

Comparative effects of the probiotic bacteria and carob powder on the quality properties of peanut butter

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Abstract

The present study examined the effect of carob powder and probiotic culture addition on the quality characteristics of peanut butter during a three-month storage period. Carob powder enhanced the growth of *Lactocaseibacillus rhamnosus* in MRS broth. It also demonstrated technofunctional properties such as oil retention capacity (2.48 g/g), water retention capacity (2.42 g/g), emulsion activity (47.67%), and emulsification index (45.35%). Furthermore, the addition of carob powder to peanut butter promoted a prebiotic effect on the growth of probiotic culture and reduced oil loss in the peanut butter samples. Overall, the effect of storage on the quality properties of peanut butter was negligible ($p > 0.05$), whereas the impact of probiotic culture on carob powder was significant ($p < 0.05$). Probiotic viability in peanut butter without carob powder and with carob powder ranged from 5.95 to 6.45 log CFU/g and from 6.15 to 6.82 log CFU/g, respectively. Specifically, oil loss in probiotic peanut butter with carob powder was lower compared to other peanut butter samples, owing to the oil retention capacity of carob powder. Additionally, all peanut butter samples maintained desirable sensory quality properties throughout storage.

Keywords: carob powder; peanut butter; probiotic; techofunctional properties

Introduction

As the connection between food and health continues to grow, the production of alternative functional foods using probiotic cultures has become a central focus. According to a joint report by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), probiotics are microorganisms that have a positive impact on human health as well as food quality and safety. In the development of new probiotic food

products, there is an increasing emphasis on selecting cultures that both enhance health benefits and improve the technological properties of food (FAO, 2002). The most commonly used probiotic bacteria in foods belong to the *Lactobacillus* and *Bifidobacterium* genera (Ünal Turhan *et al.*, 2017a,b; Şanlıbaba, 2023).

Dairy products such as yogurt, cheese, and ice cream have long been used as vehicles for probiotics (Coba *et al.*, 2019). However, probiotic dairy products are not

suitable for certain consumer groups, including those with lactose intolerance, hypercholesterolemia, or allergies to milk proteins. Additionally, modern lifestyles, along with vegan and vegetarian diets, have contributed to a decline in dairy product consumption. In response to increasing consumer demand for non-dairy probiotic food products, there has been a growing trend in the food industry towards plant-based probiotic options (Perez *et al.*, 2021). Given the rising importance of the food-health relationship, developing alternative probiotic products beyond dairy-based options has become a key focus. The application of probiotic cultures in novel food matrices has broadened the range of probiotic products. Modern consumers now seek foods that not only meet nutritional needs but also provide health-protective or disease-preventive benefits. Plant-based products, known for their biological activity and active ingredients, are valuable sources of functional components (Ünal Turhan *et al.*, 2017a; 2017b; Kalkan *et al.*, 2020). As a result, developing plant-based functional food products has become an innovative area of focus within functional food research. One of the main challenges in designing non-dairy probiotic food products is ensuring the viability of probiotic strains during storage and in the gastrointestinal tract. Probiotic foods must contain a minimum of 6 log CFU/g or mL of the probiotic strain. Recent studies have focused on novel food matrices that improve probiotic viability due to their exceptional nutritional properties (Shori, 2016; Perez *et al.*, 2021; Rasika *et al.*, 2021). Food matrices such as fruits, vegetables, plant-based milk, cereals, and legumes have been successfully utilized to deliver probiotics to consumers (Rasika *et al.*, 2021). Notably, low-moisture food matrices like chocolate or peanut butter have shown promising results as probiotic carriers (Coba *et al.*, 2019). However, only a limited number of studies have explored probiotic peanut butter (Klu *et al.*, 2012; 2014; Klu and Chen, 2015; Silva *et al.*, 2022; Akkuş, 2023).

Peanut butter and carob powder are noteworthy examples of functional plant-based products due to their rich compositions. Peanut butter, for instance, is considered a functional food owing to its high content of vitamin E, folate, coenzyme Q10, minerals, resveratrol, phenolic compounds, and flavonoids (Tounsi *et al.*, 2017; Coba *et al.*, 2019). Since peanut butter has low water activity (approx. 0.35) and a slightly acidic pH value (approx. 6.3), it is a shelf-stable, low-moisture food matrix with promising properties as a probiotic carrier (Coba *et al.*, 2019). Similarly, carob powder has unique functional properties and nutritional composition, including sugars, polyphenols, proteins, vitamins, and minerals. Particularly, it is rich in dietary fiber and polyphenols, making it a valuable functional ingredient (Tounsi *et al.*, 2017; Ceylan and Muştu, 2021). Carob powder is increasingly valued as a novel food ingredient

for non-dairy functional foods due to its prebiotic and stabilizing properties. Notably, peanut butter serves as an excellent matrix for probiotic cultures, supporting probiotic viability by encapsulating them like a coating material while enhancing their survival through its prebiotic components. Peanuts can stimulate the growth of *Lactobacillus* species and other beneficial gut bacteria, a key characteristic of prebiotics. The prebiotic potential of these nuts is largely attributed to their rich polyphenol content, as well as other bioactive compounds such as dietary fibers, healthy fats, and proteins (Coba *et al.*, 2019; Das and Hyderabadwala, 2019). Probiotic strains show high stability during long-term storage and in vitro gastrointestinal tract passage when embedded in peanut butter (Coba *et al.*, 2019). Carob powder presents great potential as a stabilizing and prebiotic agent in non-dairy functional foods due to its functional properties related to its chemical structure (Tounsi *et al.*, 2017; Petkova *et al.*, 2017; Solana *et al.*, 2021). The emulsifying and stabilizing properties of carob powder are linked to the oil and water binding capacity of polysaccharides and proteins in its composition. On the other hand, the prebiotic effects of carob powder are primarily attributed to its polysaccharide content, particularly the galactomannans and other soluble fibers. These polysaccharides are not fully digested in the human gastrointestinal tract, making them an ideal substrate for fermentation by gut microbiota (Petkova *et al.*, 2017; Basharat *et al.*, 2023; Arachchi *et al.*, 2025).

As a novel probiotic product, peanut butter has been evaluated by a few researchers. However, the available data on the production of probiotic peanut butter remains limited. Previous studies on probiotic peanut butter have mainly focused on the survival of probiotic cells (Klu *et al.*, 2012; 2014; Klu and Chen, 2015; Silva *et al.*, 2022; Akkuş, 2023). However, there is also a need to assess the quality characteristics of the product during storage. Additionally, there have been no previous studies on the production of probiotic peanut butter fortified with carob powder. This study examines the quality characteristics of peanut butter enriched with probiotics and carob powder over a 3-month storage period.

