properties, *Punica granatum*, Sensory scores, Yogurt.

INTRODUCTION

Functional foods have emerged and are showing positive growth in the market due to an upsurge in scientific interest and increased consumer demand for wellness food (Al-Hindi and Abd El Ghani, 2020). These food products are created by incorporating naturally derived therapeutic compounds,or probiotics, or other specific microorganisms that can generate biogenic substances (Deepa *et al*., 2016). There is a growing demand for new functional dairy products that offer potential health benefits (Bulut *et al*., 2022; Zahid *et al*., 2023). Among dairy products, yogurt is the most favored fermented milk product, providing an excellent source of protein, fat, vitamins and minerals including calcium (Rashwan *et al*., 2023). However, yogurt naturally does not contain any phenolic compounds (Abdel-Hamid *et al*., 2020).

Pomegranate peels, constituting about 50% of the fruit's weight, are rich in bioactive compounds, including flavonoids, ellagic acid, gallic acid, punicalagin, punicalin, ellagitannins, caffeic acid, pelletierine alkaloids, kaempferol, luteolin, quercetin, *etc*. minerals (*viz*., calcium, sodium, potassium, phosphorus, iron, zinc) (Singh *et al*., 2023; Leesombun *et al*., 2022). Typically, after juice processing, pomegranate peels are considered as waste material and are often discarded in large quantities (Görgüç *et al*., 2022). The phytochemicals in pomegranate peels are recognized for their bioactivities, such as antioxidant,

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anti-inflammatory, wound-healing, anti-hyperglycaemic, anti-hyperlipidemic, cardioprotective, anti-cancer and antibacterial properties (Demir *et al*., 2019).

The objective of this study was to incorporate hydroethanolic extract of *Punica granatum* as a functional ingredient in yogurt and observe the repercussions of such inclusion on the physicochemical, sensory characteristics and microbiological quality of the resultant yogurt and monitor the storage changes in such products, stored under refrigeration (4°C). This study aimed to determine how the addition of pomegranate peel extract (PPE) impacts the overall quality of yogurt.

MATERIALS AND METHODS

Pomegranate peel preparation and extraction method

The study was carried out from October 2023 to March 2024 in the laboratories of the Higher School of Agronomy, Mostaganem, Algeria. Mature *Punica granatum* fruits were procured from the Mostaganem City market in West Algeria, with maturity determined based on their deep red color, firm texture and glossy rind, which was then processed into PPP. The peels were manually separated and such peels dried in a Memmert oven (Germany) at 40°C for 2 weeks until a constant weight was achieved, the moisture content was estimated at 6.83% and finely ground using an electric blender. The resulting fine (<1 mm) powder was stored sealed glass vials at -20°C for subsequent use (Bakhti *et al*., 2024).

For the extraction process, 10 g of PPP was mixed with 100 mL of of 70% (v/v) ethanol in water and subjected to agitation on an orbital shaker for 24 h. The mixture was then vacuum filtered and concentrated using a rotary evaporator (Buchi R-100, Switzerland), The resulting concentrate was subsequently frozen at -20°C in round-bottom flasks and lyophilized at -80°C under pressure for 72 h in a lyophilizer (Biobase, China) (Khodadadi *et al*., 2021).

Yogurt preparation

Experimental yogurts were produced to evaluate the impact of incorporating phenolic extracts on yogurt quality. The experiment utilized pasteurized semi-skimmed cow's (fat content of 1.5%, total solids (TS) content of 12% and acidity of 15° Dornic), provided by the Mostaganem subsidiary of the industrial dairy products group, GIPLAIT. The milk was heated to 45°C and divided into twelve sterile 100 mL polypropylene jars. Each batch of three jars received one of the following doses of phenolic extracts (Total phenolics content: 339.02±12.73 µg GAE/mg d'extrait): 0 mg (control), 10.0 mg, 20.0 mg and 30.0 mg per 100 mL of milk. The mixtures were then inoculated with 0.05% of CHR HANSEN starter culture (YF-L812) comprising of two strains *viz*., *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*. The jars were sealed, incubated for 4 h at 45°C using in incubator (Wisd, South Korea) and subsequently refrigerated at 4°C (Guemidi *et al*., 2024).

