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Enhancement structure probiotic yoghurt made from whole milk powder by psyllium husk

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ABSTRACT

Psyllium husk is a bioactive source that provides health benefits and is new best possible use in the dairy industry as a texture enhancer for its hydrochloride properties. The current study focuses on the optimization of a suitable amount of psyllium husk powder (PHP) with whole milk powder (WMP) to produce bioactive probiotic yoghurt and keep it stable during the storage period. The different levels of PHP (0, 0.5, 1.0, and 1.5 % w/w) with WMP were used to prepare the bioactive probiotic yoghurt as a functional food. We made four different mixtures and tested them for their chemical makeup (pH, titratable acidity, moisture, total solids, ash, fat, and protein), colour (L*, a*, b*, and C*), ability to hold water (WHC), and how they tasted after 1, 7, and 14 days at 5°C. The PHP probiotic yoghurt showed a significant decrease ($p < 0.05$) in pH, protein, and fat. While titratable acidity and ash contents were increased compared with the control. Also, the texture profile, like adhesive and hardiness, had better flow properties and more WHC than the control. Additionally, the color parameter showed an increase in a*, b*, and C* values and a decrease in the L* value. The sensory evaluators preferred the 1% PHP samples. The current study found that samples fortified with 1% PHP improved the physicochemical, texture, and sensory properties of probiotic yoghurt and maintained probiotic yoghurt structure during storage compared with the control. Probiotic yoghurt prepared from PHP has provided new dairy industrial applications.

Contribution/Originality: This study demonstrates the importance of improving the texture of probiotic yoghurt and introduces psyllium husk as an effective ingredient for achieving this. The psyllium husk enhances yoghurt consistency during storage and offers potential health benefits. This research provides valuable insights and practical applications for enhancing the quality of probiotic yoghurt and utilizing psyllium husk in the dairy industry.

1. INTRODUCTION

Yoghurt is a functional dairy product that has experienced worldwide expansion in recent years. Yoghurt can be obtained by the fermentation of lactose to lactic acid by lactic acid bacteria such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus* at a specific time and temperature (Shori, Ming, & Baba, 2021). Probiotics are active microorganisms that can promote host health when taken in certain amounts. Lactic acid bacteria (LAB) are examples of probiotics (Shori, Aljohani, Al-zahrani, Al-sulbi, & Baba, 2022). Therefore, optimal nutrition and health are linked to a bacterial population in our digestive system that is in balance (Rybka & Kailasapathy, 1995). The microbes *lactobacilli* and *bifidobacteria* are usually responsible for maintaining this state of balance (Lourens-

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Hattingh & Viljoen, 2001). It is highly encouraged to regularly consume foods containing probiotic bacteria in order to create a favorable balance of the population of beneficial and useful bacteria in the gut flora (Dahiya & Nigam, 2022). The texture of yoghurt is one of its most important sensory components, and various texture deficiencies, including poor gel consistency and syneresis, are likely to be present in a variety of yoghurts, which could reduce customer satisfaction. Various processing methods are used in industrial yoghurt production to improve its nutritional and practical properties and to avoid syneresis and problematic texture defects (Karam, Gaiani, Hosri, Burgain, & Scher, 2013). In yoghurt, water holding capacity is important after production and during storage. According to (Arab et al., 2023), there are a number of factors that affect it, including milk preheating, total solids, pH, thickening agents, and mechanical disturbances.