Materials and Methods

Probiotic culture, peanuts, carob powder

This study utilized *Lactocaseibacillus rhamnosus* GG (LGG; Chr. Hansen, Denmark) as the probiotic culture. Virginia-type NC-7 peanuts, sourced from the Republic of Türkiye Ministry of Agriculture and Forestry's Oil Seed Research Institute, served as the raw material. Carob powder was provided by a commercial supplier.

Preparation of probiotic culture

L. rhamnosus was cultured in de Man, Rogosa, and Sharpe (MRS) broth (Merck, Germany) by incubating for 24 h at 37°C. After incubation, the culture tubes containing *L. rhamnosus* were centrifuged at 7000 rpm for 5 min at 10°C. The cells were washed once with peptone water and then resuspended in peptone water to achieve the desired concentration. Optical density measurements were performed to ensure consistent cell concentration throughout the study. Peanut butter production was conducted using *L. rhamnosus* at a final concentration of 10^7 CFU/g, as described by Ünal Turhan *et al.* (2017a) described.

Peanut butter production

Peanut butter production was carried out with three replications. The samples produced in this study include plain peanut butter (K), peanut butter containing *L. rhamnosus* (A), and peanut butter containing *L. rhamnosus* and carob powder (B).

The production was conducted under aseptic conditions at the Republic of Türkiye Ministry of Agriculture and Forestry Oil Seed Research Institute. Figure 1 illustrates the steps involved in peanut butter production. The peanuts were first roasted in a peanut roasting machine (Practical Nuts-Ceselsan Makina) at a temperature of 175°C and a belt speed of 35 kg/h until the moisture content fell below 10%. After roasting, the outer membrane was peeled off using a professional blower machine, and the peanuts were then sent to the peanut butter machine (Karaerler Puree Machine) to be ground. The peanuts were ground in the butter machine until they reached a fluid paste consistency, resulting in peanut butter. This plain peanut butter was used to produce the control sample (K) and the probiotic samples (A and B). The control sample was obtained by directly filling plain peanut butter into glass jars without adding probiotics or carob powder. For the production of probiotic peanut butter with the A sample code, *L. rhamnosus* (10^7 CFU/g) was added to the plain peanut butter under aseptic conditions. The mixture was then filled into glass jars and mixed at room temperature for 15 min. In the production of probiotic peanut butter with the B sample code, 3% carob powder and *L. rhamnosus* (10^7 CFU/g) were added to the plain peanut butter in glass jars and mixed at room temperature for 15 min (Klu *et al.*, 2012; Tanrikulu, 2019). Tanrikulu (2019) reported that peanut butter was less appreciated in terms of sensory quality and consistency when carob powder was added above 5%. According to Tanrikulu (2019), adding 2.5% and 5% carob powder to peanut butter resulted in the highest sensory scores (ranging from 5.33 to 6.44) for attributes

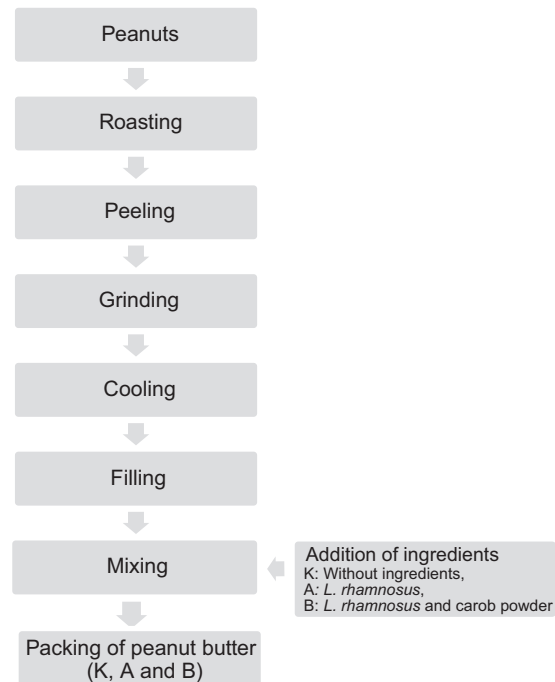


Figure 1. Flow chart of peanut butter production.

such as oiliness, viscosity, texture, stickiness, taste, and overall impression. In the preliminary experiments of this study, the addition of 5% carob powder resulted in a hard texture, while 3% carob powder provided an acceptable soft texture that allowed for homogeneous mixing of the additives (carob powder and probiotic culture). Therefore, a 3% carob powder concentration was chosen for this study. All samples were stored at 4°C for 3 months. In their previous study on probiotic peanut butter, Klu *et al.* (2012) reported that storage at 4°C was more suitable for preserving probiotic cell viability, so 4°C was selected as the storage temperature in this study.

The production processes of peanut butter, including growth, handling, storage, roasting, grinding, and stabilization (additives), significantly influence its quality attributes, such as flavor, color, and texture. Numerous efforts have been made to optimize the production processes of peanut butter, aiming to achieve superior sensory and biochemical quality (Sithole *et al.*, 2022). Roasting is one of the most critical steps in developing the flavor profile of peanut butter. The grinding process affects the texture of peanut butter, which is a key sensory attribute. To improve the flavor, consistency, and stability of peanut butter, various additives such as salt, sugar, emulsifiers, and stabilizers are often incorporated. The way peanut butter is packaged and stored can impact its sensory, biochemical, and microbiological quality, particularly over time (Sanders III *et al.*, 2014; Tanrikulu, 2019).

Microbiological and physicochemical analysis

Enumeration of *L. rhamnosus* was conducted to evaluate the survival of probiotic cells during storage and assess the prebiotic activity of carob powder. Additionally, physicochemical analyses of carob powder included measurements of pH, ash content, dry matter, oil and water binding capacities, emulsification activity, and emulsification index. In contrast, the analyses of peanut butter focused on pH, ash content, dry matter, and fat loss.

Determination of the prebiotic activity of carob powder

To determine the prebiotic activity of carob powder, 0%, 1%, 3%, and 5% carob powder were added to de Man, Rogosa, and Sharpe (MRS) broth (Merck-Germany), and the media with carob powder were sterilized at 121°C for 15 min. A 1% inoculum of the overnight culture of *L. rhamnosus*, incubated at 30°C for 18 h, was added to the sterile media with carob powder (0%, 1%, 3%, and 5%) and incubated at 30°C for 6 and 24 h. After incubation at different growth phases (6 and 24 h), samples were collected, appropriate dilutions were made, and a 0.1 mL sample was plated on MRS agar in duplicate. Plates were incubated under anaerobic conditions (Anaerocult A, Merck) for 48 h at 37°C. The results were expressed as log CFU/g (Zahid *et al.*, 2021; Luca *et al.*, 2017; Sousa *et al.*, 2015).

