Effect of gelatin, fermentation temperature, starter culture ratio on physicochemical properties of peanut kefir

Dung T. N. Dang
Faculty of Chemical and Food Technology, HCMC of Univeristy of Technology and Education, Ho Chi Minh City, Vietnam

ARTICLE INFO

Research Paper

Received: August 21, 2024
Revised: November 02, 2024
Accepted: November 15, 2024

Keywords

Rheology
SEM
Shear stress
Viscosity

***Corresponding author**

Dang Thi Ngoc Dung
Email:
dzungdang@hcmute.edu.vn

ABSTRACT

Peanuts *(Arachis hypogaea)* are highly nutritious exerting health benefits such as preventing malnutrition, reducing heart disorders, and potentially prevent certain types of cancer. Kefir is one of the fermented dairy products containing probiotics and renowned for its beneficial effects on human health. This study aimed to evaluate the effects of gelatin concentration, fermentation temperature, and starter culture ratios on pH, the rheological properties, texture properties, and SEM (scanning electron microscope) of peanut kefir. The rheological properties of Peanut kefir exhibited pseudo-plastic behavior ($0 < \eta < 1$) and weak gel properties. Peanut kefir's rheological characteristics (viscosity, shear stress) and texture properties (hardness, adhesiveness, adhesive force) changed with gelatin content, fermentation temperature, and starter culture ratio. The FTIR spectrum of the gel peanut kefir sample was similar to that of the control sample. The optimal conditions for producing peanut kefir were 0.5% gelatin content, fermentation temperature of 25°C for 13 h, and a 5% starter culture ratio, resulting in a smooth kefir surface structure and a well-bound kefir gel. The SEM images revealed that the experimental sample exhibited a stable gel texture and no layer separation compared to the control sample. Generally, gelatin content, fermentation temperature, and starter culture ratio significantly influenced the quality of peanut kefir.

Cited as: Dang, D. T. N. (2024). Effect of gelatin, fermentation temperature, starter culture ratio on physicochemical properties of peanut kefir. *The Journal of Agriculture and Development* 23(Special issue 2), 166-182.

1. Introduction

Kefir is a fermented dairy product containing probiotics, and it can be fermented by kefir grains (Saygili et al., 2022; Bourrie et al., 2023). Kefir grain, a natural starting culture, comprises several lactic acid bacteria, acetic acid bacteria, and yeasts encased in a polysaccharide matrix (Stadie et al., 2013). Kefir shows its beneficial effects on human health (Egea et al., 2022). Kefir grains' microbiota generates fatty acids, peptides, organic acids, and bacteriocins that can bind mycotoxin and have antibacterial and antifungal properties (González-Orozco et al., 2022). In addition to its antibacterial, antimutagenic, anticarcinogenic, wound-healing, cholesterol-lowering, allergy, and lactose intolerance-preventing qualities, traditional kefir is good for the immune and digestive/gastrointestinal systems. Moreover, studies using kefir have shown promise in promoting increased colon *bifidobacteria* and glycemic control because it has high α-glucosidase inhibitory activity and balances intestinal microbiota (Egea et al., 2022). Kefir's distinct flavor is derived from a complex microecological environment that is created by the breakdown of lactose, protein, and fat in milk into galactose, lactic acid, exopolysaccharides, vitamins, free amino acids, free fatty acids, volatile alcohols, aldehydes, ketones, and esters (Xiao et al., 2023). Lactic and acetic acid-producing bacteria, lactose fermentation, and alcoholic yeast produce these compounds (González-Orozco et al., 2022; Xiao et al., 2023).

Peanuts are a legume belonging to the family *Fabaceae*, the genus *Arachis*, and the scientific name *Arachis hypogaea*, originating in Central and South America (Settaluri et al., 2012). They are widely grown in India, South America, China and elsewhere. Peanuts are considered an important nutritional source because they are a rich source of protein and essential amino acids, which can help prevent malnutrition, reduced heart disorders, certain types of cancer (Syed et al., 2021). Furthermore, peanuts contain rich compounds of lipids (Dwivedi et al., 2014) and carbohydrates, capable of supplementing the energy needs of the human body (Settaluri et al., 2012). Furthermore, recent research has shown the importance of the phytonutrient content of peanuts, including phytosterols, phenolic acids, isoflavonoids, and resveratrol, which may improve general health and wellness (Dwivedi et al., 2014).

Kefir is a popular fermented milk product worldwide; however, it is fermented from plant milk and has yet to be popular. Kefir is a new product in the Vietnamese market and is less popular than yogurt. The ultimate chemical-physical qualities and sensory quality of kefir are determined by fermentation parameters, which include temperature, time, starter culture ratio, and gelatine content. Research indicates that rheological and textural characteristics significantly impact kefir quality; nevertheless, there is little research on the subject, particularly regarding nut kefir.

This study examined the effects of gelatin concentration, fermentation temperature, and kefir culture ratio on pH value and peanut kefir's rheological-textural properties. Based on the rheological behaviors of *peanut kefir*, manufacturers will choose suitable methods, techniques, and equipment for the production process.

2. Materials and Methods

2.1. Raw materials

Sucrose and mature peanut seeds were purchased from Coop supermarket. Peanuts had characteristic color, no strange smell, no mold or weevils, and an oval shape of about 4 ÷ 10 mm. Gelatin was obtained from Louis Francois, France. Kefir starter culture with 6.8 x 10^{10} CFU/g was purchased from Yogourmet, France. Chemical materials were purchased from China's Xilong Scientific Co., Ltd.

Figure 1. Peanut seeds.

2.2. Research methods

2.2.1. Preparing peanut milk

The peanuts (Figure 1) were cleaned and soaked in water for 5 h. Then, they were crushed using a blender (Philips HR3041/00, Holland) and filtered through a clean muslin cloth to remove residual hull particles. The solution was homogenized at 7000 rpm in 10 min (T18 digital ULTRA-TURRAX® – IKA, Germany). Milk from the separated peanuts was pasteurized at 85°C for 10 **min**, cooled at 25°C quickly, and stored at 4°C.

2.2.2. Preparing kefir culture

Kefir grains were obtained from commercial kefir grains with identified lactic acid bacteria strains such as *Lactococcus cremoris*, *Streptococcus cremoris*, *Lactobacillus plantarum*, and the yeast (*Saccharomyces cerevisiae)*. Kefir strains were commercial strains activated in unsweetened fresh cow's milk at 5 g/1000 mL (w/v). The inoculated culture containing commercial strains was incubated at 37°C for 24 h under sterile conditions. Afterward, the kefir culture was kept at 4°C for later use.

2.2.3. Preparing peanut kefir

Peanut milk (containing 9% total solids) mixed with 3% (w/v) sucrose. Subsequently, gelatin was added to the mixture at concentrations of 0.3, 0.5, and 0.7% (w/v). The mixture was heated at 85°C for 5 min, then cooled to 25°C. Kefir culture was added with the ratio of 1, 3, 5, 7, and 9% (v/v) of the peanut milk. The inoculum was poured into 100 mL sterilized glass bottles and incubated at 23, 25, 27, and 29°C until pH = 4.6 was reached.