Psyllium is a natural polysaccharide with amazing nutritional properties and a high concentration of water-soluble fiber, which has many health benefits and prebiotic effects (Belorio, Marcondes, & Gómez, 2020; Franco, Sanches-Silva, Ribeiro-Santos, & de Melo, 2020). Due to its large water-holding capacity, which is around 80 times its weight, this mucilage gel is well-recognized for its laxative effects (Tosif et al., 2022). Psyllium husk contains around 78% of the soluble fibers and 13% of the insoluble fibers. In addition, psyllium is widely known to reduce the risk of constipation, symptoms of irritable bowel syndrome, stomach pain, cancer prevention, diarrhea, inflammatory bowel disease, ulcerative colitis, obesity, diabetes, and hypercholesterolemia (Brum, Gibb, Peters, & Mattes, 2016; Yadav, Sharma, Kapila, Malik, & Arora, 2016). Psyllium husk has hydrocolloid properties, and hot water-extracted polysaccharide from psyllium husk has been shown to have unique gelling properties. Emulsion gel has been easily and feasibly prepared from psyllium husk, which has great potential for food and biomedical applications (Zhou et al., 2022). Recent research has shown the use of probiotics in conjunction with psyllium fibers as prebiotics can maintain a healthy intestinal microbiota (Martellet, Majolo, Ducati, Volken de Souza, & Goettert, 2022). Psyllium caused similar changes in the properties of starch as xanthan gum, which is why xanthan gum can be substituted in a variety of applications (Franco, Sanches-Silva, Ribeiro-Santos, & de Melo, 2020). In the traditional Indian medical system (Ayurveda), psyllium husk, also known as isabgol (Plantago ovate), is frequently used to treat skin irritations, hemorrhoids, constipation, and diarrhea (Yadav et al., 2016). Psyllium is well known for its useful effects in decreasing low-density lipoprotein, reducing blood sugar levels in diabetes, and preventing constipation (Alkandari et al., 2021). This study aimed to evaluate the physiochemical, microbiological, and sensory properties of probiotic yoghurt made with the addition of psyllium husk powder during refrigerated storage.

2. MATERIALS AND METHODS

Psyllium husk powder containing cured protein (1.9%), Fiber (80.9%), and Ash (2.3%) was obtained from Imtenan Health Section, Food Additives Company, Egypt. Whole milk powder (WMP) having a composition of protein (24.4%), fat (26.2%), carbohydrate (29.8%), and sodium (mg/100g) was obtained from Nestlé NIDO®, Egypt. A mixed starter culture of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *Bulgaricus*, and *Bifidobacterium bifidum* was obtained from Chr. Hansen's Laboratories in Copenhagen, Denmark.

2.1. Production of Probiotic Yoghurt

The probiotic yoghurt was made from whole milk powder according to Obi, Henshaw, and Atanda (2010), with some modifications as follow: The psyllium husk was grounding and sifted separately. Whole milk powder was reconstituted in sterilized water and homogenized in a 130:900 (w/v) ratio, then pasteurized for 15 minutes at 85 °C cooled to 43 °C. The reconstituted milk was distributed in four portions. Using a high-speed mixer at concentrations of 0%, 5%, 1.0%, and 1.5%, psyllium husk powder was added to the four portions of reconstituted milk at 43°C for 10 min. Commercial starter culture (*Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *Bulgaricus*, and *Bifidobacterium bifidum)* was added to the mixture (0.2 g/L). The inoculated milk was transferred

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into 150-gramme plastic cups and incubated at 42ºC until the pH reached 4.6. After 4 h of incubation, fermentation was stopped by rapid cooling. Yoghurt samples were taken to the refrigerator and stored at 5 ºC for 14 days.

2.2. Physicochemical Properties

Total solids (TS), moisture, ash, fat, pH, and protein of control (0% PHP) and PHP probiotic yoghurt were measured according to AOAC (2000).

2.3. Water Holding Capacity (WHC)

The determination of water holding capacity (WHC) in probiotic yoghurt samples was conducted by following the method of Isanga and Zhang (2009). A total of ten grams of yoghurt were subjected to centrifugation at 4600 rpm for 30 minutes at a temperature of 4°C. The WHC was then calculated using the following formula:

$$\text{WHC (\%)} = 1 - \frac{W1}{W2} \times 100$$

Where, W1 is the amount of whey after centrifugation (g), and W2 is the amount of yoghurt sample (g).

2.4. Texture Profile

The texture profile analysis (TPA) of four different formulas of PHP probiotic yoghurt was performed using the multi-test 1-d texture analyzer (Mecmesin Limited, Slinfold, West Sussex, UK) according to the method of Clark, Costello, Drake, and Bodyfelt (2009). Experiments were carried out using a compression test that generated a plot of force (N) versus time (sec). Samples were double-compressed at a compression speed of 2 cm/min. The analysis was carried out at room temperature. Hardness (N), springiness (mm), chewiness (N*mm), gumminess (N), and cohesiveness were calculated from the obtained TPA according to the definition given by the IDF (1991).

