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FORTIFICATION OF SET YOGURT WITH CITRUS PEEL POWDER – EFFECT ON PHYSICOCHEMICAL AND SENSORY PROPERTIES

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Abstract: *Yogurt is a widely consumed fermented dairy product valued for its nutritional and functional properties. Fortification of yogurt with fiber-rich materials, such as fruit by-products, has gained increasing attention as a means to enhance its health-promoting properties and functional value. In the present study, yogurt was fortified with lemon and lime peel powders at concentrations of 0.25%, 0.50%, and 0.75%, and the resulting samples were evaluated for their physicochemical and sensory properties. Citrus peel addition significantly affected pH and titratable acidity, which remained within acceptable ranges, while protein and fat contents were not influenced by fortification, indicating compositional stability. Yogurt samples containing lime peel powder exhibited higher total fiber content, highlighting the nutritional advantage of fortification. Incorporation of citrus peel powders improved viscosity and reduced syneresis, enhancing texture, and positively influenced color and appearance, especially at lower inclusion levels. Among the formulations, yogurt with 0.50% lemon peel achieved the highest overall sensory acceptability. These findings demonstrate that lemon and lime peel powders can serve as effective functional fortifying agents in yogurt, with 0.50% inclusion offering an optimal balance of nutritional enhancement, improved textural quality, and consumer preference.*

Keywords: *functional yogurt; fortification; citrus peel; physicochemical properties; sensory quality*

1. Introduction

Dairy products provide a complex matrix of essential nutrients, including proteins, lipids, carbohydrates, and are widely recommended as part of a healthy diet. For centuries, milk and its fermented forms have been consumed for their substantial nutritional benefits. Yogurt is one of the oldest and most valued fermented dairy products, and is produced by fermenting milk with starter culture of the lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, but may be supplemented with *Lactobacillus*, *Bifidobacterium*, or other probiotic microorganisms [1]. Due to the metabolic activity of starter cultures that convert lactose into lactic acid, yogurt contains reduced lactose levels, making it more tolerable for individuals with lactose intolerance [2]. In addition, yogurt contains many bioactive compounds that contribute to the enhancement of host immune function, such as bacteriocins, vitamins, amino acids, peptides, metabolic enzymes, short-chain fatty acids, antioxidants, anti-inflammatory and immune-modulating agents, as well as exopolysaccharides [3]. Regular consumption of yogurt has been associated with immunostimulatory effects, protection against pathogenic microorganisms, blood pressure regulation, reduced risk of obesity and colorectal cancer, and overall improvement of health status [4]. In the context of functional dairy innovation, recent research was focused on

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yogurt fortification with non-digestible carbohydrates and a range of bioactive compounds – such as quercetin, kaempferol, apigenin, and catechins – as functional ingredients aimed at enhancing gut health and overall physiological benefits [5,6]. Fruit processing by-products gained particular interest as functional yogurt ingredients owing to their high content of dietary fiber and retention of health-promoting bioactive compounds. Furthermore, by-products such as fruit peels, generated during the processing of agricultural commodities, represent cost-effective and sustainable sources of natural antioxidants, offering potential alternatives to synthetic compounds [7].

The industrial processing of citrus fruits (e.g., grapefruit, lemon, orange, lime, clementine) generates annually approximately 40 million tons of peel waste [8]. These residues are particularly abundant in phenolic compounds such as hesperidin, naringenin, kaempferol, nobiletin, tangeretin, quercetin, apigenin, luteolin, hesperetin, rutin, eriodyctiol, myricetin, tannic acid, and resveratrol [8,9]. Moreover, citrus peel also has a high content of dietary fiber, containing more water-soluble fraction by comparison to other sources, and it is regarded as an optimal source of dietary fiber when the content of its water-soluble fraction reaches 30-50% [10]. Therefore, utilizing this waste for value-added applications may contribute to reducing its environmental footprint. The role of citrus peel in the functional fortification of yogurt was investigated by a few studies published to date [7,11–13], and two of the mentioned papers considered the addition of orange peel fiber and orange-peel extract, respectively.