2.3. Methods

pH: The pH value of kefir samples was measured every hour of incubation using a previously calibrated digital pH meter (HANNA). pH meter was calibrated with a pH buffer of 7.00 and a pH of 4.00 before the kefir samples were measured; the sample's pH was determined by immersing the probe directly into a homogenized kefir sample.

Determination of protein content: Protein content was determined according to the method of Mæhre et al. (2018).

Determination of fat content: Fat content was determined based on the research method of AOAC 905.02 (2000).

Dynamic rheology measurements

The experiment was based on the method given by Gul et al. (2018). HAAKE RheoStress

RS600, USA, viscoelastic measurements were taken in a controlled strain rheometer. The plates were set up in a parallel geometric configuration (PP35, ø = 20 mm) with a measuring gap of 0.5 mm. The shear stress, and apparent viscosity test was performed at 25°C with shear rates from 0.01 ÷ 100s^{1}. As a result, Ostawwld - de Waele equations can be used to fit each set of data as follows: η_{app} = K. γ (n-1).

Whereas η_{app}: apparent viscosity (Pas); k: consistency coefficient (Pa sn); γ: shear sweep (1/s); n: flow behavior index.

Textural properties

The textural characteristics of peanut kefir samples were analyzed using the Texture Analyzer, CT3TM (BROOKFIELD, USA). The textural characteristics such as hardness (g. force), adhesiveness (mJ), and adhesive force (g. force/s) were measured using a spreadability probe with a cylinder 12.7 mm at 25 ± 1°C. The equipment was set to 0.5 mm/s test speed, 10 mm distance, and trigger force = 5 g. The instrument software examined the textural data (force vs. time) and parameters. The tests were done in triplicates, and the results were expressed as mean standard ± error.

Fourier transform infrared spectroscopy (FTIR)

The determination of FTIR was based on the research method of Chen et al. (2015). The TIR analysis aimed to identify the functional groups in kefir products. Before measuring the infrared spectrum, the samples were deep-frozen and freeze-dried for 24 h to collect dried kefir powder. FTIR spectra of kefir samples were recorded by scanning the transmittance from wavenumbers 4000 ÷ 400 cm^{-1} with a resolution of 4 cm^{-1}.

Scanning electron microscope (SEM)

Measuring kefir microstructure with SEM followed a method described by (Xiao et al., 2023) using an SEM-type TS1000PLUS (Hitachi, Japan) with 100-time magnification and 10 kV. The control sample (CS) was used to compare the differences in microstructure images of the studied peanut kefir.

2.4. Data analysis

All experiments were repeated three times. Data were analyzed and statistically processed using the ANOVA test on the Origin 2024b software platform.

3. Results and Discussion

Table 1 shows the means of the chemical composition of peanut milk samples used to produce kefir.

Table 1. Chemical composition of peanut milk

No.	Criteria	Content (g/100 mL)
1	Protein	8.2 ± 0.19
2	Lipid	13.65 ± 0.26
3	Carbohydrate	24.4 ± 0.54

Mean value from triplicate means± standard deviation.

According to Diarra et al. (2005), the quality assessment of peanut milk revealed a protein content of at least 8%, indicating its high quality. The protein analysis of the experimental samples yielded a protein content of 8.2%. Therefore, the protein content of peanut milk samples was suitable for kefir fermentation. In addition, peanut's protein content is significant for fermented products' physical and structural properties. Therefore, the peanut milk not only meets the quality criteria for further studies but also improves the structure of the finished peanut kefir product for further studies.

The effect of added gelatin content on and rheological - textural properties

Gelation addition may improve the quality of the kefir. The results showed a significant improvement in the gel texture of kefir products with added gelatin; the product did not dehydrate and prevent water separation; the gel network formed had a smooth texture; and viscosity tended to increase compared to CS (Figure 2). In particular, with samples supplemented with higher gelatin content, the coagulated gel blocks formed in the product had good adhesiveness, did not break, and the amount of whey separated was also less.

Gelatin was one of the most critical factors influencing kefir's rheological properties (Said, 2020). As a thixotropic gel, yogurt's shear thickening qualities lead its viscosity to frequently reduce during mixing, recover some of its previous structure, and then increase when shearing stops. The steady-state rheological behavior of peanut kefir at selected gelatin levels (0, 0.3, 0.5, 0.7%) is shown in Figure 2a. In contrast, the effect of gelatin levels on the apparent viscosity behavior is shown in Figure 2b. The Ostawwld - de Waele model explained the rheological behavior of kefir well.

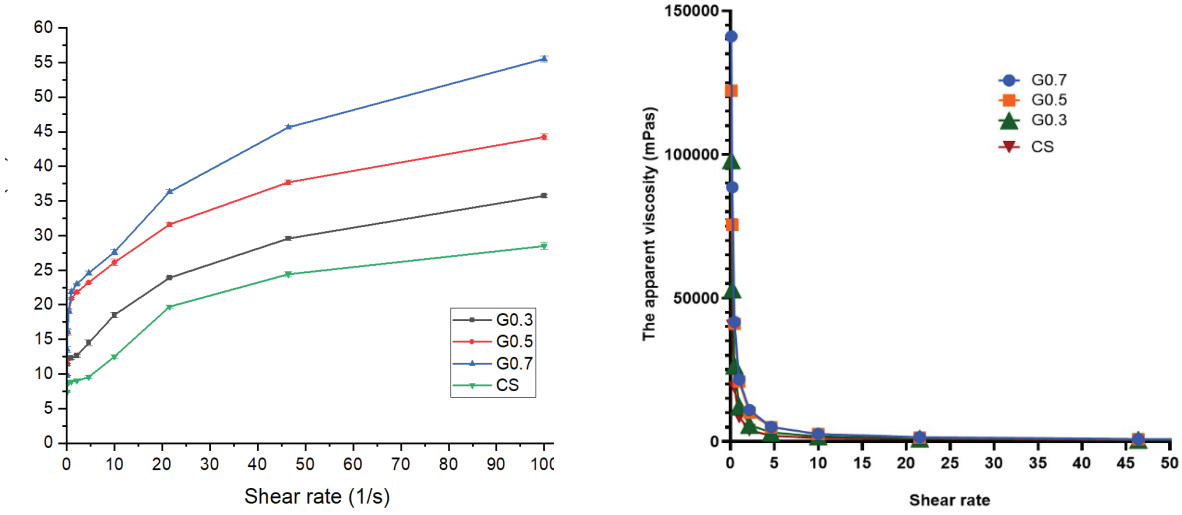

Figure 2. (a) Shear stress at various gelatin levels: 0, 0.3, 0.5, 0.7%; and (b) the apparent viscosity of peanut kefir at various gelatin levels: 0, 0.3, 0.5, 0.7%.

