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## APPLICATION OF PEA PROTEIN ISOLATE IN THE PRODUCTION OF LACTOSE-FREE GOAT MILK KEFIR

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**Abstract:** Global food industry trends are increasingly focused on the development of innovative functional products with added value, such as lactose-free and high-protein foods that offer specific health benefits to consumers. At the same time, growing consumer awareness of the environmental impact of food production, particularly animal-based raw materials, has encouraged manufacturers to explore the replacement of animal proteins with plant-based alternatives. This study investigated the application of pea protein isolate (PPI) to increase the protein content of lactose-free goat kefir by 25 and 35%. Control samples without added PPI included kefir with lactose (K) and lactose-free kefir (K1). The lactose-free samples enriched with PPI, designated as KG1 and KG2, showed an increase in protein content from 2.8 to 3.5% and 3.8%, respectively. The samples were analyzed for titratable acidity ( $^{\circ}\text{SH}$ ), pH, chemical composition, syneresis, texture and SDS-PAGE electrophoretic protein profile. The flow properties were determined by viscometry technique in the shear rate range 0 - 200  $\text{s}^{-1}$ . Sensory quality was evaluated by trained panelists using a five-point scale. The addition of PPI significantly increased the viscosity of KG1 and KG2 samples compared to control samples at all shear rates investigated, suggesting that these samples would remain stable during pumping and transportation, which is especially important as goat milk is characterized by a fragile gel texture. The hysteresis area increased in the following order:  $\text{K1} < \text{K} < \text{KG1} \ll \text{KG2}$ . Lactose removal reduced the hysteresis area by approximately 25%, indicating weaker structural resistance to shear. In contrast, the addition of pea protein isolates significantly increased the hysteresis area, with a 46% increase at the lower enrichment level (KG1) and a 160% increase at the higher enrichment level (KG2) compared to the lactose-free control, confirming the strong structure-forming effect of PPI. Texture analysis supported the viscometry results, as significantly higher viscosity index and consistency values were observed in the enriched samples. Syneresis levels in K1, KG1, and KG2 were similar. These findings indicate that enrichment of lactose-free goat kefir with pea protein isolate represents a promising strategy for the development of high-protein functional products with solid structural stability, without increasing syneresis, thereby meeting current consumer demands for both nutritional benefits and sustainable protein sources.

**Keywords:** Goat milk, Kefir, Pea protein, Sustainable protein source, Texture

### INTRODUCTION

Current trends in the food industry globally are focused on finding solutions to alleviate its negative environmental impact, and to provide sufficient quality foods for the growing population worldwide. To achieve this, the intensive research on the possibility of the application of new food processing technologies and non-thermal preservation methods are

ongoing (Señorans et al., 2003). Additionally, efforts have been made in discovering new protein sources to produce fortified and functional foods, as a part of emerging food trends and transition to sustainable food development and innovative green strategies (Hassoun et al., 2024).

Aligning with this, plant proteins are increasingly being used in innovative food products, to partly replace animal-based proteins, or to produce plant-based food analogues. Plant proteins are advantageous compared to animal proteins because their production has a lower environmental footprint, requiring less water and land, and results in reduced CO<sub>2</sub> emissions (Hertzler et al., 2020). Additionally, they are generally more cost-effective and widely available. The main plant protein sources are cereals, legumes, nuts and oilseeds. As previously reported, their protein content varies greatly and ranges from 6 to 45% (Tan et al., 2023). Plant proteins are characterized by favorable techno-functional properties in food processing, such as gelation, emulsification, and foaming, which enables their wide application in the food industry.

Consumers often connect plant-based diets with certain health benefits, which were indeed demonstrated in different studies (ex. improved weight status, energy metabolism and systemic inflammation in healthy participants, obese and type-2 diabetes patients), as reported in a systematic review by (Medawar et al., 2019). The Protein Digestibility-Corrected Amino Acid Score (PDCAAS) was developed to assess protein quality by comparing its amino acid composition with that of a theoretical reference protein that meets amino acid requirements, considering digestibility as well. Most animal proteins have a PDCAAS close to 1.0, and are commonly recognized as complete protein sources, whereas many plant proteins contain limiting essential amino acids. Although legumes are often low in sulfur-containing amino acids, pea protein ranks among the higher-quality plant proteins, with a PDCAAS of at least 0.75 (Hertzler et al., 2020). This places pea protein alongside soy, canola, potato, and quinoa as one of the most nutritionally valuable plant-based protein sources.

Kefir is a traditional fermented milk product, with an increase in production due to its specific taste and health benefits. It is obtained by sour-lactic and mild alcoholic fermentation, using lactic acid bacteria and yeasts. It is characterized by a wealth of nutrients, a diverse microbiota, natural antioxidant activity and a lower content of lactose (milk sugar) compared to yogurt (Gul et al., 2018). Kefir thus represents an ideal product for innovation and for exploring new functional formulations consistent with sustainable production principles. Among fermented dairy products, kefir is particularly recognized as a functional food and has shown substantial growth in production and consumption in recent years.

In recent years, growing attention is being directed toward lactose intolerance, as a widely present health concern. Epidemiological studies indicate that approximately 70% of the global population is affected by lactose malabsorption, with prevalence reaching as high as 95-100% in some Asian and African countries (Li et al., 2023), underlining the importance of lactose-free dairy products.

The aim of this study was to evaluate the possibility of pea protein isolate application in lactose-free goat milk kefir as a sustainable alternative to traditionally used milk proteins.

By enriching kefir with plant-based protein, the goal was to develop a product with increased protein content - aligned with current trends in the dairy industry - while simultaneously reducing its environmental footprint.