The present study aimed to examine the physicochemical, rheological, and sensory properties of set yoghurt fortified with citrus peel powder. For this purpose, fortification of yogurt was made with two types of powder, lime and lemon peel, at concentrations of 0.25%, 0.50%, and 0.75%, and the effect of the incorporation of these components on the quality of yogurt was investigated. This study presented an effective strategy for the development of high-quality yogurt fortified with citrus peel and provided valuable experimental insights for the formulation of functional food products.

2. Materials and methods

2.1. Materials

Lime, lemon, and cow's milk (4.1% fat content) were purchased from a local market in Suceava, Romania. The lime (country of origin – Peru) and lemons (country of origin – Italy) were labelled as organically grown and untreated, with peels declared safe for consumption. The probiotic culture Yogourmet® Yogurt Starter (Yogourmet, Canada) was purchased through a local distributor. Chemicals used in this study were of analytical grade and were purchased from Merck KGaA (Germany).

To prepare the citrus peel powder, citrus fruits (lime and lemon) were washed thoroughly with tap water, and the peels were manually separated and cut into small pieces. The peel was dried in an oven with air circulation at 55 °C to a constant weight, and then powdered in a food processor. Subsequently, the resulting powder was passed through an analytical sieve shaker Retsch AS 200 (Retsch GmbH, Germany), and the powder with <125 μm particle size was packed and stored at 4 °C until use for yogurt preparation.

2.2. Preparation of fortified yogurt

Yogurt was prepared according to the procedure described by Zaki et al. [7], with some modifications, using fresh cow's milk with a fat content of 4.1%. The milk was heated to 90 °C for 5 minutes to ensure pasteurization, followed by cooling to 40 °C. It was then divided into seven equal portions, one portion serving as the control sample, while the remaining six were supplemented with lime peel powder and lemon peel powder, respectively, at concentrations of 0.25%, 0.50%, and 0.75% (w/w). Citrus peel powders were pre-hydrated in a small amount of milk at 40 °C for 15 minutes before being added to ensure uniform dispersion. All samples were subsequently inoculated with Yogourmet® Yogurt Starter containing *Lactobacillus delbrueckii* subsp. *bulgaricus, Streptococcus thermophilus,* and *Lactobacillus acidophilus*.

The inoculated mixtures were transferred into clean, dry glass jars and incubated at 42 °C for 6 h. Following incubation, all yogurt samples were gradually cooled to ambient temperature (~25 °C), then to 10 °C, and finally stored under refrigeration at 4 °C.

2.3. Physicochemical measurements

The pH of the yogurt samples was measured using a Mettler Toledo pH-meter (Columbos, OH, USA). Titratable acidity was determined in accordance with the ISO 11869:2012 standard. Protein content was analyzed using the Kjeldahl method, following ISO 8968-4:2016 standard, while fat content was determined according to ISO 19662:2018 standard. Fiber content was determined gravimetrically in accordance with ISO 6865:2000, using a Fibretherm Gerhardt FT-12 analyzer (C. Gerhardt GmbH & Co. KG, Königswinter, Germany) following acid and alkaline digestions. Color measurements were conducted in triplicate at 25 °C using a CR-400 chroma meter (Konica Minolta, Tokyo, Japan), previously calibrated with a standard white reference plate.

The CIE L^*, a^*, and b^* color coordinates, along with the derived parameters hue angle (h^*_{ab}) and chroma (C^*_{ab}), were obtained from the reflection spectra of the samples using a D65 illuminant and a 2° standard observer.

Syneresis of yogurt was determined according to the method used by Ropciuc & Dabija [14]. Approximately 20 g of yogurt was transferred into a 50 mL centrifuge tube and subjected to centrifugation at 3500 rpm for 15 minutes. Syneresis was assessed in yogurt samples that had been stored at 4 °C for 24 hours. Syneresis was calculated as the percentage of whey separated relative to the total mass of the yogurt:

$$\text{Syneresis (\%)} = (\text{mass of whey} / \text{mass of yogurt}) \times 100 \quad (1)$$

The final value represents the mean of three independent measurements. Rheological determinations, more exactly steady-state rheology was studied according to the procedure described by Oroian, Codină & Dabija [15]. Apparent viscosity of the yogurt samples was determined using a Mars 40 rheometer (Thermo Haake, Germany) equipped with a parallel plate system (Ø 40 mm) at a fixed gap of 1000 µm. Following sample loading, a five minute equilibration period was allowed to enable structural recovery and temperature stabilization. All rheological measurements were performed at 4 °C. The shear rate (γ, s^{-1}) was ranged from 0 to 100 s^{-1}, while the corresponding apparent viscosity (η, Pa·s) was recorded.