The flow curves of the shear stress variation with shear rate show that all samples exhibit an exponent of $0 < \eta < 1$, demonstrating that peanut kefir samples have non-Newtonian and pseudo-liquid properties (Guénard-Lampron et al., 2020). Increasing the amount of added gelatin increases the shear stress value, with the gelatin ratio being 0.3% (τ = 35.74 Pa) < 0.5% (τ = 44.22 Pa) < 0.7% (τ = 55.51 Pa) at a shear rate 100 (s^{-1}) (Figure 2). Additionally, it has been established that protein network stability requires the unfolding and rearrangement of secondary structure, aided by forming disulfide bridges, hydrogen bonds (Luo et al., 2019), and hydrophobic interactions, making the protein block tighter and requiring greater shear force (Ahmed et al., 2019). The shear stress with added gelatin increased in the early stages, then gradually stabilized, and

gelatin developed a solid three-dimensional network in fermented yogurt (Ares et al., 2007). The higher the stress, the higher their resistance to shear force (Frengova et al., 2002). Therefore, the results showed that the investigated samples were consistent with the Ostawald-de Waele rheological model. The samples' viscosity curves with shear rates from 1 ÷ 100 s^{-1} were similar; the apparent viscosity at the shear rate ranges from 0 ÷ 20s^{-1} decreased sharply, then gradually decreased to a constant value. Specifically, sample G0.7's apparent viscosity at the shear rate range from 0 ÷ 20 (s^{-1}) decreased from 141100 mPas to 1578 mPas. According to Figure 2b, kefir samples with added gelatin had a higher viscosity than CS. CS had an apparent viscosity of 284.7 mPas, and samples G0.3, G0.5, and G0.7 had an apparent viscosity, respectively, 357.4, mPas, 442.2 mPas and 472.3 mPas at a shear rate of 100 s^{-1}. Pang et al. (2019a) stated that adding 0.1 ÷ 1% gelatin affects the structure and rheology of peanut yogurt. The increase in viscosity upon adding gelatin is reportedly due to interactions between gelatin and milk proteins.

Studies have shown that adding a stabilizer (gelatin or other hydrocolloids) that acts as a thickener or gelling agent (Said, 2020) can help achieve good texture and stability (Supavititpatana et al., 2008).

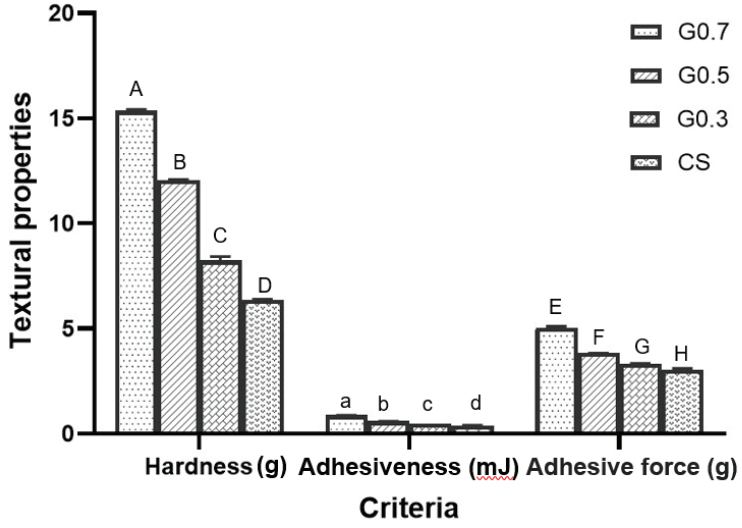

Figure 3. Effect of gelatin levels on peanut kefir's hardness, adhesiveness, and adhesive force. Mean value from triplicate means ± standard deviation. Different letters are significantly different ($P < 0.05$).

The results of the study on the effect of gelatin concentration (from 0.3, 0.5, and 0.7%) on the hardness, adhesion, and cohesive force of peanut kefir showed that the hardness increased by 46.67% and the adhesion increased by 16.27% between the G0.7 and G0.3. Figure 3 shows that all samples' hardness, adhesiveness, and adhesive force values were from 8.23 to 15.35 g, 0.45 to 0.87 mJ, and 3.33 to 5.04 g, respectively. The highest hardness, adhesiveness, and adhesive force of G0.7 were 15.35 g, 0.87 mJ, and 5.04 g; the lowest values of G0.3 were 8.23 g, 0.49 mJ, and 3.33 g, respectively. The CS showed that the gel texture was too soft, and hardness could not be determined. It was explained by the fact that gelatin contributes to stabilizing the structure

of kefir (Said, 2020) due to its ability to cut polysaccharide chains into short chains, thus increasing the gelation ability and contributing to the stability of the gel state. The gelatin ratio increased, and the firmness of kefir increased. G0.5 had a relatively harmonious firmness, making it a smooth structure suitable for kefir product properties.

The effect of fermentation temperature on pH, fermentation time, and rheological properties

The rheological characteristics are crucial in identifying various interactions in the recently developed kefir formulation (Saygili et al., 2022). The temperature at which kefir is incubated impacts its rheological characteristics (Dimitreli & Antoniou, 2011).

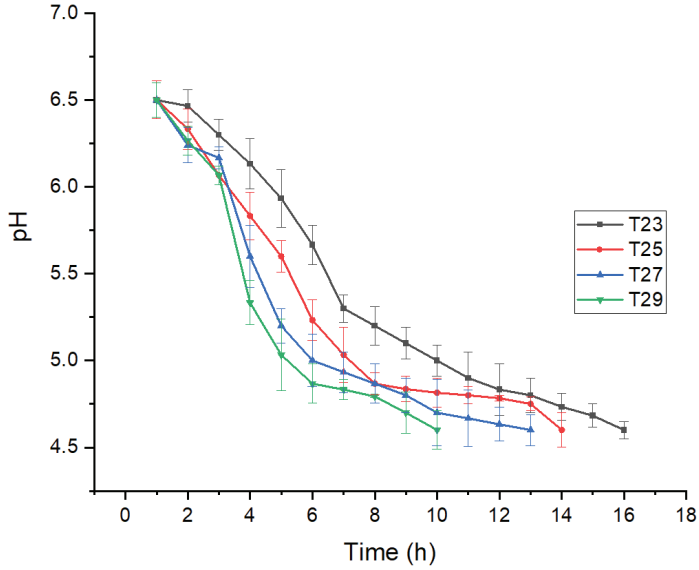

Figure 4. Various fermentation temperatures (23, 25, 27, 29°C) affect Peanut kefir's pH and fermentation time. Mean value from triplicate means ± standard deviation.

Figure 4 shows that the pH decreased while the acidity increased over fermentation time. When the fermentation temperature increases, the samples' fermentation time is different; the higher the temperature, the shorter the fermentation time. The T29 has the lowest time (10 h), with pH = 4.59; T23 has the longest fermentation time (16 h), with pH = 4.61. Fermentation temperature affects the fermentation process, as low temperature decreases the number or strength of hydrophobic bonds inside the protein gel. This result is entirely consistent with the research results of Nguyen et al. (2017) when studying the influence of ingredients (milk, gelatin, and kefir) on the quality of fruit yogurt, confirmed that the same proportion of microorganisms, fermentation temperature increases, fermentation time decreases. Lopes et al. (2019) stated that fermentation temperature significantly affects gel formation and acidification rate. High fermentation temperatures increase the ability to separate layers and weaken the protein network, thereby reducing gel hardness, viscosity smoothness, and sensory properties. Therefore, it makes the gel network susceptible to rearrangements, and these changes can lead to greater whey separation; besides, high fermentation temperatures cause rapid coagulation formation but easily lead to dehydration because acidification occurs

too quickly, and protein molecules are dense, reducing water-holding capacity (Mellema et al., 2002). The creation of acid content in kefir products depends on the growth of microorganisms and fermentation ability. The research survey and similar studies show that the peanut kefir fermentation temperature of T25 is suitable for the 13-h fermentation time, and the gel structure is stable.