2.5. Color

Color analyses were implemented on the different four formulations of PHP probiotic yoghurt samples after 1, 7, and 14 days of refrigerated storage using a Hunter colorimeter (Hunter Ultra Scan VIS). The colorimeter provided values for L*, a*, b*, and C*. The L* value represented the lightness of the yoghurt on a scale of 0-100, with higher values indicating a lighter color. The a* value indicated the degree of red and green color, with a higher positive value indicating more red color. The b* value represented the degree of yellow and blue colors, with a higher value indicating more yellow color. The chroma value (C*) was calculated as $C^* = (a^{*2} + b^{*2})^{1/2}$, representing the color intensity (Hunter & Harold, 1987).

2.6. Sensory Properties

Using a sensory rating scale of 1 (bad) to 9 (great) for certain sensory aspects such as color, odor, texture, taste, and general acceptability, PHP probiotic yoghurt samples were evaluated by 8 panelists, according to Bodyfelt, Tobias, and Trout (1988). The tasting panel was randomly selected among the staff members who were very familiar with fermented dairy products. All samples were presented to the panelists in plastic jars (80 mL) at 4-6 °C.

2.7. Statistical Analysis

The data from the physicochemical and organoleptic properties were analyzed using a two-way ANOVA using SPSS.16 statistical software. The p-value <0.05 was considered statistically significant for all analyses.

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3. RESULTS AND DISCUSSION

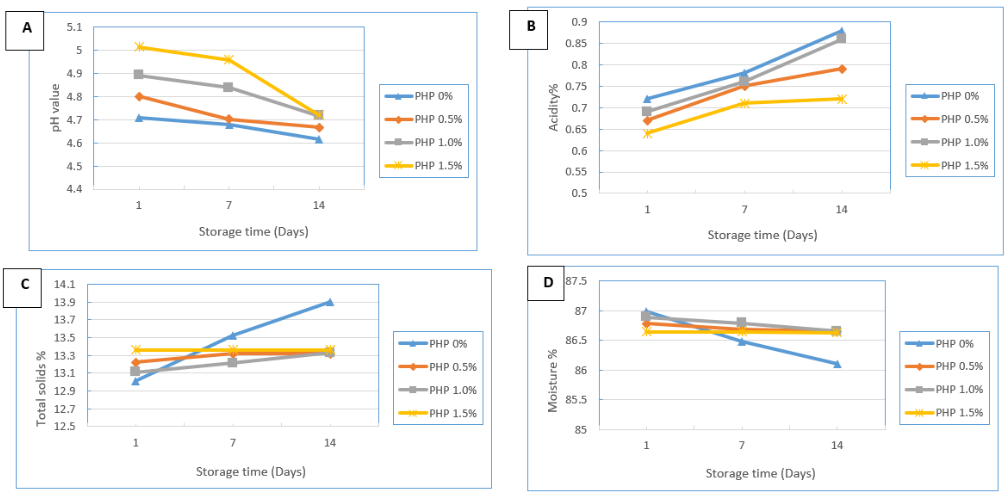
3.1. Physicochemical Characterization

The acidity and pH of PHP probiotic yoghurt during storage at 4°C for fourteen days are shown in Figure 1. The results recorded showed that the pH decreased towards more acidic conditions and the acidity of probiotic yoghurt increased during the storage period in all probiotic samples. The supplementation of PHP has not significantly (p >0.05) affected the titratable acidity in probiotic yoghurt with 1% and 1.5% PHP during the storage period. The highest titratable acidity value was recorded in control samples (0% PHP) during the storage period. So, adding a PHP case decreases the titratable acidity value of PHP probiotic yoghurt due to the PHP fiber which has an attributable diluting effect (Issar, Sharma, & Gupta, 2017). The TS increased significantly ($p < 0.05$) in probiotic yoghurt supplements with PHP; this increase in ingredients and PHP content caused a significant increase in total solids content in the PHP probiotic yoghurt (Darwish, Darwish, & Ismail, 2017; Zare, Boye, Orsat, Champagne, & Simpson, 2011).