2.4. Sensory analysis of yogurt samples

Sensory evaluation was conducted following the "generic sensory descriptive analysis" methodology. The process consisted of three main stages: (1) panel selection, (2) sample preparation, and (3) product evaluation. A total of 28 semi-trained panelists participated; 17 were female and 11 males, aged between 19 and 38 years. The semi-trained panelists were students and professors from the Faculty of Food Engineering of the Stefan cel Mare University of Suceava, Romania. All participants had successfully completed a sensory analysis course as part of their bachelor's degree curriculum, thereby possessing adequate foundational knowledge in sensory evaluation of food products. A nine-point hedonic scale was employed for rating the sensory attributes, where 1 represented "dislike extremely" and 9 denoted "like extremely." The parameters assessed included color, taste, flavor, texture, mouthfeel, appearance, and overall acceptability.

2.5. Statistical analysis

The data obtained from the analysis of the physicochemical properties of yogurt samples were subjected to analysis of variance (ANOVA) using Statgraphics Centurion XVI software (Statgraphics Technologies, Inc., The Plains, VA, USA). Differences between means were evaluated using Fisher's Least Significant Difference (LSD) test at a 95% confidence level. Principal component analysis (PCA) was performed using OriginPro 2025b software, trial version (OriginLab Corporation, Northampton, MA, USA).

3. Results and discussion

3.1. Physicochemical properties of fortified yogurt

Changes in color parameters for yogurt fortified with lemon peel powder, lime peel powder, and the control sample are shown in Table 1. Among all samples, the highest lightness (L^*) values were recorded for the control, while increasing levels of citrus peel powder in the yogurt samples resulted in a reduction of L^* values. This observation was in agreement with the findings of Rahman et al. [12] and Dinkçi et al. [16]. The control sample exhibited green and yellow hues, and as the proportion of citrus peel powder increased, the fortified samples showed lower a^* (green) and higher b^* (yellow) values. Considering the lower hue angle (h^*_{ab}) and higher chroma (C^*_{ab}) values, these color shifts were particularly pronounced in the samples containing 0.50% and 0.75% lime peel powder, as expected. A similar increase in yellow hues in fortified yogurt samples was also reported by Ghanem et al. [17] as the amount of lemon peel powder increased from 0.4% to 0.8%.

The influence of lemon peel powder and lime peel powder incorporation into yogurt on its physicochemical characteristics – including pH, titratable acidity, protein content, fat content, and fiber content – is shown in Table 2.

The pH of yogurt represents a critical indicator for evaluating its quality, safety, and stability during storage. Routine assessment of pH and titratable acidity is essential to ensure microbial safety and to preserve desirable sensory attributes, such as taste and texture [18,19]. In the present study, the incorporation of citrus peel powder significantly influenced the pH of yogurt samples ($p < 0.05$).

The results indicated a progressive decrease in pH values with increasing concentrations of citrus peel powder incorporated into yogurt. This acidification effect was more pronounced in samples fortified with lime peel powder compared to those containing lemon peel powder.

Table 1.
Color parameters of yogurt samples. Mean values ± standard deviation.

Sample	L^*	a^*	b^*	h^*_{ab}	C^*_{ab}
YControl	88.38±0.04^a	-2.25±0.01^b	5.12±0.04^{de}	113.68±0.19^a	5.59±0.04^e
YLemon-0.25	88.00±0.06^b	-2.11±0.01^a	4.98±0.06^e	113.00±0.14^a	5.41±0.06^e
YLemon-0.50	87.91±0.02^b	-2.17±0.01^a	5.77±0.07^d	110.60±0.23^b	6.16±0.07^d
YLemon-0.75	87.51±0.01^b	-2.27±0.01^b	7.35±0.01^c	107.14±0.04^d	7.69±0.01^c
YLime-0.25	87.69±0.06^b	-2.46±0.02^c	7.03±0.01^c	109.29±0.18^b	7.44±0.02^c
YLime-0.50	87.11±0.03^c	-3.04±0.02^d	9.32±0.08^b	108.03±0.04^c	9.80±0.09^b
YLime-0.75	86.59±0.05^d	-3.28±0.02^e	10.50±0.08^a	107.33±0.05^d	11.00±0.07^a
F-value	9.14	7.02	40.48	18.11	40.53

$^{a-e}$ Different letters in the same column indicate significant differences among samples ($p < 0.05$)

Table 2.