Physicochemical analysis

Assessment of yogurt pH and acidity

The titratable acidity and pH of the experimental yogurts (even of control yogurt) were measured in accordance with AOAC (2000) methods. Dornic acidity, expressed in Dornic degrees (°D), was determined by titrating 10 mL of well mixed yogurt sample incorporating a few drops of phenolphthalein as an indicator, using a 1/9 N sodium hydroxide solution for titration (Zahan *et al*., 2020). The pH was measured using a pH meter (model STARTER 2100 PH BENCHST2100-F) calibrated with standard acidic and basic buffer solutions (Remya *et al*., 2019).

Syneresis measurement

Syneresis in yogurt was assessed following the method described by Alsaleem and Hamouda (2024). An aliquot of 5.0 g yogurt sample (4°C) was centrifuged at 230 gravity force for 15 min in a centrifuge tubes of 10 mL using a Sigma centrifuge. After centrifugation, the clear supernatant was separated and weighed. The proportion of such liquid out of the weight of yogurt sample taken was recorded as per cent syneresis.

Viscosity measurement

Viscosity of yogurt was measured using a falling ball viscometer Type C (Thermo Scientific, HAAKE, Germany). The procedure involved filling a 35 mL yogurt sample in a glass tube and then dropping a ball of nickel-iron alloy with a diameter of 11 mm into the tube. The time (seconds) it took for the ball to travel a distance of 10 cm through the tube filled with product was recorded with a stopwatch. Dynamic viscosity was then calculated using the formulas:

$$\mu = K. (\xi \text{ bille} - \xi \text{ yaourt}). t.$$

$$K = 2r^2g/9.x$$

Where

- μ = dynamic viscosity (kg, m^{-1}, s^{-1}).
- K = 6.59×10^{-4}; a constant.
- r = Radius of the ball, r=D/2=5.5 mm.
- x = Distance travelled by the ball, x=10 cm.
- g = 9.81 m/s^2; gravitational force.
- ξ bille = Density of the ball.
- ξ yaourt = Density of the yogurt.
- t = Time taken for the ball to travel a distance of 10 cm.

Microbiological analysis

Under aseptic conditions, 1 g of yogurt sample was weighed and transferred to a sterilised jar. Then, 9 mL of sterile physiological solution (0.9% NaCl). was added and mixed thoroughly to achieve a 1:10 dilution; such solution was in turn used to prepare a series of further dilutions. The count of *Lactobacillus delbrueckii ssp. bulgaricus* was determined using MRS agar medium (Condalab, Spain), while the count of *Streptococcus thermophilus* was enumerated using M17 agar medium (Condalab, Spain) (Shehata *et al*., 2016).

Evaluation of yogurt sensory quality

To evaluate the sensory quality of yogurts incorporated with or without PPE, 25 panelists (12 males, 13 females) agedbetween 23 and 52 from the Higher School of Agronomy in Mostaganem, Algeria rated the product for color, odor, taste, consistency, texture, acidity and overall acceptability on a 5-point scale (Meghwar *et al*., 2024).

Statistical analysis

The experimental trials were conducted in triplicate. Statistical analysis was performed on the data generated using ANOVA with IBM SPSS software® (version 20). The data are presented as mean ±SD and differences were considered significant at $p<0.05$.

Results and Discussion

Physicochemical properties

pH and acidity of freshly prepared yogurts and changes in such products during storage

Fig 1 presents the changes in the pH and titratable acidity in the yogurt samples (YC, Y10, Y20, Y30) over a storage period of 21 days. Initially, the pH values for all the four yogurt samples were ranging from 4.7 to 4.48, with YC exhibiting the highest initial Ph. As anticipated, the pH values declined over time due to the fermentation process. Concurrently, the titratable acidity values increased across all samples with progressive storage period. YC, which initially was associated with the highest pH value, attained an acidity level of 99°D by the end of the storage period (*i.e*. 21st day). The trend noted was comparable across all samples, demonstrating similar fermentation rates despite slight initial differences.