The PHP probiotic yoghurt has not significantly ($p > 0.05$) affected the moisture content during the storage period due to psyllium husk, which has a significant water retention capacity, making it an important ingredient in curdled yoghurt, and that will solve the problem of water release from yoghurt during the storage period. The control probiotic yoghurt recorded a significant decrease ($p < 0.05$) in moisture during the storage period, and this decrease may be due to evaporation or a decrease in pH during the storage period (Atwaa et al., 2022). The content of ash recorded significantly ($p < 0.05$) increased with increasing the percentage of PHP and during the storage period compared with the control because psyllium husk has a content of fiber, protein, and ash (Noguerol, Igual, & Pagan, 2022). The protein content of PHP probiotic yoghurt decreased with an increased percentage of PHP, thus the protein content in PHP (1.9%) being lower than WMP (24.4) (Ahmad, Xiong, Hanguo, Khalid, & Khan, 2022). Whereas, the protein content recorded no significant ($p > 0.05$) differences in PHP probiotic yoghurt during storage compared with control probiotic yoghurt. In the study by (Atwaa et al., 2022; Ladjevardi, Gharibzahedi, & Mousavi, 2015), adding PHP to probiotic yoghurt significantly ($p < 0.05$) lowered the fat content of the PHP yoghurt compared to the control yoghurt. This decrease in fat content in PHP probiotic yoghurt might be due to the minute amount of fat content of psyllium husk compared to WMP (Noguerol et al., 2022).

3.2. Water Holding Capacity (WHC)

The WHC of yoghurt refers to its capacity to hold water. The results show the WHC of PHP probiotic yoghurt samples in Figure 1.



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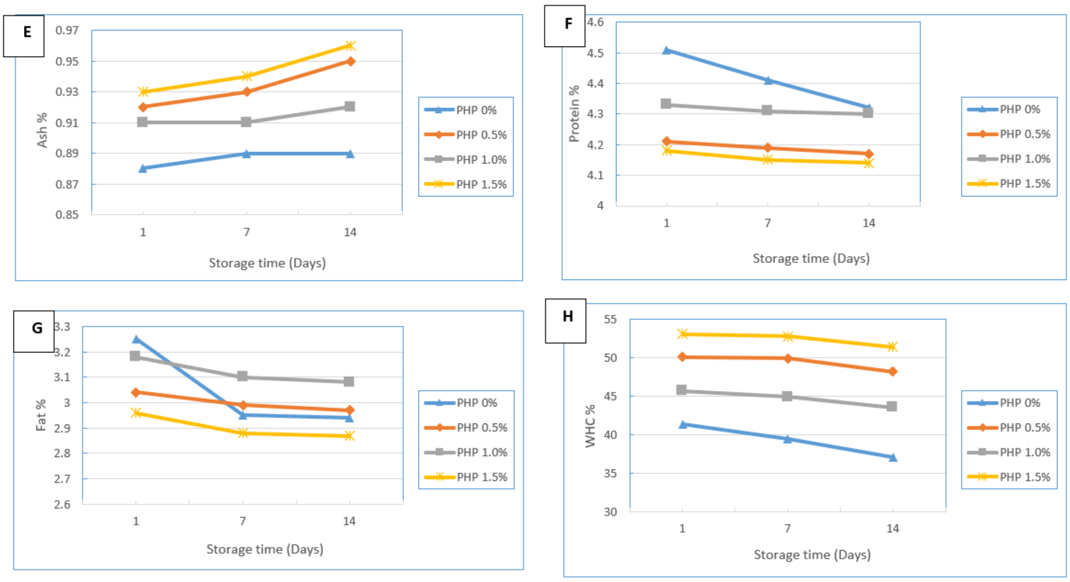

Figure 1. Changes in (A) pH, (B) acidity %, (C) total solids%, (D) moisture%, (E) ash %, (F)protein%, (G) fat % and (H) water holding capacity (WHP) % of probiotic yoghurt supplemented with different concentrations of psyllium husk powder (0 % PHP, 0.5 % PHP, 1.0 % PHP, 1.5 % PHP) during storage at 4 ºC for 14 days.

It is recorded that WHC was significantly ($p < 0.05$) increased from 42.5% (0% PHP) to 53.07% (1.5 % PHP) in probiotic yoghurt samples. During the storage of 14 days at 4 °C the WHC decreased ($p < 0.05$) in the control probiotic samples, whereas there was a significant ($p < 0.05$) increase with the increased presence of PHP in probiotic yoghurt. The increase in WHC I with the addition of PHP probiotic yoghurt compared to control may be due to protein and the amount of fiber present in the psyllium husk. In food systems, the interaction between water and proteins is most significant, and it has an impact on food texture. The results of this study's WHC are consistent with those of earlier studies (Ahmad et al., 2022; Darwish et al., 2017).