Physicochemical properties of yogurt samples. Mean values ± standard deviation.

Sample	pH	Titratable acidity (g lactic acid/100 g)	Protein content (g/100 g)	Fat content (g/100 g)	Fiber content (g/100 g)
YControl	4.61±0.01[a]	8.74±0.06[e]	3.61±0.01[a]	3.95±0.02[a]	n.d.
YLemon-0.25	4.50±0.02[b]	9.29±0.05[d]	3.59±0.01[a]	3.93±0.02[a]	0.05 ± 0.00[c]
YLemon-0.50	4.42±0.01[c]	9.44±0.04[c]	3.60±0.01[a]	3.93±0.02[a]	0.09 ± 0.00[c]
YLemon-0.75	4.30±0.01[de]	10.14±0.04[b]	3.59±0.01[a]	3.93±0.02[a]	0.15 ± 0.00[b]
YLime-0.25	4.49±0.02[c]	10.25±0.06[b]	3.61±0.01[a]	3.94±0.03[a]	0.09 ± 0.00[c]
YLime-0.50	4.38±0.02[d]	10.67±0.05[a]	3.60±0.01[a]	3.94±0.03[a]	0.19 ± 0.00[b]
YLime-0.75	4.25±0.01[e]	10.92±0.06[a]	3.59±0.01[a]	3.94±0.03[a]	0.28 ± 0.00[a]
F-value	5.80	14.51	1.13	1.25	10.40

[a-e] Different letters in the same column indicate significant differences among samples ($p < 0.05$)

These findings are consistent with previous studies that have reported similar pH reductions following the addition of lemon peel powder to yogurt formulations [12].

Titratable acidity serves as a critical parameter for evaluating both the quality and the progression of the fermentation process in yogurt [18]. Therefore, in this study, the titratable acidity of yogurt samples fortified with varying concentrations of lemon peel powder and lime peel powder was systematically assessed. Comparative analysis was conducted to elucidate the influence of each citrus peel type and concentration on acid development, thereby providing insights into their fermentative behavior and contribution to the overall biochemical profile of the yogurt matrix. An increase in titratable acidity was observed with the incorporation of higher concentrations of citrus peel powder into the yogurt. This finding is consistent with previous studies that reported similar trends in yogurts fortified with lemon peel powder [13] and yogurt enriched with orange pomace powder [20]. The observed increase in titratable acidity following the addition of citrus peel powder can be attributed to the presence of naturally occurring organic acids, such as citric acid, malic acid, and oxalic acid in lemon peel and lime peel [21]. These compounds contribute to the acidification of the product when the citrus peel powder is incorporated or blended into the formulation. In contrast to other physicochemical properties evaluated in the fortified yogurt samples, the incorporation of citrus peel powder did not significantly influence the protein and fat contents ($p > 0.05$). Yogurt retains the whey and casein proteins in proportions similar to those found in raw milk, although partial proteolysis occurs during fermentation [22]. Similar to proteins, milk lipids undergo significant biochemical transformations during the fermentation process. These include the hydrolysis of triglycerides and the generation of both short- and long-chain fatty acids. Beyond the intrinsic characteristics of raw milk, the fatty acid profile of yogurt is also influenced by processing conditions, including heat treatment, homogenization, fermentation parameters, and storage duration [23]. Considering the chemical composition of lemon and lime peel, as well as the low concentrations of peel powder incorporated into the yogurt (0.25-0.75%), the similarity in protein and fat content between the control sample and fortified yogurt samples was expected. These results corroborate previous findings on yogurt fortification [15].