At the onset of fermentation in yogurt preparation, the pH decreases due to the growth of *Streptococcus* species, which utilize free amino acids in milk or those derived from casein hydrolysis. This leads to production of formic acid and CO_2, which serves as growth factors for *Lactobacillus* (Yamauchi *et al*., 2019). Consequently, *Lactobacillus spp*. proliferate rapidly as the pH decreases, releasing additional lactate and further reducing the pH of yogurt (Chandan and Kilara, 2013; Naibaho *et al*., 2022). As per documented literature, yogurt bacteria continue to ferment lactose during cold storage, producing more lactate

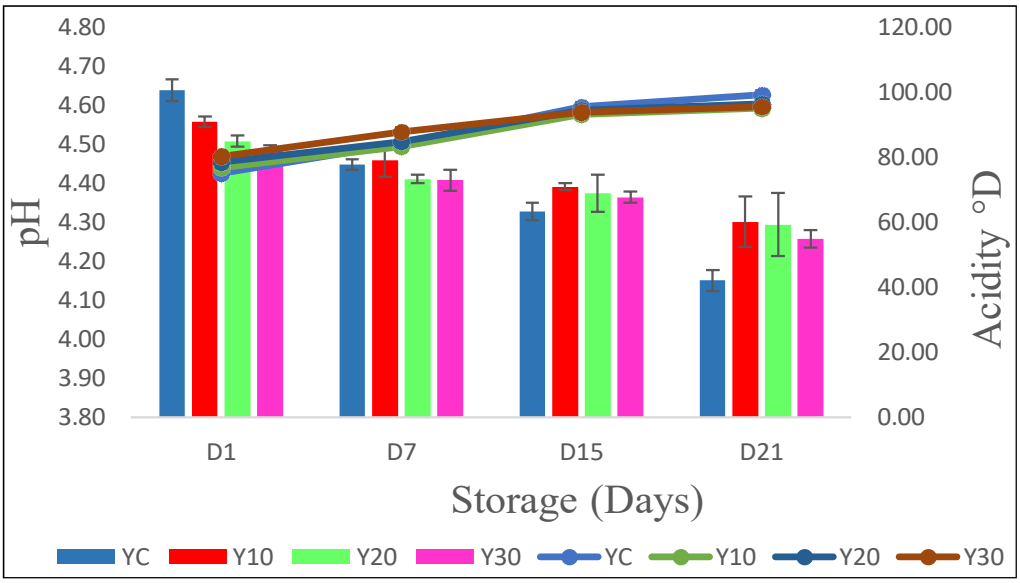


Fig 1: Changes in the pH and titratable acidity of yogurts during storage.

Table 1: Changes in the syneresis value (%) in yogurt samples during refrigerated storage.

Yogurt samples	Syneresis value (%) in yogurt during storage period (days, D)				Mean (Y)
	D1	D7	D15	D21	
YC	37.61±0.81	39.96±0.70	46.52±0.75	39.22±0.62	40.82[a]
Y10	36.66±0.89	38.76±1.13	43.47±1.60	39.34±1.38	39.55[a]
Y20	30.48±1.90	33.44±2.39	42.71±4.34	35.98±1.52	35.65[b]
Y30	33.42±1.61	35.83±1.22	41.91±2.50	37.88±2.29	37.25[b]
Mean (D)	34.54[c]	36.99[b]	43.65[a]	38.10[b]	

Mean values followed by different letters (a, b, c) within the same row indicate significant differences ($p<0.05$) among the samples at differing storage periods, while different superscript letters within the same column indicate significant differences ($p<0.05$) among the samples.

(Korbekandi *et al*., 2015), mainly by the activity of *Lactobacillus spp*., thereby increasing the yogurt's acidity (Gallina, 2022). Such post-acidification changes are considered unfavorable due to its negative impact on the product quality, particularly through the accumulation of lactic acid, which results in an unpleasant sour taste (Han *et al*., 2022). Guemidi *et al*. (2024) reported a significant decrease in pH ($p<0.01$) in yogurts enriched with different incorporation rates of peppermint hydroethanolic extract (PHE) during refrigerated storage. On Day 1, the pH ranged from 4.58 (0% PHE) to 5.27 (6% PHE), decreasing to 4.45-4.77 on Day 10 and further to 4.30-4.76 on Day 20. In contrast, acidity increased significantly ($p<.01$) with storage time and PHE incorporation. On Day 1, acidity ranged from 80.33°D (0% PHE) to 70.67°D (6% PHE), increasing to 87.89-72.00°D by Day 10 and reaching 94.48°D (0% PHE) to 73.39°D (6% PHE) by Day 20.