Enumeration of *L. rhamnosus*

Peanut butter was placed in 90 mL of peptone water (0.1%, Oxoid, UK) in a sterile stomacher bag and homogenized with a stomacher for 1 min at 10 strokes per second (Bagmixer, Interscience, France). The number of viable *L. rhamnosus* cells was determined by plating onto MRS agar and incubating under anaerobic conditions (Anaerocult A, Merck) for 48 h at 37°C (Ünal Turhan *et al.*, 2017a).

pH

For the pH measurement of carob powder, 1 g of the sample was dissolved in 20 mL of pure water by stirring, and the pH was then measured with a pH meter (Hanna Instruments) (Kasimoğlu, 2014). For the pH measurement of peanut butter, 5 g of the sample was dissolved in 50 mL of pure water, and the pH was measured (Silva *et al.*, 2022).

Ash

To determine the ash content in carob powder, 5 g of the sample was placed in a muffle furnace at 550°C for 5 h

and then weighed. To determine the ash content in peanut butter, 2 g of the sample was placed in a muffle furnace at 525°C for 4 h and then weighed (AOAC, 2005).

Dry matter

To determine the dry matter in carob powder, 0.5 g of the sample was weighed and left at 70°C. For peanut butter, 2 g was weighed and left at 105°C (AOAC, 2005).

Water and oil retention capacity

The water retention capacity of carob powder was determined by mixing 0.5 g of carob powder with 20 mL of distilled water, followed by centrifugation at 5000 rpm for 30 min. After centrifugation, the supernatant was discarded, and the water retention capacity was expressed in grams of water per gram of dry matter. Similarly, the oil retention capacity was measured by mixing 0.5 g of carob powder with 10 mL of sunflower oil and centrifuging at 5000 rpm for 20 min. After centrifugation, the supernatant was removed, and the oil retention capacity was recorded in grams of oil per gram of dry matter (Tounsi *et al.*, 2017).

Emulsion activity and emulsification index

Two methods were employed to evaluate carob powder's emulsifying properties: emulsion activity and emulsification index. Emulsion activity was measured by suspending 1 g of carob powder in 10 mL of distilled water and adding 10 mL of sunflower oil. The water/oil mixture was homogenized for 1 min and centrifuged at 4500 rpm for 20 min. The emulsion activity (%) was calculated as the ratio of the emulsion layer's height to the total height of the mixture (Chandra *et al.*, 2013).

For the emulsification index, 10 g of carob powder was mixed with 10 mL of water for 20 min and then centrifuged. Equal volumes of the resulting supernatant and sunflower oil were mixed and homogenized using a Waring blender. The homogenized mixture was transferred into a measuring cylinder and left undisturbed for 24 h. The emulsification index (%) was determined as the ratio of the emulsified layer's height to the total height of the mixture (Çağlar *et al.*, 2012).

Oil loss

Peanut butter was placed into centrifuge tubes and centrifuged (10,000 g, 15 min) at 25°C. The surface oil was removed after centrifugation, and the residual peanut

butter was weighed. The oil loss (%) was calculated as the ratio of the weight of the surface oil to the initial weight of the peanut butter (Huang *et al.*, 2020).

Sensory analysis

Peanut butter was assessed based on sensory attributes, including aroma, color, taste, texture, spreadability, and overall impression. The trained panelists consists of 5 females and 5 males, aged between 20 and 40, from the staff of the Department of Food Technology at University of Osmaniye Korkut Ata. During the sensory assessment, the room was maintained at a temperature of 20–25°C and relative humidity of 50–55%. A 10 g sample of each peanut butter was placed in a plastic plate and served with water for sensory evaluation. Panelists evaluated the samples independently with random blinding codes (three-digit random numbers) using quantitative descriptive analysis. A 9-point hedonic scale, ranging from 1 (dislike extremely) to 9 (like extremely), was employed to measure sample acceptability, where 1 indicated a very poor rating and 9 indicated an excellent rating. The total attribute scores for each sample were tallied, and the mean values were determined (Degon *et al.*, 2021).

Statistical analysis

The analysis was performed with three replications and two parallels. All results are presented as averages and were evaluated using the SPSS Version 15.0 statistical software (SPSS Inc., USA). Differences between results were assessed through variance analysis (Duncan's Multiple Comparison Test) at a 95% confidence level. Pairwise comparisons were conducted using the t-test.

Results and Discussion

The food matrix is one of the most effective factors in the viability of probiotic cultures during storage and in the gastric system following consumption (Ünal Turhan *et al.*, 2017a, b). Peanut butter is recognized as an effective probiotic carrier due to its low water activity (0.35), near-neutral pH value (6.3), and nutrient composition, which includes lipids (40%–50%), proteins (27%–29%), carbohydrates (16%), and dietary fiber (8.5%) (Coba *et al.*, 2019). Similarly, carob powder exhibits prebiotic properties that support the viability of probiotics due to its dietary fiber content, including oligosaccharides, galactooligosaccharides, fructooligosaccharides, and fructones. Carob powder also has the potential to enhance the technofunctional properties of foods (Solana *et al.*, 2021). In this study, probiotic peanut butter and peanut butter containing carob powder were developed as

functional foods, as both peanut butter and carob powder are suitable matrices for promoting the growth of probiotics. First, the quality characteristics of the carob powders intended for incorporation into probiotic peanut butter were evaluated. Subsequently, the quality characteristics of all peanut butter samples were determined during a three-month storage period. In addition to its prebiotic effects, carob powder was also considered a stabilizing agent that contributes to a more stable emulsion structure in peanut butter (Petkova *et al.*, 2017).

Chemical and technofunctional quality properties of carob powder

Carob powder is widely incorporated into the production processes of various food products as a functional ingredient to enhance their nutritional value. It has been utilized as a food additive in bakery items, ice cream, dessert bars, and confectionery. For instance, adding carob powder to biscuits results in a product with improved health benefits. Moreover, carob powder has been used as a novel ingredient in pasta, tarhana, and specific diet products as a food supplement (Şener, 2022). The polysaccharide isolated from carob flour has been characterized as galactomannan. This carob galactomannan demonstrates promising water solubility (84%) and a higher oil-holding capacity (4.0 g oil/g sample) compared to its water-holding capacity (1.4 g water/g) (Petkova *et al.*, 2017). Table 1 presents some of the chemical and technofunctional properties of carob powder.

The average chemical composition of carob powder used in this study was 5.16 pH, 2.57% ash, and 95.46% dry matter. This low moisture content, in particular, supports the stability and extends the shelf life of the product (Eldeeb and Mosilhey, 2022). In accordance with these data, previous studies have reported the pH to range from approximately 5.2 to 5.7, dry matter content to vary between 90% and 97%, and ash content to range from 2.20% to 7.2% in carob powder samples (Çağlar *et al.*, 2012; Kasımoğlu, 2014; Petkova *et al.*, 2017; Eldeeb

Table 1. Chemical and technofunctional properties of carob powder.