The incorporation of citrus peel powders resulted in an increase in the total fiber content of the yogurt formulations relative to the control sample, in which fiber was not detected. Both lemon and lime peel powders provided quantifiable amounts of dietary fiber, consistent with the high levels of structural polysaccharides characteristic of citrus by-products [24]. A clear dose-dependent effect was observed for both powders, as higher addition levels (0.50 and 0.75%) resulted in higher fiber content in the yogurt. At the same inclusion levels, lime peel powder produced slightly higher fiber values than lemon peel powder, indicating a somewhat greater fiber contribution from lime peel. Overall, these results show that incorporating citrus peel powders is an effective way to increase the fiber content of yogurt, with the degree of increase depending on the amount of peel added. Figure 1 illustrates the syneresis values of yogurt samples fortified with varying concentrations of lemon peel and lime peel powders. Syneresis, or whey separation, is a major technological defect in yogurt, characterized by the release of liquid onto the surface, which impairs sensory properties (particularly mouthfeel) and diminishes overall consumer acceptance [25].

A lower syneresis value, indicating a greater capacity to retain whey, is generally linked to higher consumer acceptability. The extent of syneresis may be influenced by the fat content of the milk used; yogurts produced from milk with lower fat levels typically exhibit higher syneresis compared to those made from milk with higher fat content [12, 26]. As illustrated in Figure 1, the syneresis values decreased with the incorporation of lemon and lime peel powders, a trend consistent with findings reported in previous studies [12, 20, 27]. This reduction may be attributed to the high dietary fiber content of citrus peels, particularly pectin, which accounts for over 20-30% of the composition in both lemon and lime peels, thereby enhancing water retention within the yogurt matrix.
The reduction in syneresis was slightly more pronounced in yogurt samples fortified with lime peel powder, indicating a potentially greater water-binding capacity associated with its compositional characteristics.

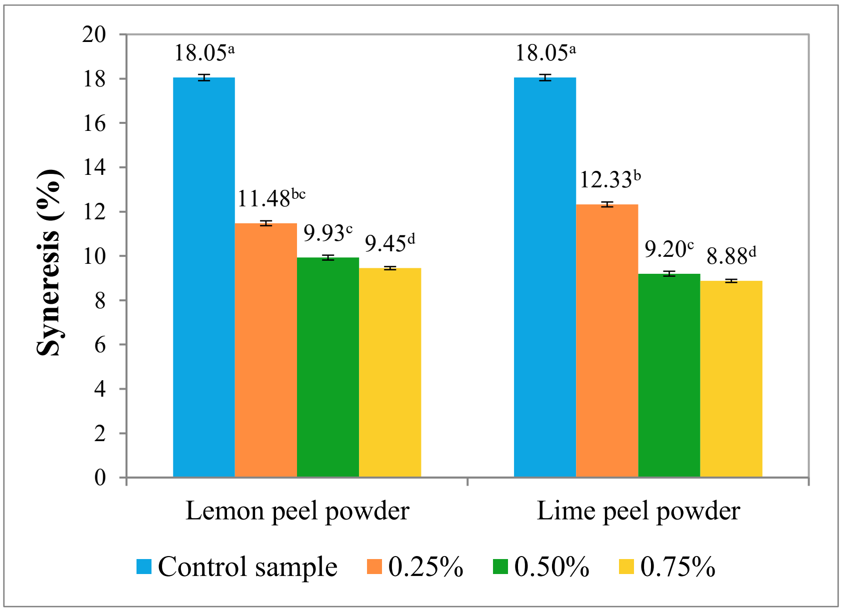

Fig. 1. Syneresis of yogurt. Different letters (a-d) beside the values indicate significant differences among samples ($p < 0.05$)

Yogurt is rheologically characterized as a non-Newtonian viscoelastic material [28]. Its rheological properties are influenced by various factors throughout the production, storage, and consumption stages. Consequently, rheological analyses are extensively employed to predict, monitor, and control the final texture and quality of yogurt. Figure 2 shows the apparent viscosity of yogurt samples.

For all yogurt samples, including both the control and those fortified with citrus peel powder, a decrease in apparent viscosity was observed with increasing shear stress, indicative of non-Newtonian, shear-thinning behavior.

The incorporation of citrus peel powder led to an overall increase in apparent viscosity, which was positively correlated with the concentration of the added powder; this finding was consistent with previous studies [12]. This increase in apparent viscosity is likely attributed to interactions between the components of citrus peel powder, particularly pectin, and casein micelles, resulting in a reinforced gel network. Notably, only the yogurt samples fortified with 0.50% and 0.75% lemon peel powder exhibited significantly higher viscosity compared to the control sample.