Syneresis in yogurt

Table 1 presents the syneresis (expressed in per cent) in yogurt samples as noted during its refrigerated storage. The syneresis values demonstrated a notable ($p<0.05$) increase from day 1 to day 15, irrespective of the four types of yogurt samples. Following this peak, a decline in syneresis value was observed on 21st day of storage, samples YC and Y10 exhibited significantly ($p<0.05$) higher mean syneresis values compared to the other two samples (*i.e*. Y20, Y30) suggesting that the latter two samples exhibited better water-holding capacity. As compared to at other storage intervals, indicative of the dynamic nature of yogurt's physical properties during post-acidification. All the yogurt samples exhibited a notable ($P<0.05$) increase in syneresis, as the storage period progressed. This trend is possibly associated with the decreasing pH in the yogurts during storage, resulting from post-acidification in product by the starter cultures. The rising acidity caused greater curd shrinkage and induced further whey expulsion (Hamdy *et al*., 2021).

Ahmed *et al*. (2022) reported maximum syneresis level (1.82 g/100 g) exhibited by control yogurt as noted at 21st day of refrigerated (4°C) storage, while the least syneresis value (1.32 g/100 g) was noted in freshly prepared

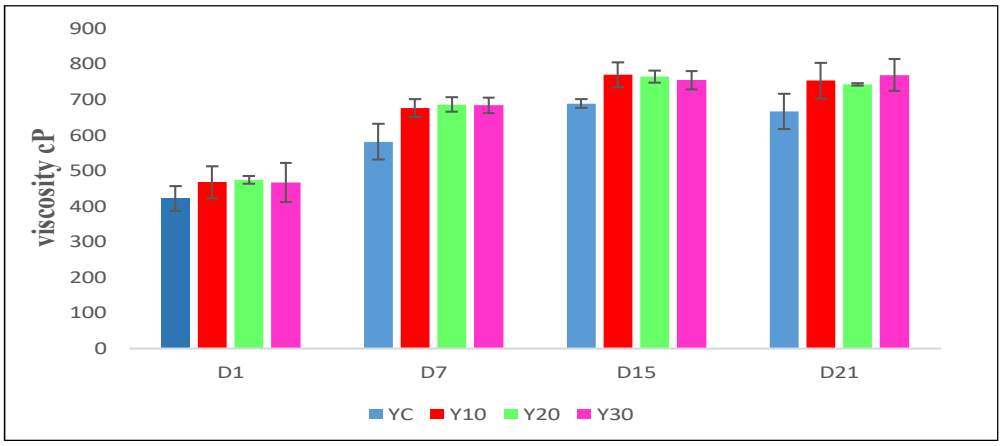


Fig 2: Changes in the viscosity of yogurt samples during storage.

Table 2: Changes in the two specific yogurt starter culture counts as influenced by refrigerated storage.

Starter	Yogurt samples	Count (log_{10}CFU/g) of yogurt starter during storage period (days, D)			
		D1	D7	D15	D21
Streptococcus thermophilus	YC	8.09±0.04	8.32±0.02	8.28±0.09	7.99±0.09
	Y10	8.13±0.05	8.39±0.02	8.33±0.01	8.03±0.08
	Y20	8.17±0.06	8.36±0.06	8.33±0.03	8.01±0.16
	Y30	8.15±0.07	8.36±0.04	8.33±0.04	8.02±0.07
	Mean (D)	8.13^{b}	8.35^{a}	8.31^{a}	8.01^{c}
Lactobacillus delbrueckii ssp. *Bulgaricus*	YC	7.74±0.06	7.91±0.03	7.88±0.05	7.74±0.08
	Y10	7.83±0.07	7.99±0.04	8.00±0.07	7.80±0.12
	Y20	7.79±0.03	7.99±0.03	8.00±0.06	7.77±0.04
	Y30	7.85±0.04	8.04±0.03	8.00±0.14	7.72±0.20
	Mean (D)	7.8^{b}	7.98^{a}	7.97^{a}	7.75^{b}

Mean values followed by different letters (a, b, c) within the same row indicate significant differences ($p<0.05$) among the samples at differing storage periods.

experimental yogurt sample (containing 9.0% PPP. The incorporation of 2.5% apple pomace powder and 2.5% PPP in yogurt was found favourable in retarding syneresis in yogurt, even during refrigerated storage till 21st Notably, treatments containing PPP in yogurt showed markedly lower syneresis compared to that made employing apple pomace powder on 21st day of storage.