Chemical and technofunctional properties	Results
pH	5.16±0.00
Ash (%)	2.57±0.01
Dry matter (%)	95.46±0.02
Oil retention capacity (g/g)	2.48±0.69
Water retention capacity (g/g)	2.42±0.33
Emulsion activity (%)	47.67±1.64
Emulsification index ₂₄ (%)	45.35±1.65

and Mosilhey, 2022). The chemical properties of carob powder generally depend on the variety and the roasting process (Eldeeb and Mosilhey, 2022). Carob powder has been commercially evaluated as a food supplement due to its nutritional value (Petkova *et al.*, 2017).

Emulsions are a critical component in processed food formulations. Forming an emulsion requires energy to break and mix the oil and water phases. Emulsifying properties are assessed by measuring emulsion activity, emulsification index, emulsification stability, and emulsification stability index (Nartea *et al.*, 2023). In this study, carob powder exhibited an oil retention capacity of 2.48 g/g, a water retention capacity of 2.42 g/g, an emulsion activity of 47.67%, and an emulsification index of 45.35% (Table 1). The emulsion activity and emulsification index are commonly used to evaluate the emulsifying properties of various food-grade powders or flours (Çağlar *et al.*, 2012; Chandra *et al.*, 2013). The emulsion activity and emulsification index analyses were similar, supporting that both methods can assess emulsifying properties. Emulsion and oil retention capacity may vary depending on the flour or powder derived from plant products (Çağlar *et al.*, 2012; Chandra and Samsher, 2013). For example, emulsion activities of wheat flour, rice flour, green gram flour, and potato flour were stated to be 43.88%, 4.119%, 41.48%, 41.17% and 39.05%, respectively (Chandra and Samsher, 2013).

Carob gum (E 410), derived from carob, is used as a thickener, gelatin, and water binder due to its mannose and galactose content (Uyandran, 2020). Legume flours, characterized by relatively low starch and high protein content, exhibit high oil-binding and emulsification properties. Emulsification activity refers to the ability of these flours to form stable emulsions through protein dispersion in the presence of both oil and water phases. On the other hand, emulsion stability denotes the strength or durability of the formed emulsion. Legumes and other plant-based flours are frequently added to various food products because of their emulsifying properties (Nawaz *et al.*, 2021). Moreover, mixtures of legume flours can be used to impart specific technofunctional properties to foods. For instance, a mixture of wheat flour, corn flour, and chickpea flour resulted in an emulsification activity of 34.57% (Nawaz *et al.*, 2021).

Effect of carob powder on *L. rhamnosus* growth

Various plant-based products serve as sources of dietary prebiotics, or nondigestible oligosaccharides and polysaccharides, which stimulate the growth of beneficial bacteria, including Lactobacilli and Bifidobacteria. The primary components of plant-based prebiotics include fructooligosaccharides, glycoligosaccharides, and inulin.

Many plant-derived products, such as cereals, legumes, seeds, roots, fruits, vegetables, and leaves, contain prebiotic components that support the growth of probiotics (Althubiani *et al.*, 2019). The prebiotic effect of carob was attributed to dietary fiber and galactomannan (Basharat *et al.*, 2023; Arachchi *et al.*, 2025).

Table 2 presents the effect of carob powder at different concentrations on the survival of *L. rhamnosus*. Probiotic viability in media without carob powder ranged from 7.54 to 9.32 log CFU/mL, whereas media containing carob powder exhibited values between 8.04 and 12.47 log CFU/mL. Adding carob powder significantly enhanced probiotic viability ($p<0.05$). Samples incubated for 24 h showed greater probiotic growth than those incubated for 6 h, with the incubation time exerting a statistically significant effect on probiotic growth ($p<0.05$). The highest differences (additional 4.36 log CFU/mL growth for 24-h-incubated sample) in probiotic cell viability between samples for 6- and 24-h incubation were obtained from samples grown in MRS broth with 5% carob powder ($p<0.05$). Among the 6-h incubation samples, the 1% and 3% carob powder concentrations resulted in higher probiotic growth than the 5% and 10% concentrations ($p<0.05$). At the 6-h incubation, the differences between adding 1% and 3% carob powder, as well as the differences between 5% and 10% carob powder, were statistically insignificant ($p>0.05$). Among the 6-h incubation samples, the lowest probiotic cell growth (7.54 log CFU/mL) was observed in MRS broth ($p<0.05$). Similarly, after a 24-h incubation, the probiotic cell growth in MRS broth was significantly lower (9.32 log CFU/mL) compared to MRS broth supplemented with carob powder ($p<0.05$). At the 24-h incubation, there were no statistically significant differences ($p>0.05$) among the samples containing carob powder, except for the sample with 3% carob powder, which showed a statistically significant difference ($p<0.05$).

Table 2. Effect of carob powder on the growth of *L. rhamnosus*.

Media	Viable cell counts of <i>L. rhamnosus</i> (log CFU/mL)	
	6-h incubation	24-h incubation
MRS broth	7.54±0.09 ^{Ac}	9.32±0.03 ^{Bc}
MRS broth with 1% carob powder	8.91±0.09 ^{Aa}	12.44±0.05 ^{Ba}
MRS broth with 3% carob powder	8.82±0.06 ^{Aa}	12.19±0.04 ^{Bb}
MRS broth with 5% carob powder	8.04±0.03 ^{Ab}	12.40±0.10 ^{Ba}
MRS broth with 10% carob powder	8.18±0.09 ^{Ab}	12.47±0.01 ^{Ba}

A-B: Differences between values shown by different letters within the same row are statistically significant ($p<0.05$).

a-b: Differences between values shown by different letters within the same column are statistically significant ($p<0.05$).

High concentrations of carob powder may initially inhibit probiotic growth during the lag phase, but adaptation to the environment may occur over time (Turhan *et al.*, 2017a,b; Broeckx *et al.*, 2020). As shown in the current data, the promoting effect of carob powder on cell growth at 6-h incubation was lower than that at 24-h incubation ($p < 0.05$). The probiotic bacterial population in growth media increased in MRS broth, growing from 7.54 log CFU/mL to 9.32 log CFU/mL as incubation time increased from 6 to 24 h. Similarly, in a previous study, when *L. rhamnosus* GG was grown to mid-log phase (6–7 h) and stationary phase (17–24 h), cell viability of bacteria grown for 6 and 17 h in MRS broth was found to be 8.71 and 9.12 log CFU/mL, respectively (Broeckx *et al.*, 2020).