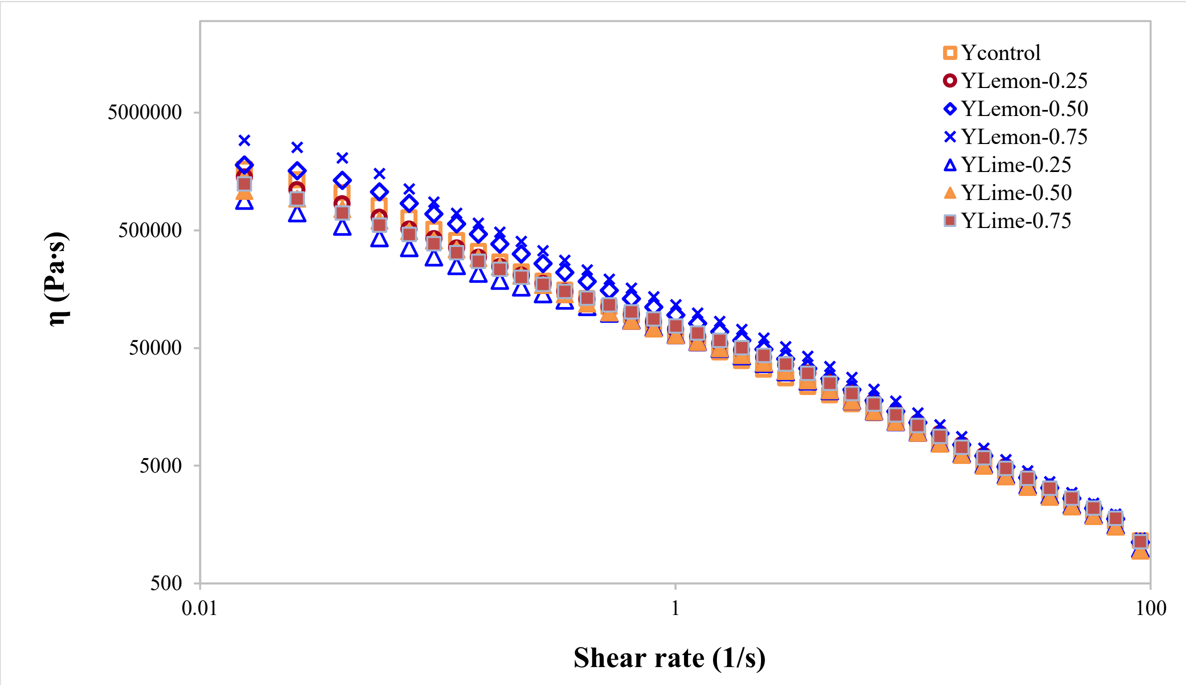

Fig. 2. Apparent viscosity of yogurt samples

Regardless of the type or concentration of citrus peel powder, the most pronounced reduction in apparent viscosity occurred within the shear rate range of 0.01-1 s^{-1}.

3.2. Sensory evaluation of fortified yogurt

The results of the sensory evaluation of the yogurt samples are presented in Figure 3. The control yogurt, along with samples fortified with 0.25%, 0.50%, and 0.75% lemon peel powder, received significantly higher scores for appearance, color, and overall acceptability. In particular, yogurt samples containing 0.25% and 0.50% lemon peel powder, and 0.25% lime peel powder achieved higher preference scores for texture, taste, and flavor attributes.

Texture is a critical physical attribute influencing the sensory quality and consumer acceptance of yogurt.

The observation that samples fortified with citrus peel powder up to a concentration of 0.50% were rated more favorably than the control suggests that consumers appreciated the textural enhancement imparted by the addition of citrus peel powder. Substantial variation in color scores across different citrus peel powder concentrations was