Fermentation temperature is one of the critical factors influencing peanut kefir rheology. The steady-state rheological behavior of peanut kefir at selected gelatin levels (23, 25, 27, 29°C) is shown in Figure 5a. In contrast, the effect of gelatin substitution on the apparent viscosity behavior is shown in Figure 5b. The Ostawwld – de waele model explained the rheological behavior of kefir well.

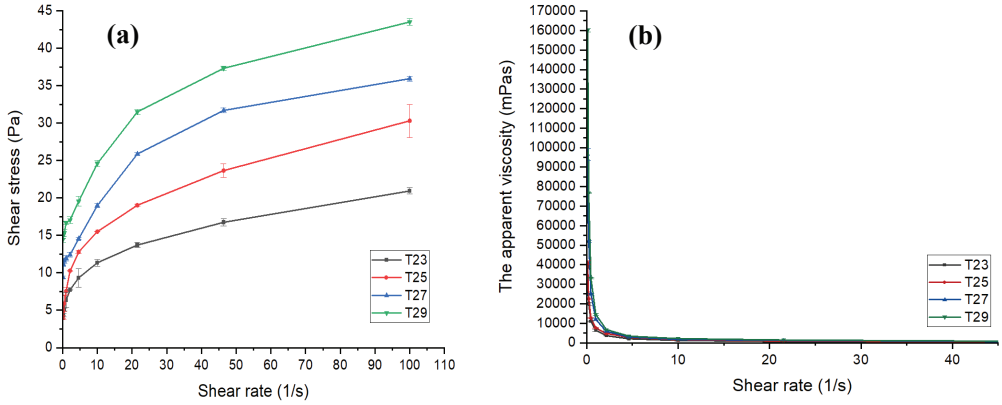

Figure 5. (a) Shear stress at various fermentation temperature; and (b) The apparent viscosity of kefir at various fermentation temperature: 23; 25; 27; 29oC. Mean value from triplicate means ± standard deviation.

According to Figure 5a, the peanut kefir samples surveyed are all pseudo-liquid because $0 < \eta < 1$. The samples tend to withstand low shear stress at a specific low shear rate, with T23 having a shear stress of 3.92 Pa at a shear rate of 0.9969 s^{-1}. After that, the increased the shear rate leads to a gradual increase in shear stress, and at a certain point, when shear rate continues to increase, the shear stress remains constant (Figure 5a). However, there are some differences in shear stress between the samples when changing the shear rate from 0 ÷ 100s^{-1}. When increasing the fermentation temperature from 23°C to 29°C, shear stress, according to the kefir's shear rate, gradually increases from 20.93 Pas to 43.51 Pas at a shear rate of 100s^{-1}; in contrast, the viscosity decreases because the water hol capacity decreases as the shear rate increases. According to Saygili et al. (2022), examining the apparent viscosity values of fermented cow's milk, it was determined that the viscosity values also increased with increasing fermentation temperature. Li et al. (2014) stated that the fermentation process of soy milk products has reduced viscosity with increasing shear stress because water molecules are well dispersed in the network structure. Besides, polysaccharides contribute to fermented milk products' increased viscosity and pseudo-liquid properties due to their ability to bind water and interact with proteins (Dimitreli & Antoniou, 2011). Bensmira and Jiang (2012) suggested that increasing fermentation temperature promotes hydrophobic interactions and creates

stronger gels. Besides, Man (2010) said that the kefir fermentation process is at its optimal temperature of 23 ÷ 25°C, the appropriate temperature for lactic bacteria and yeast to grow well. Low fermentation temperatures require longer fermentation times, affecting research performance, so we chose a kefir fermentation temperature of 25°C as the appropriate temperature for further research.

The effect of kefir culture ratio on pH, fermentation time, rheological properties, and SEM

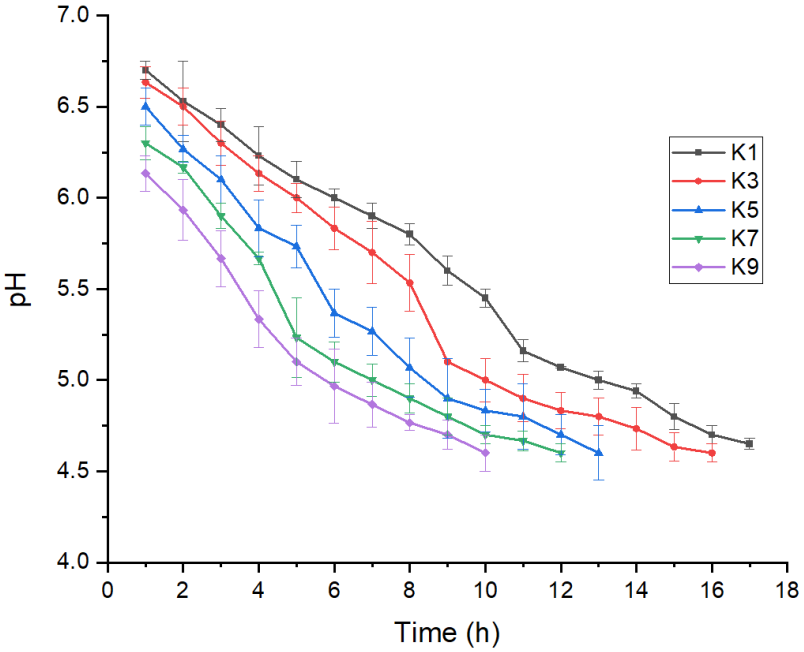

Figure 6. pH and fermentation time with various kefir culture ratios: 1, 3, 5, 7, 9%. Mean value from triplicate means ± standard deviation.

Figure 6 shows that the pH of kefir samples decreased over fermentation time with starter culture ratios. The pH in the first five h of K1, K3, and K5 decreased slowly, from 6.8 to 5.7. From 5 ÷ 10 h, pH decreased rapidly. After 10 h, the pH of the samples continued to decrease (K1 reached 4.65 at 17 h, K3 reached 4.6 at 16 h, and K5 reached 4.6 at 13 h). The pH in the first 5 h of K7 and K9 fermentation decreased rapidly, from 6.8 to 5.2 and 5.1. After 5 h, the pH of the samples continued to decrease slowly (K7's pH reached 4.6 with 12 h, and K9's pH reached 4.6 with 10 h). Figure 6 shows that increasing the kefir culture ratio, the fermentation time between samples is different; the fermentation end time of K9 is at least 10 h, pH = 4.6, and K1 has the longest fermentation time, 18 h with pH = 4.6. The kefir culture ratio increases, and the fermentation time decreases. However, the shorter the fermentation time, the more holes and water separation in the sample. When the microbial density is higher, the process of converting lactose into lactic acid occurs faster; the amount of acid formed increases, reducing the pH of the product and quickly reaching the end of the fermentation process (pH = 4.6), thereby shortening the fermentation time. Currently, the curd will not be durable, affecting the structure and feel of the product. The kefir culture ratio affects the pH value and acidity, showing that a higher kefir

culture ratio reduces fermentation time. The acidity of kefir may be developed due to organic acids; acid production in kefir products depends on microbial growth and fermentation capacity (Saygili et al., 2022). Research results show that a starter culture ratio of 5% has the most stable change in pH.