3.3. Texture Profile Analysis (TPA)

Texture Profile Analysis (TPA) of different PHP probiotic yoghurt samples was estimated during storage at 4 ºC for 14 days. The parameters included hardness, springiness, cohesiveness, gumminess and chewiness (Figure 2). PHP probiotic yoghurt samples (0.5%, 1%, and 1.5%) recorded a significant ($p < 0.05$) increase in hardness compared with control. Hardness is directly proportional to the increase in PHP. There was a gradual decrease ($p > 0.05$) in hardness during the storage period at 4 °C for 14 control ($p < 0.05$) and PHP probiotic yoghurt samples ($p > 0.05$), due to the firmness of PHP probiotic yoghurt, which has a high protein content (Ahmad et al., 2022) or from PHP (Alkandari et al., 2021). Springiness is the speed at which the sample resumes to return to its original shape after being deformed (Kong et al., 2022). There are various factors that affect springiness, like heat treatment, protein interaction, elasticity, and the degree of protein unfolding (Delikanli & Ozcan, 2017; Mousavi, Heshmati, Daraei Garmakhany, Vahidinia, & Taheri, 2019). There were significant ($p < 0.05$) differences in springiness between the 1% and 1.5% PHP samples and the control. However, the effectiveness of adding PHP percentage on the springiness of probiotic yoghurt samples was greater than the effectiveness of the storage period at 4 ºC for 14 days. The springiness of yoghurt samples was reported to have the maximum effect at a level of 1.5% of PHP and a storage time of 14 days. Springiness indicates the texture integrity of yoghurt, so the addition of PHP to probiotic yoghurt increases texture integrity. As a result, it is a suitable reason for the higher springiness observed in yoghurt containing PHP. These results were in agreement with those recorded that the springiness of the yoghurt samples increased by the increase in partially hydrolyzed guar gum level (Mudgil, Barak, & Khatkar, 2017) and the addition of carrot to the yoghurt during the 1st and 10th days of storage time (Ayar and Gurlin,2014). Cohesiveness, which shows structural integrity, is frequently described in terms of bond strength. In the current

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study, the cohesiveness of PHP probiotic yoghurt samples showed a significant ($p < 0.05$) increase; this increase in cohesiveness was directly proportional to the percentage of PHP. Additionally, cohesiveness continued to increase throughout a 14-day storage period at 4 °C (Figure 2). The cohesiveness and springiness of the yoghurt may be attributed to stronger gel structures, indicating better structural integrity. This is likely due to the denaturation of whey protein, which increases the charged groups on the amino acid groups (Magenis et al., 2006). A formulation containing 1, 1.5% PHP was found to have superior textural qualities, making it a high-quality yoghurt.

Gumminess is the energy needed to break up a semisolid food into bite-sized pieces until it is ready to be swallowed (Dar & Light, 2014; Mousavi et al., 2019). Gumminess ranged from 0.25 to 0.69 N Figure 2. Probiotic yoghurt with 0% PHP (0.25 N) and probiotic yoghurt with 1.5% PHP (0.69 N) had the lowest and highest gumminess values, respectively. The results showed that supplementation by 1.0% and 1.5% PHP has a significant ($p < 0.05$) effect. While 0.5% for gumminess was an insignificant value. During storage period, there was insignificant value in gumminess. The texture and appearance are adversely affected by gumminess, because the PHP had significant effects on texture, a high PHP percent had a negative effect on gumminess and we recognized an increase in gumminess in yoghurt that had been enriched with PHP. In the study by Mudgil (2018), it was found that the addition of guar gum as soluble fiber to yoghurt caused enhanced gumminess. These researchers discovered the relationship between gumminess and fermentation (Azari-Anpar, Soltani Tehrani, Aghajani, & Khomeiri, 2017; Darwish et al., 2017). Chewiness, which is related to hardness, cohesiveness, and elasticity, is the amount of time or effort required to masticate a sample to make it suitable for consumption (Dar & Light, 2014; Mousavi et al., 2019). Responses of chewiness for PHP probiotic yoghurt samples during a storage period of 14 days at 4 °C are shown in Figure 2.