The presence of carob powder in the medium induced the growth of *L. rhamnosus*, exhibiting a prebiotic effect. Prebiotic additives added to the MRS medium have increased the viability of probiotic cultures such as *L. rhamnosus* (Luca *et al.*, 2017). In addition to artificial additives, the inclusion of natural plant-based ingredients, such as chamomile (*Smallanthus sonchifolius*) tuber flour, fig powder, fruit peel flours (e.g., apple, banana, and mango peel), and fruit extracts (e.g., blueberry, banana), also produces a prebiotic effect on lactic acid bacteria (Sousa *et al.*, 2015; Thakkar and Preetha, 2016; Zahid *et al.*, 2021). In the study of Sousa *et al.* (2015), yacon tuber flour promoted the growth of *Enterococcus faecium*, *Bifidobacterium animalis*, *Lactobacillus acidophilus*, and *Lactobacillus casei*, probably due to its fructooligosaccharides (FOS) content. In another study, fig powder was utilized as a potential prebiotic for *Lactobacillus* spp., and an increased viability was attributed to the presence of resistant starch (Thakkar and Preetha, 2016). According to Zahid *et al.* (2021), fruit peel (apple, banana, and mango) powder at different concentrations (2% and 4%) enhanced the number of probiotic strains, including *L. rhamnosus*, *L. casei*, and *Bifidobacterium lactis* (>10 logs) after 24 h of incubation. Similarly, in this study, adding carob powder, a natural food ingredient, to the MRS medium led to higher probiotic viability (max. 12.47 log CFU/mL). In accordance with this, Hariri *et al.* (2017) reported that carob powder caused an increase in the cell mass of *L. bulgaricus*.

Chemical and technofunctional properties of peanut butter during storage

Additives derived from plant sources are commonly incorporated into food formulations, providing various technofunctional properties such as water retention, oil retention, foam formation, and emulsion stability. These properties are crucial for maintaining food quality and safety throughout storage (De Angelis *et al.*, 2024). Stabilizers are widely utilized in the production of bakery,

dairy, meat, vegetable, and fruit products to enhance shelf life and improve textural attributes. Natural stabilizers can be sourced from seaweeds (e.g., agar, alginate, carrageenan), seed gums (e.g., guar gum, carob seed gum, tara gum), tree exudates (e.g., gum arabic, karaya gum), plant extracts (e.g., pectins and glucomannans), animal products (e.g., gelatin, caseinate), and microbial sources (e.g., xanthan gum, gellan gum). Modified stabilizers are produced through the acid, alkali, enzymatic, or alcoholic modification of starch and cellulose. Examples of modified celluloses include carboxymethyl cellulose, hydroxypropyl cellulose, methylcellulose, microcrystalline cellulose, and dextrins, while modified starches include acid- or alkali-treated starch, enzyme-treated starch, and oxidized starch (Kumar *et al.*, 2021).

Table 3 illustrates the impact of storage period and production methods on peanut butters' chemical and technofunctional properties.

The pH values of plain peanut butter, peanut butter with carob powder, and those without varied in the range of 6.60–6.72, 6.43–6.58, and 6.53–6.69, respectively. These results suggest that probiotic culture led to lower pH values. Furthermore, a decrease in pH was observed in samples A and B, which contained probiotic cultures, as storage time increased. The effect of carob powder and probiotic culture addition was found to be significant ($p < 0.05$) on pH values of peanut butter samples. In line with these findings, Akkuş (2023) reported a pH range of 6.27–6.66 in peanut butter containing probiotic culture and noted a decline in pH over time. This pH reduction was attributed to the probiotic activity affecting the chemical composition of the peanut butter (Akkuş, 2023). The effect of storage time on pH values was found to be significant ($p < 0.05$) for samples A and B but not for sample K ($p > 0.05$). The inclusion of carob powder in sample B provided a carbohydrate source for the probiotic *L. rhamnosus*, leading to a reduction in pH due to the fermentation of this carbohydrate, albeit at a limited level. Carob powder contains approximately 45% carbohydrate (Tanrikulu, 2019). At the onset of storage, the pH values of all samples were statistically different, a result attributed to the varying chemical compositions of the peanut butter samples. During the first month of storage, sample B was statistically distinct from samples A and K. In the second and third months, sample K was statistically different ($p < 0.05$) from samples A and B.

Ash content is a quality characteristic that typically remains stable during storage, averaging around 2.5% (Davis and Dean, 2016). In this study, it was assumed that ash content would not change throughout the storage period. Consequently, the analysis values for ash were reported based on the first day of storage (0th month). The ash content of the peanut butter samples ranged

Table 3. Chemical and technofunctional properties of peanut butter.

Quality Properties	Sample	0 th month	1 st month	2 nd month	3 rd month
pH	A	6.68±0.01 ^{aA}	6.67±0.01 ^{aA}	6.69±0.01 ^{aA}	6.53±0.12 ^{baA}
	B	6.56±0.01 ^{cBA}	6.53±0.01 ^{bb}	6.58±0.01 ^{cA}	6.43±0.03 ^{bc}
	K	6.60±0.00 ^{bc}	6.66±0.04 ^{aB}	6.72±0.01 ^{ba}	6.67±0.01 ^{aB}
Ash (%)	A	2.57±0.02 ^{aA}	2.57±0.02 ^{aA}	2.57±0.02 ^{aA}	2.57±0.02 ^{aA}
	B	2.60±0.05 ^{aA}	2.60±0.05 ^{aA}	2.60±0.05 ^{aA}	2.60±0.05 ^{aA}
	K	2.59±0.06 ^{aA}	2.59±0.06 ^{aA}	2.59±0.06 ^{aA}	2.59±0.06 ^{aA}
Dry matter (%)	A	98.37±0.18 ^{aBA}	98.20±0.74 ^{aBA}	97.31±0.21 ^{cB}	98.92±0.29 ^{aA}
	B	97.85±0.45 ^{aA}	98.17±0.28 ^{aA}	97.86±0.14 ^{ba}	98.32±0.06 ^{ba}
	K	98.32±0.66 ^{aA}	98.51±0.15 ^{aA}	98.61±0.16 ^{aA}	99.07±0.06 ^{aA}
Oil loss (%)	A	4.95±0.29 ^{aA}	4.07±0.71 ^{aA}	3.69±0.10 ^{baA}	3.86±0.71 ^{baA}
	B	2.79±0.40 ^{ba}	3.26±0.49 ^{ba}	2.80±0.44 ^{ba}	2.53±0.10 ^{ba}
	K	3.69±0.15 ^{baA}	4.06±1.00 ^{aA}	4.18±0.31 ^{aA}	4.30±0.59 ^{aA}

A–B: The difference between the values shown by different letters in the same line is statistically significant ($p<0.05$).

a–b: The difference between the values shown by different letters in the same column is statistically significant ($p<0.05$).

from 2.57% to 2.60%. According to Turkish standards, peanut butter's maximum allowable ash content is 3%. The addition of carob powder did not alter the total ash content. However, it is possible that the mineral composition of the samples varied, a hypothesis that should be verified through mineral analysis in future studies.