Changes in shear stress according to the shear rate of kefir samples with different kefir culture ratios (1, 3, 5, 7, 9%) are presented in Figure 7.

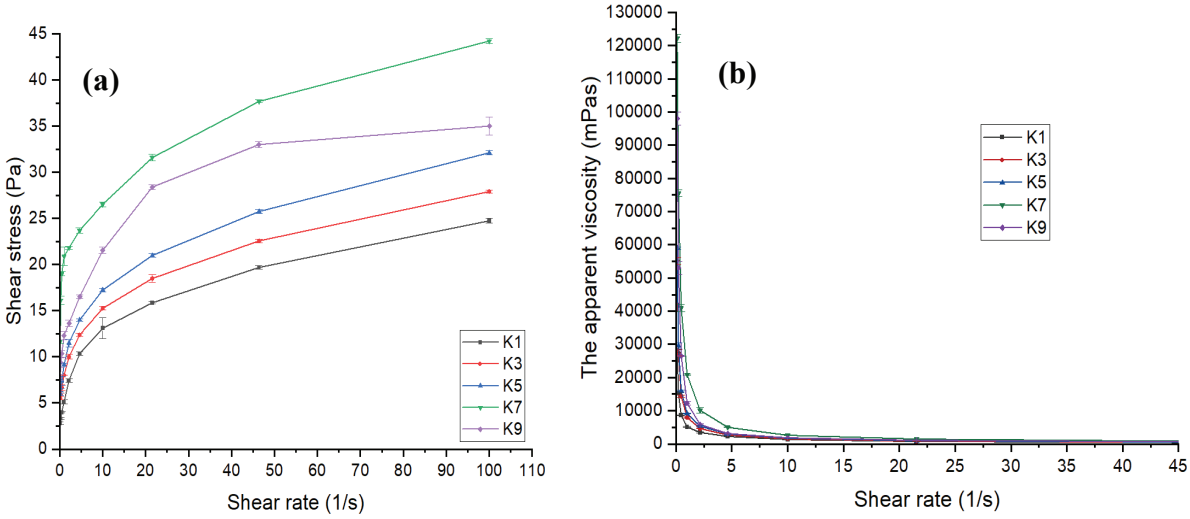

Figure 7. (a) Shear stress at various kefir culture ratios: 1, 3, 5, 7, 9%; and (b) The apparent viscosity of kefir at various kefir culture ratios: 1, 3, 5, 7, 9%. Mean value from triplicate means± standard deviation.

Figure 7 shows that the flow curve of the change in shear stress with the shear rate of the peanut kefir samples all shows an exponent of $0 < \eta < 1$, indicating that the samples are in pseudo-liquid form (Guénard-Lampron et al., 2020). Samples tend to increase the shear stress according to the shear rate from $0 \div 46s^{-1}$; K1's shear stress was 13.09 Pas at $10s^{-1}$; then, the shear rate continues to increase, the constant shear stress from the shear rate of $46 \div 100s^{-1}$, K1's shear stress was 21.98 to 22.87 Pa.

The K7 has the highest viscosity, leading to higher shear stress than the other samples. The fermentation process takes place too quickly, and the pH suddenly drops quickly, causing the protein in peanut milk to coagulate; the curd will not be stable, affecting the texture of the product. The apparent viscosity depends significantly on the existence and development of microorganisms. The highest viscosity of K7 was 442.2 mPas, and the lowest viscosity of K1 was 247.4 mPas, at $100s^{-1}$. Too high a kefir culture ratio will cause the gel structure to become loose, quickly causing layer separation and significantly affecting viscosity. K9 created acidification too quickly, reducing water retention. The decrease in viscosity was independent of the fermentation parameters and the source of the kefir starter microflora in the present study; this decrease can be explained by the hydrolysis of EPS into its monomers by glycol hydrolases (Purwandari et al., 2007). Structure scanning electron microscope of samples in Figure 8.