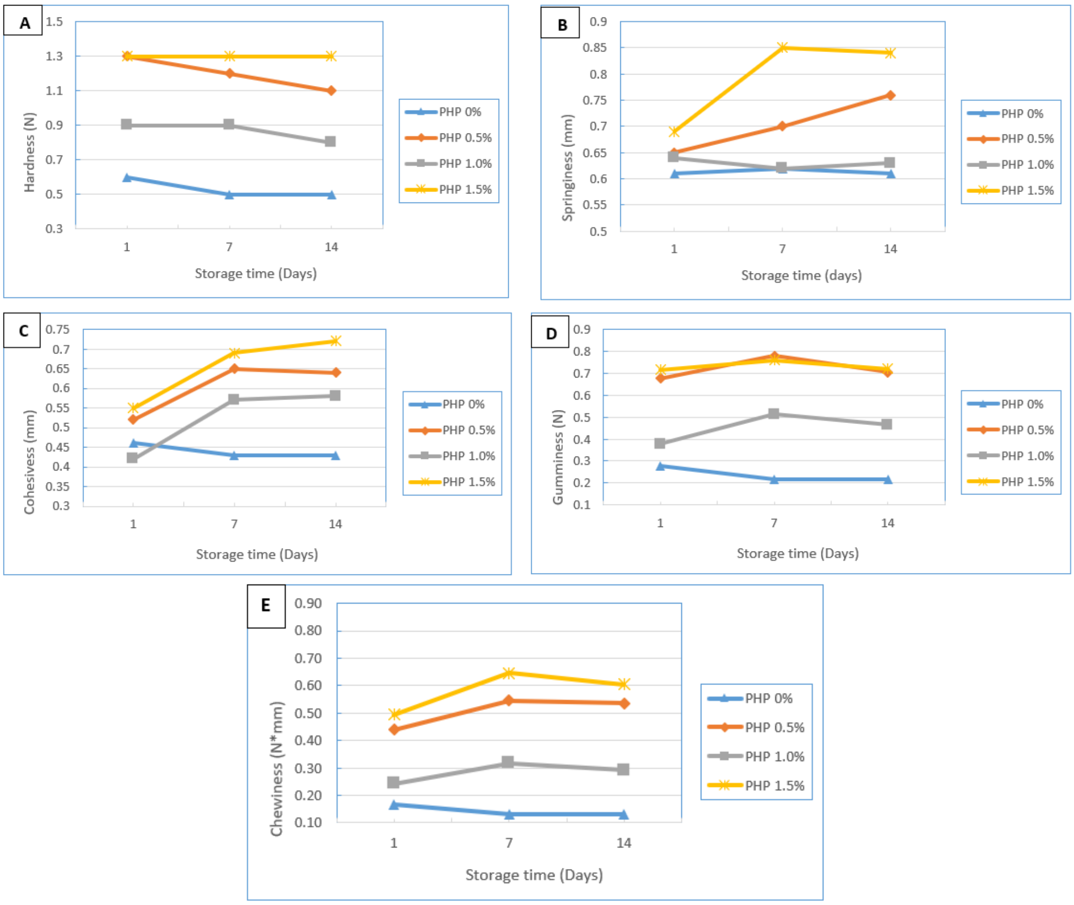

Figure 2. Changes texture profile analysis: (A) Hardness, (B) Springiness, (C) Cohesivess, (D) Gumminess, and (E) Chewiness of probiotic yoghurt supplemented with different concentrations of psyllium husk powder (0 % PHP, 0.5 % PHP, 1.0 % PHP, 1.5 % PHP) during storage at 4 °C for 14 days.

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The chewiness significantly increased with increasing levels of PHP, while storage time did not affect this parameter. The Chewiness of our samples ranged from 0.28 to 0.62 N, depending on PHP levels and storage time. The amount of PHP has a significant effect on chewiness. The chewiness increase of the PHP probiotic yoghurt samples containing PHP could be due to the effect of PHP viscosity, which may have improved the structure of the PHP probiotic yoghurt samples.