Moisture content is a critical factor influencing food products' stability and shelf life (Akkuş, 2023). Peanut butter is one of the shelf-stable low-moisture food products. The low-moisture peanut butter matrix also presents promising properties as a probiotic carrier (Coba *et al.*, 2019). In this study, the dry matter content of samples K, A, and B varied in the range of 98.32–99.07%, 97.85–98.32%, and 97.31–98.92%, respectively. The effect of carob powder and probiotic culture addition was found to be significant ($p<0.05$) on dry matter of peanut butter samples for 2- and 3-month storage, while it was insignificant ($p>0.05$) for 0- and 1-month storage. The effect of the storage period was mostly found to be insignificant ($p>0.05$) on dry matter for all peanut butters. While no significant change in dry matter was observed in samples B and K during storage, a change was noted in sample A. Additionally, no statistically significant difference was found in the dry matter content of the samples between the 0th and 1st months. However, by the second month, the dry matter content of all samples differed significantly. By the third month, the dry matter content of sample B was significantly different from samples A and K. Similar findings were reported by Akkuş (2023), who found the dry matter content of peanut butter with psychobiotics to range between 97% and 98.5%.

The lowest oil separation and spreadability are the main characteristics prioritized in the development and

formulation of peanut butter products for consumer satisfaction. In the food industry, especially in the production of peanut butter, researchers have explored various stabilizers to improve the product's spreadability and minimize oil separation (Ferdaus *et al.*, 2022). Storage conditions such as temperature and time may affect oil separation of peanut butter. Rozalli *et al.* (2016) stated that oil separation was less at 10°C (maximum 0.35%) storage than at 25 and 35°C storage. In this study, oil loss values in samples K, A, and B ranged from 3.69–4.30%, 3.69–4.95%, and 2.53–3.26%, respectively. No statistically significant changes in oil loss were observed across storage time ($p>0.05$). However, the effect of carob powder and probiotic culture addition was found to be significant ($p<0.05$) on oil loss of peanut butter samples. The lowest oil loss was consistently observed in sample B, which caused poor spreadability. The present data revealed that carob powder acted as a natural stabilizer and provided less oil loss compared with the control sample. The high emulsifying capacity of additives indicates that they also have good oil-binding properties. Carob powder, a plant-derived flour, possesses oil retention and emulsification properties (Petkova *et al.*, 2017). In the presence of carob powder, the oil in peanut butter was likely bound by the powder, resulting in reduced oil loss. Similar results have been reported by other researchers who used natural waxes (such as rice bran and carnauba wax) as stabilizers in peanut butter, achieving lower oil loss (1.09–4.04%) in these samples (Huang *et al.*, 2020; Ferdaus *et al.*, 2022). In a study of Ferdaus *et al.* (2022), 1%, 1.5%, and 2% of three natural waxes (rice bran, carnauba, and beeswax) were added to natural peanut butter. Bran wax provided the lowest oil separation (0.87%) and demonstrated the potential replacement of fully hydrogenated oil as a stabilizer. Similarly, Huang *et al.* (2020) reported that the

addition of rice bran wax (RBX) into peanut butter as a stabilizer improved stability. The oil loss of peanut butter with RBX decreased from 12.19% to 4.04%. It has also been noted that adding food-derived natural polymers such as methylcellulose and hydroxyethylcellulose improves peanut butter's stability and textural properties (Tanti *et al.*, 2016). Probiotic cultures also play a role in emulsification, mainly through their ability to produce exopolysaccharides (Angelin and Kavitha, 2020). When examining the oil loss data, lower oil loss was observed in samples A and B containing probiotic cultures than in samples without probiotic cultures during the second and third months of storage. This observation is likely due to the exopolysaccharides produced by the probiotic cultures during storage, which function as emulsifying agents (Angelin and Kavitha, 2020). Rajoka *et al.* (2018) reported that exopolysaccharides produced by *L. rhamnosus* possess emulsifying properties. Additionally, Dikkala *et al.* (2024) found that incorporating peanut shells into peanut butter enhanced its functional and nutritional properties without negatively affecting sensory quality. Their research also indicated that adding peanut shells increased viscosity and hardness while reducing spreadability and oil separation compared to the control. In another study, *Curcuma Longa* (turmeric) industrial byproducts used as stabilizers demonstrated an effective decrease in oil separation compared to control peanut butter (Praveen *et al.*, 2024).

Survival of *L. rhamnosus* in peanut butter during storage

Fat content, concentration, type of proteins, sugars, and pH of the product are some factors that could affect probiotic growth and food survival (Ranadheera *et al.*, 2010). Due to their nutritional or compositional properties, peanut butter and carob powder are beneficial probiotic carrier food matrices for delivering probiotic bacteria to the human gastrointestinal tract (Coba *et al.*, 2019; Das and Hyderabadwala, 2019).

Peanut butter is an excellent medium for supporting probiotic cultures, offering a dual benefit to both the probiotics and the gut microbiome. The rich, creamy texture of peanut butter can encapsulate probiotic cultures, acting

as a protective coating that helps shield the probiotics from harsh digestive conditions such as stomach acidity. This ensures that a higher proportion of the probiotics reach the gut alive, where they can provide their health benefits (Coba *et al.*, 2019; Klu *et al.*, 2012 and 2014). Moreover, peanut butter contains a variety of prebiotic components that can stimulate the growth and activity of beneficial gut bacteria, such as *Lactobacillus* species. Prebiotics are non-digestible compounds that serve as food for these probiotics, promoting their proliferation and activity in the environment (the intestines or food products). The prebiotic potential of peanuts is largely attributed to their bioactive compounds, which include polyphenols, dietary fibers, healthy fats, and proteins (Das and Hyderabadwala, 2019; Silva *et al.*, 2022).

Carob powder, derived from the pod of the carob tree (*Ceratonia siliqua*), contains a variety of polysaccharides, with galactomannans being the most notable. These polysaccharides, which include galactose and mannose sugar units, are considered a type of soluble fiber. They contribute to the prebiotic effects of carob powder. The galactomannans and soluble fibers from carob are especially effective at promoting the growth of beneficial bacteria such as Bifidobacteria and Lactobacilli (Petkova *et al.*, 2017; Basharat *et al.*, 2023; Arachchi *et al.*, 2025).

In this study, the number of *L. rhamnosus* in peanut butter samples during storage is shown in Table 4. While no probiotic cell viability was detected during the storage process in the plain peanut butter, the number of *L. rhamnosus* was found to be 5.95–6.45 log CFU/g in peanut butter without carob powder and 6.15–6.82 log CFU/g in peanut butter with carob powder. A critical limit regarding probiotic viable cell number is 6 log CFU/g or mL for probiotic food products (Shori, 2016). As seen from results, probiotic peanut butter samples (A and B) contained approx. 6 log CFU/g of probiotic *L. rhamnosus*. The effect of addition of carob powder into peanut butter was found insignificant in the 1st and 2nd months ($p>0.05$) while found significant in the 3rd month ($p<0.05$). The storage period had a significant effect on probiotic cell viability for sample B ($p<0.05$). In contrast, the effect of storage period on sample A was insignificant ($p>0.05$).