Viscosity of set yogurt

Fig 2 displays the viscosity of the prepared yogurt sample as well as the changes in such aspect for all the yogurt samples (YC, Y10, Y20, Y30) over a storage period of 21 days. The viscosity (expressed in cP) of all the yogurt samples exhibited a notable (P<0.05) increase with progressive storage period. The maximum viscosity was noted for all yogurt samples on 15th day of storage, with the following values, Y10 (*i.e.* 769.63 cP), Y20 (764.06 cP) and Y30 (753.80 cP). By 21st day, the viscosities of YC, Y10 and Y20 decreased slightly. This trend suggests that Y30 slightly increased its viscosity over time, whereas the other yogurt samples showed notable (P<0.05) slight decline in their viscosity, after reaching their peak at the 15th day.

A study by Al-Hindi and Abd El Ghani (2020) demonstrated that presence of PPE increased the viscosity of yogurt to a greater extent compared to control product during the storage period at 4°C for 30 days. Such effect was ascribed to the impact of PPE on the aggregation of the casein network in yogurt through electrostatic interactions increasing the resistance of yogurt matrix to flow (Rice-Evans *et al*., 1996, Tamime and Tamime, 2007). Contrary to our findings, Ramaswamy and Basak (1992) reported that incorporation of tamarind aqueous extract at usage rate of 1.54 % tended to lower the viscosity of yogurt.

Microbiological quality of yogurts

Table 2 depicts the viable counts of *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus* in yogurt during refrigerated storage up to 21 days. Bacterial counts were monitored at weekly intervals (D1, D7, D15 and D 21) and the count was expressed as log CFU/g. The results indicate a significant change (p<0.05) in starter counts across all samples during the storage period. However, the incorporation rate of pomegranate peel extract (PPE) in yogurt preparation did not show a statistically significant effect on starter counts (P>0.05).

In case of *Streptococcus thermophilus*, the initial count increased from D1 to D7 across all yogurt samples. The highest count was associated with yogurt made incorporating 10 mg of PPE on 7th day of storage (Y10), reaching 8.39 log CFU/g by D21. Similarly, *Lactobacillus delbrueckii ssp. bulgaricus* count of yogurt displayed a similar trend during storage, with their count peaking at D7, especially at higher PPE usage rate (30.0 mg). At PPE usage rate of 30 mg (Y30), the resultant yogurt recorded highest count of 8.04 log CFU/g of *L. bulgaricus* on D7.

In contrast to our findings, Khelifi *et al*. (2018) demonstrated antibacterial effect of phenolic extracts from *Thymus vulgaris* L. against *Streptococcus* and *Lactobacillus* cultures in yogurt when used at 8% level. The possible mechanisms for the exerted antibacterial effects by phenolic compounds include damaging the bacterial cell walls, blocking the production of cellular energy, or destroying their genetic material (Yang *et al*., 2008).

Sensory scores of yogurts

Looking closely on the sensory scores of the different yogurt samples (YC, Y10, Y20, Y30) with or without PPE, as shown in Fig 3, revealed non-specific preference for the yogurt samples in terms of their overall acceptability scores. Yogurt samples Y20 and Y30 scored superior for overall acceptability, with mean values of 3.43 and 3.30 (out of maximum score of 5.00), respectively. Sample Y20 also performed well, particularly in terms of sensory odor and texture, with scores of 3.70 and 3.61 respectively.