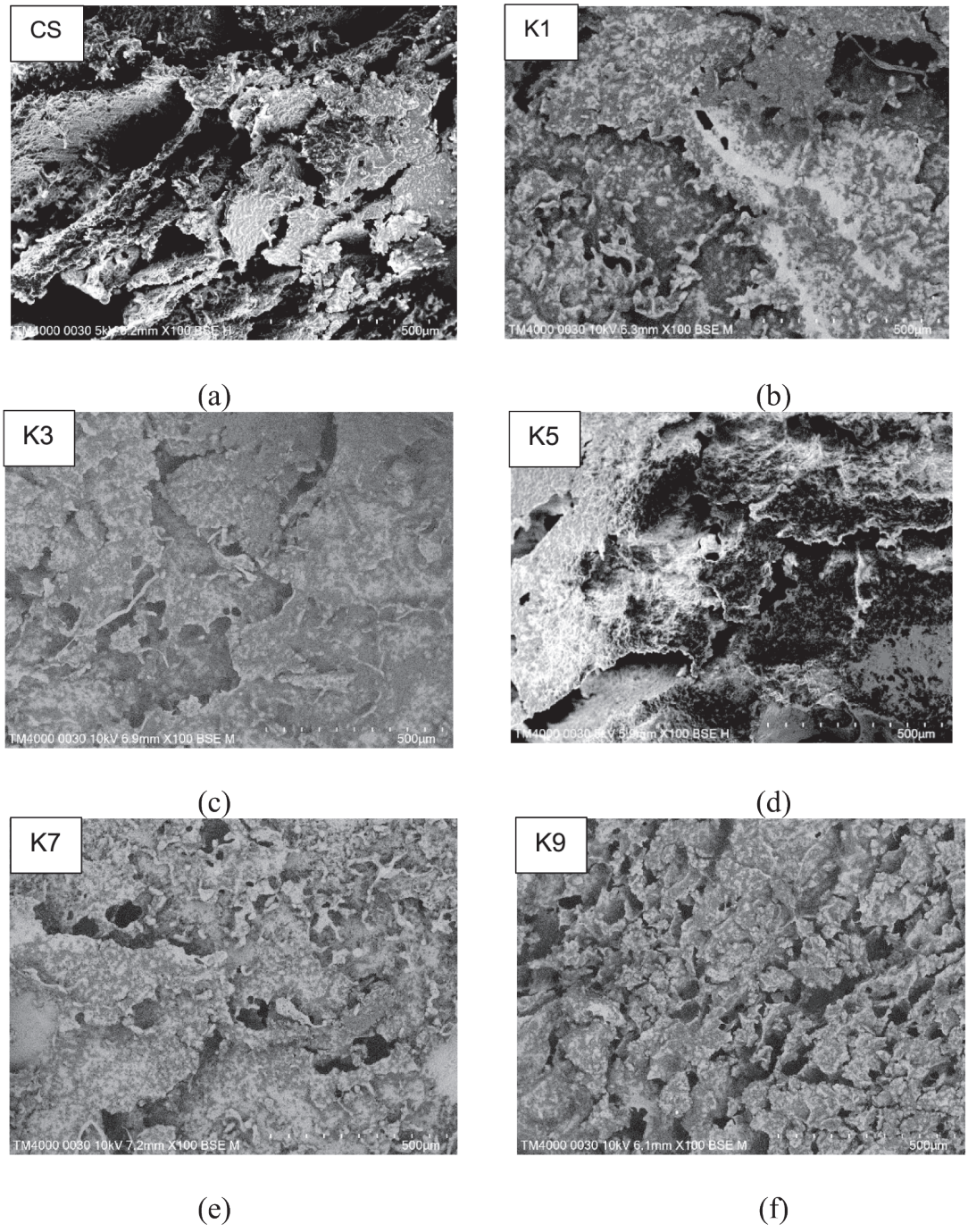

Figure 8. The SEM micrographs of kefir samples at various kefir culture ratios: 0, 1, 3, 5, 7, and 9% under a magnification of 100. (a) CS with 0% kefir culture ratio; (b) K1 with 1% kefir culture ratio; (c) K3 with 3% kefir culture ratio; (d) K5 with 5% kefir culture ratio; (e) K7 with 7% kefir culture ratio; (f) K9 with 9% kefir culture ratio.

The sample's SEM showed that a weak cheese structure had formed, the pores were spread out unevenly, and the cross-linking between kefir protein clusters was very strong, making tiny chains. K5 has the most uniformly distributed kefir product structure. The tight bonds in the gel network created a gel surface structure with very few holes; the structure of kefir was not dehydrated, the structure was tight, and the protein was evenly distributed. Therefore, after freezing and freeze-drying, the gel network did not create grooves and was not significantly broken. For CS, each gel block can be seen, but the gel network structure was uneven, porous, and had a water separation phenomenon; the gel network forms grooved and was broken, creating many holes; EPS exists in the pores in the gel network due to incompatibility with proteins (Hassan et al., 2003). K1 showed that, although the structure was tighter and fewer grooves appeared, the kefir structure was uneven and porous. K3 had an improved kefir structure, which was more uniform and less porous, but grooves still appeared. K7 had tight bonds with each other and minor porosity, proving that the structure of kefir was tight and had uniform protein mass. The gel network was also tighter; no grooves were created, and the gel network was not broken. K7 showed that the structure was much tighter and more uniform, but more grooves appeared, possibly because the pH rapidly decreased when the kefir culture ratio was increased. Acidity increases, causing the protein blocks to thicken, but the gel network is broken. K9 had a slightly looser structure, and the gel network was strongly destroyed. Shiva Dadkhah et al. (2011) stated that the kefir rate affected the pH value and the acidity, affecting the gel structure and network. The acidity of kefir products depended on the growth of microorganisms and fermentation ability. According to Hassan et al. (2003), EPS may exist in the pores in the network due to protein incompatibility. During the acidification stage, the incompatibility between EPS and peanut protein can cause phase separation, disrupting the formation and consolidation of the gel network and causing high protein retention, which reduces gel strength. Therefore, the structure is less dense (Pang et al., 2019b). However, if the fermentation process takes place too quickly, the sudden pH decrease quickly causes the peanut milk protein to coagulate. The curd will not be durable, affecting the structure and feel of the product. With the inoculum ratio of 5%, the structure is the tightest, less porous, and does not appear.

Some criteria for finished peanut kefir are in Table 2, the spectra of CS and study kefir samples are shown in Figure 8.

Table 2. Chemical properties for peanut kefir

No.	Criteria (%)	K5
1	Carbohydrate	22.12 ± 0.93
2	Lipid	8.46 ± 0.33
3	Protein	4.09 ± 0.21
4	Ash	0.61 ± 0.08

Mean value from triplicate means ± standard deviation.

According to Codex Standard 243-2003, the quality of kefir yogurt requires a protein content of not less than 2.7% and a fat content of less than 10%. This result shows that our peanut kefir research sample meets the nutritional value criteria.

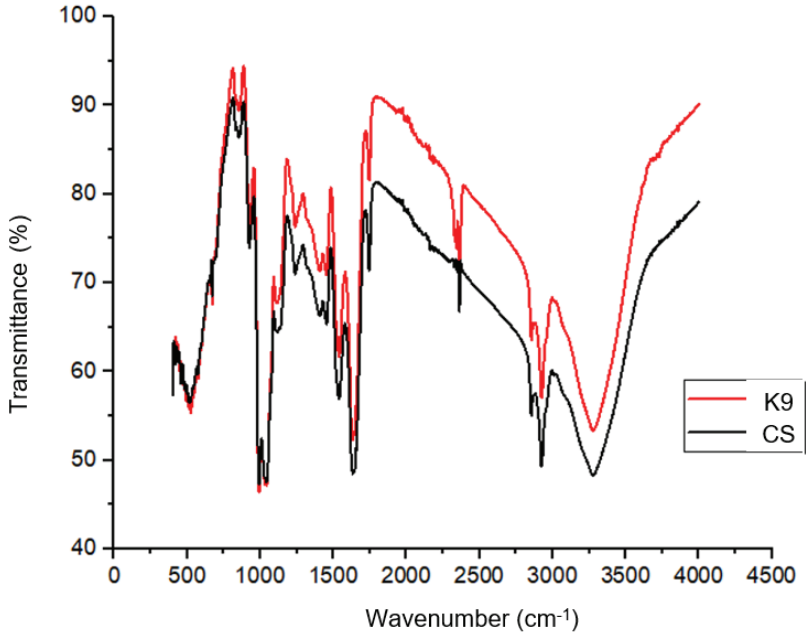

Figure 9. Fourier transform infrared spectroscopy (FTIR) spectra of the control sample (CS) and research sample (K5).

Figure 9 shows the wavelengths the functional groups of kefir samples absorb the most. Analysis related to the concentration regions of functional groups representing the presence of proteins was amide I (1600 ÷ 1800 cm^{-1}), amide II (1470 ÷ 1570 cm^{-1}), amide III (1250 ÷ 1350 cm^{-1}), and amide A (3300 ÷ 3500 cm^{-1}), which were the peaks of the amide infrared absorption characteristics. The protein leading band was the amide I band with the most vital absorption; the peak was obtained at 1744.54 cm^{-1} for both samples. It was related to the C=O stretching vibration (80%), the stretching vibration of the C-N bond (10%), and the hydrogen bond associated with COO- in the range between 1600 and 1800 cm^{-1} (Temiz & Çakmak, 2018; Vaishanavi & Preetha, 2021). In the amide II region, the maximum absorption at 1453.58 cm^{-1} arises from the bending vibration of the N–H group (60%), the stretching vibration of the CN group (30%), and the C–C stretching vibration (10%) (Long et al., 2015). In the amide III region, the most prominent absorption peak was at 1239.04 cm^{-1}; the vibrations existing in this region were mainly related to the bending in the vibrational plane of CN and NH groups in the amide or the vibrations of CH_2 groups (Temiz & Çakmak, 2018). The FTIR spectrum shows peanut protein was found in the higher range from 853.35 to 1042.10 cm^{-1}, and the lower range was 2924.28 to 3609.05 cm^{-1}. In the amide A region (3300 ÷ 3500 cm^{-1}), the absorption of these two peaks reaching a maximum at 3279.6 cm^{-1} was due to the presence of this related to the extended NH group bond and the interaction with each other by the O–H bond. The region in 3000 ÷ 3500 cm^{-1} was also due to molecular chains interacting by intra-and intermolecular hydrogen bonds (Salari et al., 2019). In addition, 900 ÷ 1200 cm^{-1} was dominated by polysaccharides related to the vibration of the characteristic C–O group and aromatic N–O compounds. In particular, there was a peak at the wave number 925.664 cm^{-1} related to additional sucrose (Mechmeche et al., 2019). The shape of the spectra of the two kefir samples was similar, indicating that gelatin was added to the sample without any change in