3.4. Color Analysis

Similar to texture, consumers' visual perception of color has a crucial influence on their opinions. The white color of milk and yoghurt results in the light dispersion of fat globules and casein micelles (Chandan, 2006). The current study recorded that the addition of PHP significantly ($p < 0.05$) changed the color of probiotic yoghurt compared with the control. Although storage time had no significant effect on the color intensity of yoghurt samples, Figure 3. The L* value of yoghurt samples ranged from 85.16 to 73.61 Figure 3. The lightest (85.16) and darkest (73.61) samples were recorded at the beginning of storage in control and PHP enriched (1.5%) probiotic yoghurt, respectively. he high fiber content of PHP cased displayed a darkening effect; this is due to fiber absorbing water and decreasing the L* value of the PHP probiotic yoghurt samples (Darwish et al., 2017; Mousavi et al., 2019). Furthermore, the results of b* values indicated that the addition of PHP to probiotic yoghurt had a significant effect on the product's fiber content. It was discovered by a colorimeter, even though it was invisible to the human eye. The fiber in the PHP may have contributed to the probiotic yoghurt's a* values being significantly (p 0.05) higher than those of the control sample. The result of our study was similar to García-Pérez et al. (2005), who reported that the addition of orange fiber in yoghurt samples increased a* and b* values and decreased L* values. Chroma (C*) is an indicator of color saturation; this parameter is increased with the concentration of PHP for probiotic yoghurt, which is higher than control Figure 3.

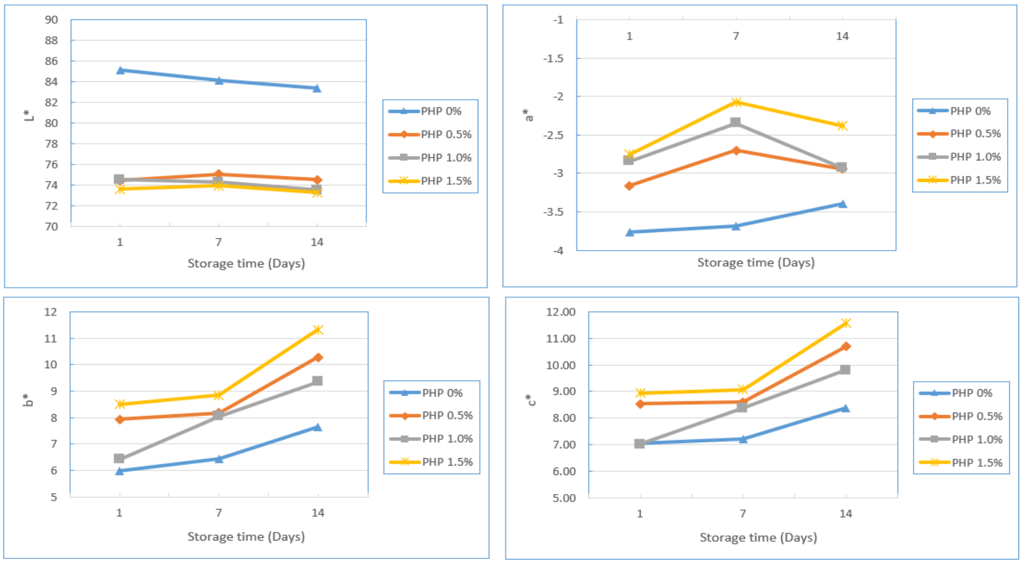

Figure 3. Changes color analysis: A Lightness (L*), B redness/ greenness (a*), C yellowness b*, and (D) C* Chroma of probiotic yoghurt supplemented with different concentrations of psyllium husk powder (0 % PHP, 0.5 % PHP, 1.0 % PHP, 1.5 % PHP) during storage at 4 °C for 14 days.

3.5. Sensory Evaluation

Sensory evaluation aids in defining the product characteristics that are important for the customer's acceptance of the product (Mousavi et al., 2019). In this study, the sensory evaluation was conducted for the PHP probiotic yoghurt samples after 1, 7, and 14 days Table 1. Sensorial evaluations such as odor, colour, taste, body texture, and overall acceptability were considered to analyze the end product's sensory quality. PHP concentration affects the sensory properties of PHP probiotic yoghurt samples significantly ($p < 0.05$) compared with control. The panelists