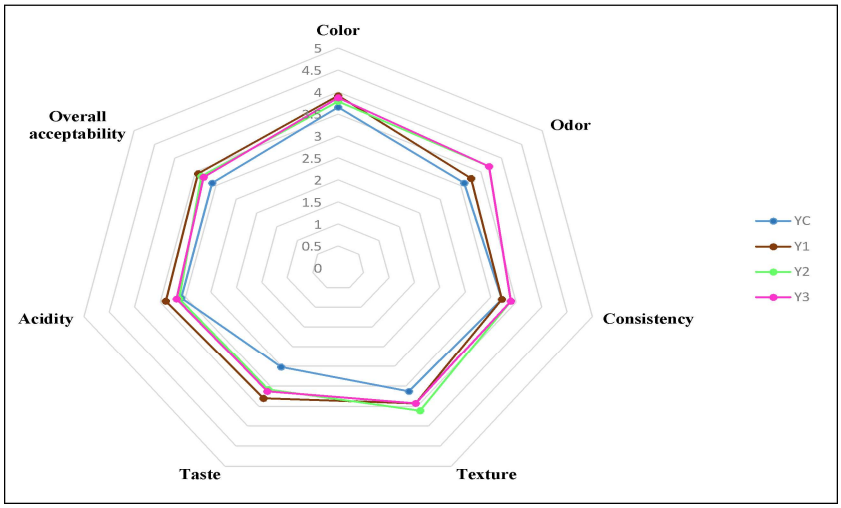


Fig 3: Effect of pomegranate peel extract on the sensory scores of freshly prepared yogurts.

These results suggest that the addition of PPE, irrespective of its usage level, enhanced the sensory quality of resultant yogurt, especially in terms of color, odor and texture, leading to better consumer satisfaction compared to the control sample.

Principal component analysis (PCA) of sensory quality of yogurt

The Principal 3D Component Analysis (PCA) (Fig 4) revealed distinct groupings among the yogurt samples containing varying levels of PPE, based on their sensory traits. The control yogurt (YC) formed a distinct cluster, characterised by lower sensory scores in terms of color, odor, taste and overall acceptability compared to the experimental samples bearing PPE. The yogurts containing varying PPE levels (Y10, Y20, Y30) got clustered into several groups, showing gradients based on the usage levels of PPE. Y10 formed a cluster close to that shown by YC, indicating moderate improvements in the sensory quality parameters, but not pronounced as was evident at higher

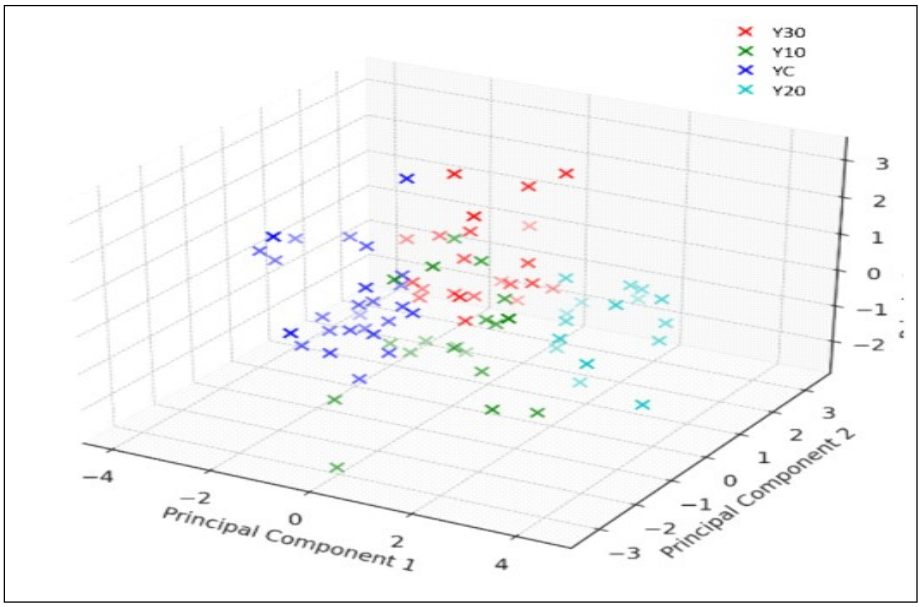


Fig 4: Principal component analysis of sensory score with 3D clusters of yogurts samples.