nutritional composition. During heating, the protein underwent structural changes related to disrupting hydrogen bonds in the molecules that stabilize the α-helical structure (Tian et al., 2020). Botelho et al. (2014) stated that FTIR to determine the nutritional characteristics of fermented soy kefir, which showed a peak in the amide I was 1629 cm^{-1}, the amide II region at 1419 cm^{-1}; our research result was 1744.54 cm^{-1}, and 1453.58 cm^{-1}, respectively. In the amide III region, the peak was 1239.04 cm^{-1}, and the peak was 3279.60 cm^{-1} - there is a prolonged N-H group bond of amide A. Therefore, K5 had no change in chemical groups in the kefir product.

4. Conclusions

The current study describes the effects of gelatin concentration, fermentation temperature, and kefir culture ratio on peanut kefir's pH, textural properties, and rheological properties. The study found that gelatin content increased the hardness, adhesiveness, and adhesive force compared to unaided gelatin content. Moreover, the results indicated that the suitable gelatin content, fermentation temperature, and kefir culture ratio for peanut kefir was 0.5% gelatin content, and fermentation temperature was 25°C with an added kefir culture ratio of 5%. The peanut kefir product has carbohydrate content (22.12 ± 0.93%), lipid content (8.46 ± 0.33), protein content (4.09 ± 0.21%), ash content (0.61 ± 0.08%) with peanut kefir's rheological behavior was a pseudo-liquid form.

Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

Acknowledgements

Thank you, HCMUTE, for supporting facilities to the author to finish the research. The author sincerely thanks Ms. Son Thi Ve Ry and Ms. Mai Ngoc Son Tuyen for their assistance with data processing.

References

Ahmed, J., Mulla, M., Al-Ruwaih, N., & Arfat, Y. A. (2019). Effect of high-pressure treatment prior to enzymatic hydrolysis on rheological, thermal, and antioxidant properties of lentil protein isolate. *Legume Science* 1(1), e10. https://doi.org/10.1002/leg3.10.

AOAC (Association of Official Analytical Chemists). (2000). *Official methods of analysis* (17th ed.). Maryland, USA: AOAC Intl.

Ares, G., Gonçalvez, D., Pérez, C., Reolón, G., Segura, N., Lema, P., & Gámbaro, A. (2007). Influence of gelatin and starch on the instrumental and sensory texture of stirred yogurt. *International Journal of Dairy Technology* 60(4), 263-269. https://doi.org/10.1111/j.1471-0307.2007.00346.x.

Bensmira, M., & Jiang, B. (2012). Effect of some operating variables on the microstructure and physical properties of a novel Kefir formulation. *Journal of Food Engineering* 108(4), 579-584. https://doi.org/10.1016/j.jfoodeng.2011.07.025.

Botelho, P. S., Maciel, M. I. S., Bueno, L. A., Marques, M. D. F. F., Marques, D. N., & Silva, T. M. S. (2014). Characterisation of a new exopolysaccharide obtained from of fermented kefir grains in soymilk. *Carbohydrate Polymers* 107(1), 1-6. https://doi.org/10.1016/j.carbpol.2014.02.036.

Bourrie, B. C. T., Diether, N., Dias, R. P., Nam, S. L., De La Mata, A. P., Forgie, A. J., Gaur, G., Harynuk, J. J., Gänzle, M., Cotter, P. D., & Willing, B. P. (2023). Use of reconstituted kefir consortia to

determine the impact of microbial composition on kefir metabolite profiles. *Food Research International* 173(Pt.2), 113467. https://doi.org/10.1016/j.foodres.2023.113467.

Chen, Z., Shi, J., Yang, X., Nan, B., Liu, Y., & Wang, Z. (2015). Chemical and physical characteristics and antioxidant activities of the exopolysaccharide produced by Tibetan kefir grains during milk fermentation. *International Dairy Journal*, *43*, 15–21. https://doi.org/10.1016/j.idairyj.2014.10.004.

Diarra, K., Nong, Z. G., & Jie, C. (2005). Peanut milk and peanut milk based products production: A review. *Critical Reviews in Food Science and Nutrition*, *45*(5), 405–423. https://doi.org/10.1080/10408390590967685

Dimitreli, G., & Antoniou, K. D. (2011). Effect of incubation temperature and caseinates on the rheological behaviour of Kefir. *Procedia Food Science* 1, 583-588. https://doi.org/10.1016/j.profoo.2011.09.088.

Dwivedi, S., Puppala, N., Maleki, S., Ozias-Akins, P., & Ortiz, R. (2014). Peanut improvement for human health. In Janick, J. (Ed.). *Plant breeding reviews: Volume 38* (1st ed., 143-186). New Jersey, USA: John Wiley & Sons. https://doi.org/10.1002/9781118916865.ch04.

Egea, M. B., Dos Santos, D. C., de Oliveira Filho, J. G., da Costa Ores, J., Takeuchi, K. P., & Lemes, A. C. (2022). A review of nondairy kefir products: Their characteristics and potential human health benefits. *Critical Reviews in Food Science and Nutrition* 62(6), 1536-1552. https://doi.org/10.1080/10408398.2020.1844140.

Frengova, G. I., Simova, E. D., Beshkova, D. M., & Simov, Z. I. (2002). Exopolysaccharides produced by lactic acid bacteria of kefir grains. *Zeitschrift Für Naturforschung C* 57(9-10), 805-810. https://doi.org/10.1515/znc-2002-9-1009.

González-Orozco, B. D., García-Cano, I., Jiménez-Flores, R., & Alvárez, V. B. (2022). Invited review: Milk kefir microbiota - direct and indirect antimicrobial effects. *Journal of Dairy Science* 105(5), 3703-3715. https://doi.org/10.3168/jds.2021-21382.

Guénard-Lampron, V., Bosc, V., St-Gelais, D., Villeneuve, S., & Turgeon, S. L. (2020). How do smoothing conditions and storage time change syneresis, rheological and microstructural properties of nonfat stirred acid milk gel? *International Dairy Journal* 109, 104780. https://doi.org/10.1016/j.idairyj.2020.104780.

Gul, O., Atalar, I., Mortas, M., & Dervisoglu, M. (2018). Rheological, textural, colour and sensorial properties of kefir produced with buffalo milk using kefir grains and starter culture: A comparison with cows' milk kefir. *International Journal of Dairy Technology* 71(S1), 73-80. https://doi.org/10.1111/1471-0307.12503.