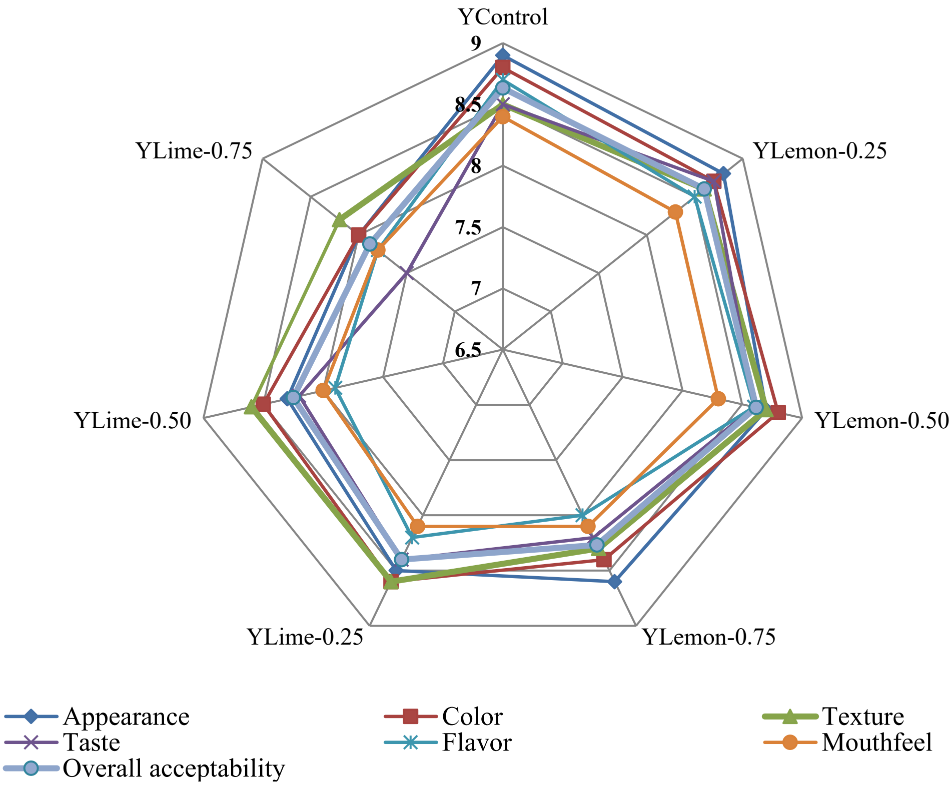

Fig. 3. Sensory evaluation of yogurt samples

observed, aligning with the instrumental color measurements reported in Table 1. Specifically, the yellow and green hues associated with samples containing 0.50% and 0.75% citrus peel powder, as indicated by the color parameter analysis, were perceptible to panelists and were rated less favorably. Lower scores for taste and flavor were recorded for yogurts fortified with 0.50% and 0.75% lime peel powder, likely due to increased sourness. These findings suggest that lower inclusion levels of citrus peel powder are preferred by consumers to maintain optimal sensory properties. Overall, the yogurt sample fortified with 0.50% lemon peel powder was found to be the most acceptable among the tested formulations. This observation is consistent with findings reported in a previous study [12].

3.3. Principal component analysis

Principal component analysis (PCA) was employed to assess the effects of incorporating citrus peel powders (lemon and lime peel) on the quality attributes of yogurt, specifically focusing on physicochemical, rheological, and sensory properties. The PCA bi-plot, illustrating both the score and loading plots, is presented in Figure 4. The first principal component (PC1) explained 71.45% of the total variance, while the second principal component (PC2) accounted for an additional 15.49%, cumulatively representing 86.94% of the overall variability in the dataset. The yogurt samples were distinctly separated according to the type of citrus peel powder incorporated, with lime and lemon peel formulations positioned in distinct quadrants of the PCA plot. The samples containing 0.50% and 0.75% lime peel powder were positioned in close proximity, primarily influenced by titratable acidity, fiber content, and color parameters such as b* and C*ab.