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recommended the higher score of odor, colour, and taste for the control probiotic yoghurt on the first day of age. Results from the body and texture score recorded that panelists preferred 1% PHP probiotic yoghurt in comparison with the control and other PHP formula samples at the end of the storage period. The overall acceptability of PHP probiotic yoghurt samples was significantly ($p < 0.05$) decreasing after 14 days of storage. Although the sensory attribute score of PHP probiotic yoghurt samples is lower than that of the control sample at the beginning of the storage period, however, overall acceptance was higher at the end of the storage period for yoghurt fortified with PHP compared to the control. The maximum values of overall acceptance were obtained for 1% PHP fortified probiotic yoghurt with statistically significant ($p < 0.05$) differences, which were 8.95 ± 0.35 at 1 day and 8.47±0.39 at 14 days, respectively. As nutritional knowledge increased, it appeared that customers favored functional foods with possible health benefits (Diez-Sánchez, Quiles, & Hernando, 2023). As well, the addition of PHP to probiotic yoghurt as a texture-enhancing agent could improve the sensory characteristics of these products.

Table 1. Sensory evaluation control PHP probiotic yoghurt during 14 days at 4 °C (mean ± standard deviation).

Treatments	Storage days	Odor	Color	Taste	Body & texture	Overall acceptance
PHP 0%	1	$8.95±0.54^{a}$	$9.12±0.71^{a}$	$8.96±0.64^{a}$	$8.71±0.33^{bc}$	$8.94±0.55^{a}$
	7	$8.58±0.31^{a}$	$8.91±0.24^{ab}$	$8.40±0.53^{abc}$	$8.65±0.25^{bc}$	$8.64±0.52^{bcd}$
	14	$7.61±0.14^{b}$	$8.44±0.37^{abc}$	$7.49±0.52^{bc}$	$7.22±0.29^{cd}$	$7.54±0.33^{d}$
PHP 0.5%	1	$8.91±0.18^{a}$	$8.86±0.23^{ab}$	$8.88±0.74^{ab}$	$9.17±0.47^{a}$	$8.96±0.40^{a}$
	7	$8.57±0.35^{a}$	$8.67±0.45^{abc}$	$8.28±0.51^{abc}$	$8.69±0.32^{bc}$	$7.63±0.40^{bcd}$
	14	$7.55±0.42^{ab}$	$7.96±0.31^{cd}$	$7.64±0.65^{abc}$	$7.24±0.21^{cd}$	$7.75±0.39^{cd}$
PHP 1%	1	$8.92±0.13^{a}$	$8.71±0.34^{abc}$	$8.87±0.62^{ab}$	$9.29±0.34^{ab}$	$8.95±0.35^{ab}$
	7	$8.68±0.24^{ab}$	$8.62±0.41^{abc}$	$8.50±0.64^{abc}$	$9.20±0.29^{ab}$	$8.85±0.34^{ab}$
	14	$8.18±0.35^{ab}$	$7.68±0.52^{d}$	$7.96±0.49^{bc}$	$8.95±0.21^{b}$	$8.47±0.39^{b}$
PHP 1.5%	1	$8.89±0.64^{ab}$	$8.70±0.48^{ab}$	$8.94±0.72^{abc}$	$9.35±0.31^{ab}$	$8.97±0.53^{bcd}$
	7	$8.79±0.35^{a}$	$8.44±0.62^{abc}$	$7.65±0.68^{bc}$	$8.59±0.29^{bc}$	$8.37±0.48^{bc}$
	14	$8.17±0.27^{ab}$	$7.66±0.74^{d}$	$7.51±0.81^{c}$	$7.92±0.25^{c}$	$7.82±0.51^{c}$

Note: abcd means in the same column followed by different lowercase letters indicates significant differences ($P < 0.05$).

4. CONCLUSION

In this study, probiotic yoghurt was fortified with different amounts of PHP (psyllium husk powder), and the probiotic yoghurt sample fortified with 1% of PHP recorded improved physicochemical, rheological characteristics, and overall acceptance of sensory evaluation compared with other concentrations. The current study supports the idea that added PHP can be used as a potential ingredient in probiotic yoghurt due to its nutritional functions, which include high fiber content as well as texture enhancement. Therefore, it is recommended that adding PHP improves yoghurt acceptance until the end of the storage period, which may then be scaled up further and serve as a foundation for future research on fermented dairy products. This study can guide other plant-based components, particularly those that include significant levels of dietary fiber for yoghurt production. Yoghurt's enhanced dietary fiber and protein content, a feature that promotes health, was made possible with the inclusion of PHP.

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Competing Interests: The author declares that there are no conflicts of interests regarding the publication of this paper.

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