Hassan, A. N., Ipsen, R., Janzen, T., & Qvist, K. B. (2003). Microstructure and rheology of yogurt made with cultures differing only in their ability to produce exopolysaccharides. *Journal of Dairy Science* 86(5), 1632-1638. https://doi.org/10.3168/jds.S0022-0302(03)73748-5.

Li, C., Li, W., Chen, X., Feng, M., Rui, X., Jiang, M., & Dong, M. (2014). Microbiological, physicochemical and rheological properties of fermented soymilk produced with exopolysaccharide (EPS) producing lactic acid bacteria strains. *LWT - Food Science and Technology*, *57*(2), 477–485. https://doi.org/10.1016/j.lwt.2014.02.025

Long, H. G., Ji, Y., Pan, B. H., Sun, W. Z., Li, T. Y., & Qin, X. G. (2015). Characterization of thermal denaturation structure and morphology of soy glycinin by FTIR and SEM. *International Journal of Food Properties* 18(4), 763-774. https://doi.org/10.1080/10942912.2014.908206.

Lopes, R. P., Mota, M. J., Pinto, C. A., Sousa, S., Silva, J. A. L. D., Gomes, A. M., Delgadillo, I., & Saraiva, J. A. (2019). Physicochemical and microbial changes in yogurts produced under different pressure and temperature conditions. *LWT* 99, 423-430. https://doi.org/10.1016/j.lwt.2018.09.074.

Luo, W. Y., Liu, Q. X., & Pang, H. Z. (2019). Tribo-rheological properties of acid milk gels with different types of gelatin: Effect of concentration. *Journal of Dairy Science* 102(9), 7849-7862. https://doi.org/10.3168/jds.2019-16305.

Man, L.V.V. (2010). *Textbook of technology for manufacturing dairy products and mixed drinks*. Ho Chi Minh City, Vietnam: Ho Chi Minh City National University publishing House.

Mæhre, H., Dalheim, L., Edvinsen, G., Elvevoll, E., & Jensen, I.-J. (2018). Protein determination-method matters. *Foods*, *7*(1), 5. https://doi.org/10.3390/foods7010005.

Mechmeche, M., Ksontini, H., Hamdi, M., & Kachouri, F. (2019). Production of bioactive peptides in tomato seed protein isolate fermented by water kefir culture: Optimization of the fermentation conditions. *International Journal of Peptide Research and Therapeutics* 25(1), 137-150. https://doi.org/10.1007/s10989-017-9655-8.

Mellema, M., Walstra, P., Van Opheusden, J. H. J., & Van Vliet, T. (2002). Effects of structural rearrangements on the rheology of rennet-induced casein particle gels. *Advances in Colloid and Interface Science* 98(1), 25-50. https://doi.org/10.1016/S0001-8686(01)00089-6.

Nguyen, P. T. M., Kravchuk, O., Bhandari, B., & Prakash, S. (2017). Effect of different hydrocolloids on texture, rheology, tribology and sensory perception of texture and mouthfeel of low-fat pot-set yoghurt. *Food Hydrocolloids 72*, 90-104. https://doi.org/10.1016/j.foodhyd.2017.05.035

Pang, H. Z., Cao, N. J., Zheng, M. Y., Luo, W. Y., Liu, Q. X., & Xiao, L. (2019a). Effects of different types of hydrocolloids on texture and rheological properties of soymilk yogurt. *Food and Fermentation Industries* 45(3), 1-6. https://doi.org/10.13995/j.cnki.11-1802/ts.018305.

Pang, H. Z., Xu, L. R., Zhu, Y., Li, H., Bansal, N., & Liu, Q. X. (2019b). Comparison of rheological, tribological, and microstructural properties of soymilk gels acidified with glucono-δ-lactone or culture. *Food Research International* 121, 798-805. https://doi.org/10.1016/j.foodres.2018.12.062.

Purwandari, U., Shah, N. P., & Vasiljevic, T. (2007). Effects of exopolysaccharide-producing strains of Streptococcus thermophilus on technological and rheological properties of set-type yoghurt. *International Dairy Journal* 17(11), 1344-1352. https://doi.org/10.1016/j.idairyj.2007.01.018.

Said, M. I. (2020). Role and function of gelatin in the development of the food and non-food industry: A review. *IOP Conference Series: Earth and Environmental Science* 492(1), 012086. https://doi.org/10.1088/1755-1315/492/1/012086.

Salari, M., Khiabani, M. S., Mokarram, R. R., Ghanbarzadeh, B., & Kafil, H. S. (2019). Preparation and characterization of cellulose nanocrystals from bacterial cellulose produced in sugar beet molasses and cheese whey media. *International Journal of Biological Macromolecules* 122, 280-288. https://doi.org/10.1016/j.ijbiomac.2018.10.136.

Saygili, D., Döner, D., İÇiEr, F., & Karagözlü, C. (2022). Rheological properties and microbiological characteristics of kefir produced from different milk types. *Food Science and Technology* 42, e32520. https://doi.org/10.1590/fst.32520.

Settaluri, V. S., Kandala, C. V. K., Puppala, N., & Sundaram, J. (2012). Peanuts and their

nutritional aspects - A review. *Food and Nutrition Sciences* 3(12), 1644-1650. http://dx.doi.org/10.4236/fns.2012.312215.

Shiva Dadkhah, R. P., Mahnaz Mazaheri Assadib and Ali Moghimic. (2011). Kefir production from soymilk. *Annals of Biological Research*, 293-299.

Supavititpatana, P., Wirjantoro, T. I., Apichartsrangkoon, A., & Raviyan, P. (2008). Addition of gelatin enhanced gelation of corn-milk yogurt. *Food Chemistry* 106(1), 211-216. https://doi.org/10.1016/j.foodchem.2007.05.058.

Syed, F., Arif, S., Ahmed, I., & Khalid, N. (2021). Groundnut (peanut) (*Arachis hypogaea*). In Tanwar, B., & Goyal, A. (Eds.). *Oilseeds: Health attributes and food applications* (93-122). Beach Road, Singapore: Springer. https://doi.org/10.1007/978-981-15-4194-0_4.

Temiz, H., & Çakmak, E. (2018). The effect of microbial transglutaminase on probiotic fermented milk produced using a mixture of bovine milk and soy drink. *International Journal of Dairy Technology* 71(4), 906-920. https://doi.org/10.1111/1471-0307.12521.

Tian, R., Feng, R. J., Huang, G., Tian, B., Zhang, Y., Jiang, Z. L., & Sui, N. X. (2020). Ultrasound driven conformational and physicochemical changes of soy protein hydrolysates. *Ultrasonics Sonochemistry* 68, 105202. https://doi.org/10.1016/j.ultsonch.2020.105202.

Xiao, R., Liu, M., Tian, Q., Hui, M., Shi, X., & Hou, G. X. (2023). Physical and chemical properties, structural characterization and nutritional analysis of kefir yoghurt. *Frontiers in Microbiology* 13, 1107092. https://doi.org/10.3389/fmicb.2022.1107092.