Table 4. Viable cell counts of *L. rhamnosus* in peanut butter (log CFU/g).

Sample	0 th month	1 st month	2 nd month	3 rd month
A	6.17±0.10 ^{Aa}	5.96±0.16 ^{Aa}	5.95±0.35 ^{Aa}	6.45±0.05 ^{Ab}
B	6.15±0.04 ^{Ba}	6.24±0.11 ^{Ba}	6.20±0.08 ^{Ba}	6.82±0.11 ^{Aa}
K	nd	nd	nd	nd

A–B: The difference between the values shown by different letters in the same line is statistically significant ($p<0.05$).

a–C: The difference between the values shown by different letters in the same column is statistically significant ($p<0.05$).

A: Probiotic peanut butter without carob powder; B: Probiotic peanut butter with carob powder, K: Plain peanut butter powder, nd: not detected.

As mentioned before, probiotic food must contain at least 6 log CFU/g of probiotic microorganisms (Turhan *et al.*, 2017b). During the 3-month storage period, the number of live *L. rhamnosus* in our samples changed from 5.95 to 6.82 log CFU/g. The number of live probiotic bacteria in sample A showed a very slight decrease in the 1st and 2nd months and a very slight increase in the 3rd month. However, these changes were statistically insignificant ($p>0.05$). There are no significant differences among *L. rhamnosus* counts of sample B in the 0th, 1st, and 2nd months. In contrast, sample B had a higher *L. rhamnosus* viability in the 3rd month compared to the other months. It was considered that the presence of carob powder in peanut butter supported the probiotic viability. Similarly, in a study by Abeer *et al.* (2014), probiotic frozen yogurt was produced by adding different levels of carob powder (1, 3, 5, 7, and 9%). In that study, treatment with 3% carob powder caused higher probiotic viability.

There are limited studies specifically focusing on probiotic peanut butter. Most of the research conducted thus far has concentrated on the survival of probiotic strains throughout storage. Klu *et al.* (2014) observed a decrease in probiotic viability of peanut butter-containing probiotic cultures by approximately 0.20 log CFU/g in samples stored at 4°C, approximately 0.50 log CFU/g in samples stored at 25°C, and 1 log CFU/g in samples stored at 37°C after 12 weeks of storage. In line with our results, very few drops in probiotic cell viability were detected at 4°C. Similarly, various previous researchers confirmed that storage conditions at 4°C provided better protection for probiotic bacteria in peanut butter compared to those at 25°C and 37°C (Klu *et al.*, 2012; Akkuş *et al.*, 2023; Kruk *et al.*, 2024).

According to Salaheen *et al.* (2014), peanut fractions positively affected the growth of *L. casei* due to the high oleic acid content of peanuts. Min *et al.* (2017) investigated the stability of *Lactobacillus acidophilus* ATCC4356T, *Lactobacillus plantarum* RC30, and *Bifidobacterium longum* ATCC15707T in various plant products (rice, wheat, coconut, oat, raisin, peanut) and determined that peanut was the best matrix in terms of preserving probiotic viability under all storage conditions (20 and 30°C, 20% and 50% humidity conditions). In another study, peanut butter's quality improved due to adding *Bifidobacterium animalis* and guarana peel extract (Silva *et al.*, 2022).

Sensory properties of peanut butter during storage

Plain peanut butter exhibits a color ranging from light yellow to dark yellow, with a characteristic peanut-like odor and a strong peanut flavor. The texture varies from a small to a completely crushed appearance. The sensory

properties of both plain and probiotic peanut butter during storage are presented in Table 5. Overall, no significant differences were observed among the peanut butter samples in terms of sensory quality ($p>0.05$). In particular, the effect of storage duration on texture and overall impression was insignificant ($p>0.05$). All peanut butter samples received sensory scores ranging from 6.88 to 8.50, indicating that both probiotic peanut butter, with and without carob powder, are equally acceptable to consumers as plain peanut butter.

In terms of aroma, no significant difference ($p>0.05$) was observed between peanut butter samples except for B-0 and B-2 samples. In general, the storage period, the addition of carob powder, and the probiotic did not affect the aroma properties of peanut butter samples. Sample B had the lowest score (6.88) for aroma among the peanut butter samples in the 0th month. Interestingly, however, sample B achieved the highest score (8.75) for taste in the third month. The aroma properties of sample B improved over time, becoming more favorable as the storage duration increased. The initially low aroma score for sample B in the 0th month was attributed to a strong aroma specific to the carob powder. As storage time progressed, the aroma of sample B became more integrated with the peanut butter, resulting in an overall improvement. As shown in the current data, the addition of carob powder caused the lowest score in terms of aroma, meaning that plain peanut butter is generally perceived as more favorable. Similarly, in a previous study, onion skin powder (6% and 3%) decreased the flavor, sweetness, and overall acceptance compared to the control (Kırkın *et al.*, 2021).

Peanut butter color has important quality implications due to its association with both desirable and undesirable aroma and taste. The characteristic golden-brown color attributed to melanin produced due to the Maillard browning reaction is associated with desirable flavors and aromas, whereas dark colors are associated with burnt flavors (Sanders III *et al.*, 2014). In terms of color, there were no statistical differences ($p>0.05$) among peanut butter samples except for the B0 sample. The color of peanut butter with carob powder changed to a favorable brown color due to the increased dispersion of brown pigments during the 3-month storage period. Carob flour ranges from pale to dark brown pigments depending on Maillard and caramelization reactions during roasting and storage (Eldeeb and Mosilhey, 2022). Particularly, sugar caramelization can contribute to the browning reaction of peanuts. Tannins and catechol-type compounds also contribute to the color (Ma *et al.*, 2013). In line with the present study, Ma *et al.* (2013) stated that incorporation of peanut skin into peanut butter created a darker, browner peanut butter. Similarly, Sanders III *et al.* (2014) confirmed that peanut butter with added peanut skin was darker with less saturated color. The brown color

Table 5. Sensory properties of peanut butter during storage.