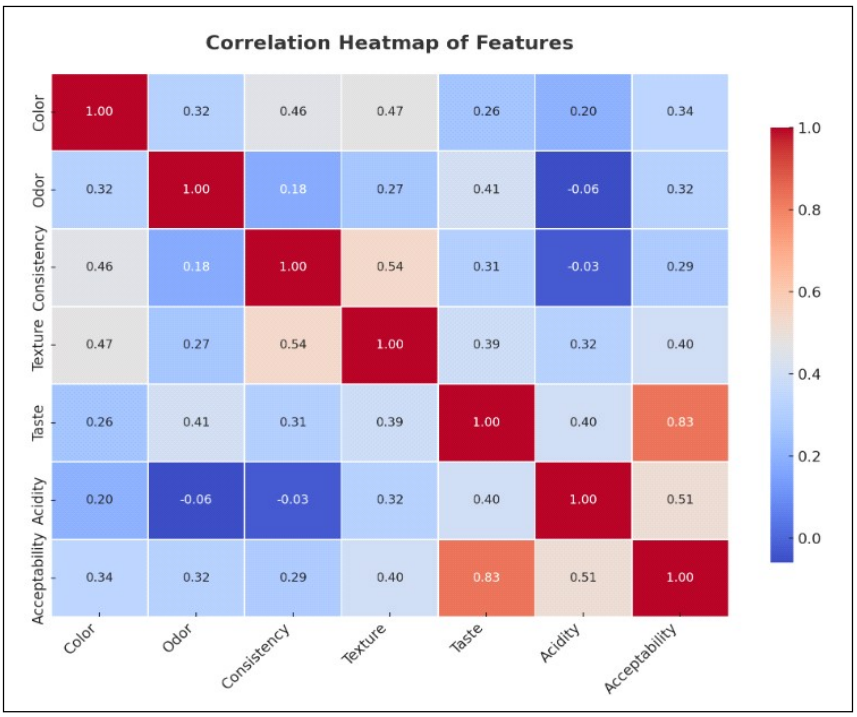


Fig 5: Principal component analysis with heatmap of features.

usage levels. Y20 and Y30 clusters were distinctly different from that of YC and Y10, showing significant ($p<0.05$) improvements in their sensory scores; Y30 displayed the highest scores for most of the sensory attributes studied. The distribution of clusters indicated a progressive increase in the sensory scores of yogurts with increasing usage levels of PPE, particularly for aspects such as color, taste and overall acceptability. The PCA analysis demonstrated that enriching yogurt with PPE significantly ($p<0.05$) altered the sensory scores, creating distinct profiles based on the varying usage levels adopted. This provided a solid foundation for optimizing the formulation of PPE enriched yogurt to maximize the perceived sensory benefits.

The heatmap (Fig 5) pertaining to the sensory analysis of yogurts enriched with PPE revealed correlations between the varying usage level of PPE and the sensory scores evaluated. There was a progressive improvement in the scores for color, odor, consistency, texture, taste and acidity with increasing usage levels of PPE extract. Notably, Y30 yogurt, incorporated with the highest usage level of PPE extract was associated with the highest scores for almost all the sensory attributes evaluated, indicating a substantial enhancement in the sensory acceptance of the product. Taste showed a strong correlation ($r = 0.83$) with overall acceptability score of the product, suggesting that these attributes played a crucial role in the acceptance for the developed product by the sensory panelists. The sensory scores for consistency and texture ($r = 0.54$) also showed an improvement when employing PPE at higher usage levels, increasing the products sensory acceptability. In summary, the heatmap demonstrated that incorporating PPE, especially at higher concentrations (*i.e.* 20 and 30 mg/100 g of milk) enhanced the sensory acceptability of the resultant yogurt markedly.

Conclusion

The findings of this study signifies the potential of *Punica granatum* hydroethanolic extract as a valued functional ingredient in yogurt formulation. The incorporation of such plant extract positively influenced the physicochemical properties, microbiological quality and sensory score of the resultant yogurt, demonstrating its potential application in enhancing both the nutritional value and sensory acceptance of yogurt. Notably, the pomegranate peel extract was beneficial in enhancing the viscosity of product, improved the pH stability as noted up to 21 days, reduced syneresis and afforded viability of yogurt starter up to 21 days of refrigerated storage. The pomegranate peel as hydroethanolic extract is recommended at a usage level of 30.0 mg per 100 mL of milk which afforded several benefits such as viscosity buildup, retarded syneresis of yogurt and greater number of viable starter bacteria during refrigerated storage, without impairing the yogurt's sensory acceptability.

Conflict of interest

No conflict of interest amongst the authors associated with this work.

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