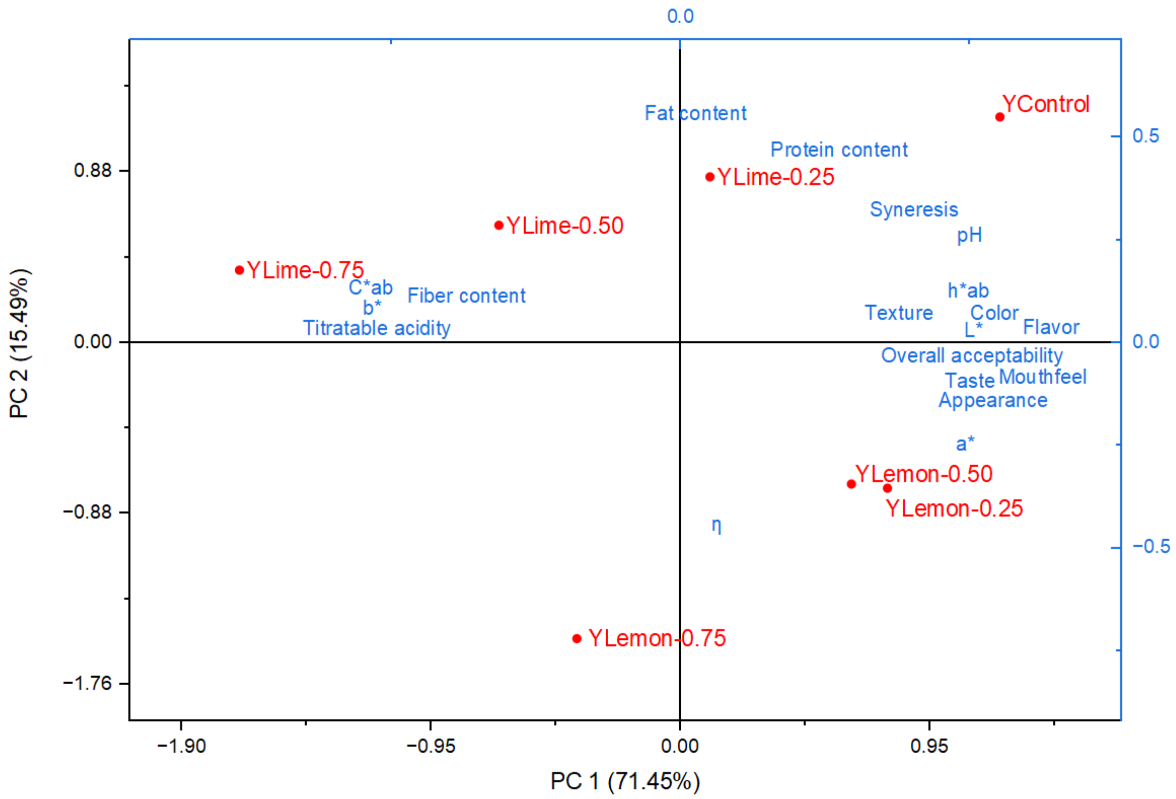

Fig. 4. Principal component bi-plot showing scores of yogurt samples (in red) and loadings of physicochemical and sensory parameters (in blue)

In contrast, the control sample and the yogurt sample with 0.25% lime peel powder were located in the same quadrant, differentiated from other samples by loading vectors associated with protein content, syneresis, pH, and fat content.

The positioning of yogurt samples containing lemon peel powder was largely influenced by apparent viscosity and the a^* color parameter. A pronounced separation was observed for the yogurt sample containing 0.75% lemon peel powder, which was distinctly positioned apart from the samples with 0.25% and 0.50% lemon peel powder, likely due to its markedly higher apparent viscosity.

4. Conclusions

In this study, yogurt was fortified with lemon and lime peel powders at concentrations of 0.25%, 0.50%, and 0.75%, and the effects of these additions on the physicochemical and sensory properties of the product were systematically evaluated.

The results demonstrated that the incorporation of citrus peel powders influenced key quality parameters, particularly pH and titratable acidity, which remained within acceptable ranges and were consistent with previously reported findings, while having no effect on the protein and fat content of the yogurt samples.

Citrus peel powder addition increased the fiber content of the yogurt samples overall, with the improvement being more pronounced in yogurts fortified with lime peel. In addition to these compositional effects, the incorporation of citrus peel powders contributed to improved viscosity and reduced syneresis, both of which are desirable textural attributes in yogurt. Improvements in texture, appearance, and color were observed, particularly at lower inclusion levels.

Yogurt samples fortified with 0.50% lemon peel powder achieved the highest overall sensory acceptability among the tested formulations. Based on both physicochemical and sensory evaluations, the findings of this study support the use of lemon and lime peel powders as functional fortifying agents in yogurt, particularly at levels up to 0.50%, to develop a more nutritious and consumer-preferred product.

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