Sample	Aroma	Colour	Taste	Texture	Spreadability	Overall impression
K-0	8.50±0.76 ^a	8.00±0.76 ^{ba}	8.25±1.04 ^{ba}	8.13±0.99 ^a	8.38±0.38 ^{ba}	8.25±0.89 ^a
A-0	8.63±0.52 ^a	8.38±0.92 ^a	8.00±0.76 ^{ba}	8.25±0.89 ^a	8.38±0.74 ^{ba}	8.00±0.76 ^a
B-0	6.88±0.83 ^c	7.50±1.20 ^b	8.00±0.93 ^{ba}	7.88±0.35 ^a	7.38±0.74 ^c	7.75±1.16 ^a
K-1	8.50±0.53 ^a	8.25±0.89 ^{ba}	8.25±0.89 ^{ba}	7.75±1.16 ^a	8.25±0.89 ^{cba}	7.88±1.25 ^a
A-1	8.25±0.89 ^{ba}	8.25±0.89 ^{ba}	8.00±1.07 ^{ba}	8.00±0.93 ^a	8.50±0.53 ^a	8.13±0.83 ^a
B-1	8.00±0.00 ^{ba}	8.25±0.89 ^{ba}	8.50±0.53 ^a	8.25±0.46 ^a	7.75±0.89 ^{cba}	8.25±0.46 ^a
K-2	8.50±0.53 ^a	8.25±0.46 ^{ba}	8.50±0.53 ^a	8.25±0.46 ^a	8.25±0.88 ^{cba}	8.25±0.88 ^a
A-2	8.50±0.53 ^a	8.25±0.46 ^{ba}	8.50±0.53 ^a	8.25±0.46 ^a	8.25±0.46 ^{cba}	8.25±0.88 ^a
B-2	7.50±0.92 ^{cb}	8.25±0.46 ^{ba}	7.50±0.92 ^b	7.75±0.88 ^a	7.50±1.19 ^{cb}	8.00±1.06 ^a
K-3	8.25±0.46 ^{ba}	8.50±0.53 ^a	8.50±0.53 ^a	8.25±0.46 ^a	8.50±0.53 ^a	8.00±0.76 ^a
A-3	8.25±0.46 ^{ba}	8.50±0.53 ^a	8.50±0.53 ^a	8.25±0.89 ^a	8.25±0.89 ^{cba}	8.00±1.07 ^a
B-3	8.00±1.06 ^{ba}	8.25±0.46 ^{ba}	8.75±0.46 ^a	8.25±1.39 ^a	7.50±1.20 ^{cb}	8.00±0.76 ^a

a–b: The difference between the values shown by different letters in the same column is statistically significant ($p < 0.05$).

A: Probiotic peanut butter without carob powder; B: Probiotic peanut butter with carob powder, K: Plain peanut butter powder. 0: 0-month storage, 1: 1-month storage, 2: 2-month storage, 3: 3-month storage.

of sample B, attributed to the presence of carob powder, was not well-received by some panelists. Carob powder is commonly used as a chocolate substitute due to its color and taste and is frequently included in cocoa-like products (Youssef *et al.*, 2013; Cepo *et al.*, 2014). Additionally, carob powder is used in gluten-free food production for its flavoring, nutritional, and coloring properties (Ceylan and Muştu, 2021). However, in this study, the color of sample B, which deviated from the typical appearance of peanut butter, led to a negative consumer perception.

In terms of taste, there were no statistical differences ($p > 0.05$) among peanut butter samples except for the B2 sample. In the 2nd month, peanut butter with carob powder (B2 sample) received lower acceptance due to perceptions of bitterness and off-flavors. Carob has nutty and chocolate-like aromas. It contains high amounts of dietary fiber and polyphenols (hydrolyzable tannins, derived from gallic acid and condensed tannins, derived from flavan-3-ol, anthocyanidines, and flavan-3,4-diol) (Petkova *et al.*, 2017). Phenolic compounds in carob powder contribute to its bitterness and astringency. On the other hand, carob powder is a natural sweetener due to its high sugar content and is also notable for its high dietary fiber content. While compounds specific to carob powder may not appeal to all consumers, they are preferred by some due to their cocoa-like qualities and natural sweetening properties (Petkova *et al.*, 2017; Eldeeb and Mosilhey, 2022). This means that taste perception of consumers may be variable for the developed peanut butter products (Praveen *et al.*, 2024).

Texture is a multi-parameter attribute that is primarily evaluated in the mouth. Textural properties of peanut

butter include spreadability, firmness, and adhesiveness. The perception of product texture is noticed during mastication and swallowing, whereas spreadability is the ease with which a sample spreads over a surface in a thin, uniform layer. A soft texture of peanut butter ensures an easily spreadable product (Sanders III *et al.*, 2014). Although there were no statistical differences ($p > 0.05$) among all peanut butter samples in terms of texture, the values of spreadability were significantly ($p < 0.05$) different. Since peanut butter is typically consumed by spreading on bread, its spreadability is critical. It is expected that peanut butter should be spreadable without damaging the bread (Tanrikulu, 2019). Regarding spreadability, peanut butter samples received scores ranging from 7.38 to 8.50. Sample B consistently received the lowest score across all months. Adding carob powder made Sample B's spreadability more difficult, as it increased the consistency and solidified the texture of the peanut butter. As mentioned before, carob powder has oil-binding properties (Petkova *et al.*, 2017). Therefore, lower oil loss was observed in peanut butter samples with carob powder (Table 3), which resulted in a firmer texture in peanut butter. The firm texture of peanut butter can make spreading difficult. In contrast, Sample A received the highest score for spreadability. Including probiotic cultures may improve spreadability, as these cultures can enhance the consistency and viscosity of food products (Miranda *et al.*, 2019; Angelin and Kavitha, 2020).

Conclusions

The present study revealed that carob powder has probiotic and technofunctional effects. Specifically, the

combination of peanut butter and carob powder has proven to be an effective matrix to promote the survival and viability of *L. rhamnosus*. This study demonstrates that probiotic peanut butter, both with and without carob powder, can serve as suitable alternatives to non-dairy functional foods due to their favorable quality properties for the food industry. Additionally, the results of this study reveal a novel association of functional ingredients for fortifying peanut butter, leading to the development of standardized products that were well-received by panelists. However, further research in these areas could significantly enhance both the functional and sensory properties of peanut butter enriched with carob powder and probiotics, offering new opportunities for healthier, shelf-stable, and consumer-friendly products.

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Data Availability Statement

Data is unavailable due to privacy restrictions.

Author Contributions

Conceptualization, E.U.T., E.K, N.İ.Ç., and S.C.; methodology, E.U.T., E.K, N.İ.Ç., and S.C.; software, E.U.T. and E.K.; validation, E.U.T., and E.K., formal analysis, E.U.T., E.K, N.İ.Ç., and S.C.; investigation, E.U.T., E.K, N.İ.Ç., and S.C.; resources, E.U.T., E.K, N.İ.Ç., and S.C.; data curation, E.U.T., E.K, N.İ.Ç., and S.C.; writing—original draft preparation, E.U.T., E.K, N.İ.Ç., and S.C.; writing—review E.U.T., E.K, N.İ.Ç., and S.C.; visualization, E.U.T; supervision, E.U.T.

Conflicts of Interest

The authors declare no conflict of interest.

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