## MATERIALS AND METHODS

### Kefir Production

UHT goat milk (Z'bregov, Vindija) containing 2.8% milk fat and 2.8% protein was purchased from a local supermarket. To enrich the kefir, pea protein isolate with a protein content of 80.7% (Pisane™ LS, Belgium) was employed. As the starter culture, a DVS freeze-dried 'eXact Kefir1' culture (Chr. Hansen, Germany), containing the strains *Debaryomyces hansenii*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*, *Lactococcus lactis* subsp. *lactis*, *Leuconostoc* sp., and *Streptococcus thermophilus*, was used. The lactase enzyme (NolaFit 5500, Chr. Hansen / Novonesis) was applied to hydrolyze lactose.

Based on the known chemical composition of goat milk and pea protein isolate (PPI), the required amounts of each component were calculated to achieve a 25 and 35% increase in total milk protein content, resulting in fortified kefir samples containing 3.5 and 3.8% total protein. Four sample variants were produced: (1) lactose-containing control (K), (2) lactose-free control (K1), (3) lactose-free kefir fortified with 25% additional protein (KG1), and (4) lactose-free kefir fortified with 35% additional protein (KG2). The production process included the following steps: weighing milk and PPI; warm hydration of the PPI in milk using a Thermomix (Vorwerk, Germany) at 40°C for 1 h with continuous mixing (speed 3.5); pasteurization at 90 °C for 5 min; rapid cooling to 25°C; addition of lactase enzyme (1 mL/L) to the samples intended for lactose hydrolysis; simultaneous inoculation with starter culture (0.2 g/L); fermentation at 25°C for 18-19 h until pH 4.6 was reached; mixing, filling into PET bottles and final cooling (4-8 °C). Kefirs were produced two times.

### PH and Titratable Acidity of Produced Kefir Samples

One day after production, pH of kefir samples was measured using digital pH-meter (Consort, Belgium). Titratable acidity was determined by titration with 0.1 M NaOH using phenolphthalein as an indicator until the titration endpoint was reached. The results were then calculated and expressed in Soxhlet-Henkel degrees (°SH).

### Texture Analysis

The textural properties of kefir were analyzed one day after production using a TA. XT Plus Texture Analyzer (Stable Micro Systems Ltd., UK) equipped with a 5 kg load cell. A single compression test was applied using a back extrusion probe (A/BE; diameter 35 mm; distance 30 mm; speed 0.001 m/s) at 8°C. The data obtained were analyzed using EXPONENT software (Stable Micro Systems Ltd., UK), from which texture parameters were calculated: firmness (g), consistency (gs), cohesiveness (g), and viscosity index (gs). These parameters describe the mechanical properties of kefir.

## **Rheological Analysis**

The rheological properties of kefir were analyzed one day after production using a Kinexus Pro+ rheometer (Malvern, UK) with a vane geometry at 8°C. To examine the viscosity and flow behavior under different shear conditions, a shear rate ramp-up and ramp-down test was performed over the range of 0-200 s<sup>-1</sup>, with continuous monitoring of shear stress. Similar to yogurt and other fermented dairy products, kefir exhibits thixotropic behavior, manifested as hysteresis, the area enclosed between the ascending and descending flow curves. The magnitude of hysteresis reflects the structural stability and strength of intermolecular interactions. Hysteresis was calculated as the difference between the areas under the ascending and descending curves using Microsoft Excel. Viscosity values in the shear rate range of 10-100 s<sup>-1</sup> represent the viscosity perceived during consumption, whereas changes in the range of 100-200 s<sup>-1</sup> indicate the product's behavior and stability under processing conditions, such as transport through pipes and filling (Hovjecki et al., 2025).

## **Syneresis Analysis**

Syneresis, the separation of whey from the protein matrix, is an undesirable phenomenon in fermented dairy products. The tendency of kefir to undergo syneresis was assessed one day after production using centrifugation method (Eppendorf 5430, Eppendorf AG, Hamburg, Germany) at 3000 × g for 10 min. The degree of syneresis was calculated as the ratio of the mass of separated whey to the initial sample mass multiplied by 100 (Setyawardani et al., 2020; Wang et al., 2019).

## **SDS PAG Electrophoresis**

For SDS-PAGE analysis, kefir samples were taken one day after production. All samples were immediately prepared for SDS-PAGE under reducing and non-reducing conditions by mixing with the appropriate buffer solutions as previously described (Hovjecki et al., 2021, 2023) and electrophoresis was performed following the method of Laemli (1970). A standard covering a range of molecular masses (from 10 to 240 kDa) was used as the molecular mass reference.

## **Sensory Analysis**

Kefir quality was evaluated after production by a sensory panel of seven members experienced in assessing goat milk products quality. Sensory attributes, such as overall appearance, color, consistency, odor, and flavor, were scored on a five-point scale (0-5), with half-point increments allowed (Hovjecki et al., 2025).

## **Statistical Analysis**

One-way ANOVA was used to assess the effect of PPI addition on the examined variables. Mean comparisons were performed using Tukey's or Games-Howell post hoc test at  $\alpha = 0.05$ , depending on the homogeneity of variances. Homogeneity of variances was checked with Levene's test. Statistical analysis was conducted in Jamovi version 2.3.28 (The jamovi project, 2024).

## RESULTS AND DISCUSSION

### PH and Titratable Acidity of Kefir Samples

One day after production, pH values of samples were similar: for K, K1, KG1 and KG2 values 4.50, 4.50, 4.52 and 4.49, respectively. The addition of PPI affected buffering capacity of fortified samples, thus maintaining the similar pH values to that of control samples. However, titration with 0.1 M NaOH revealed that the lactose-free control (K1) had significantly higher acidity ( $52.33 \pm 1.55$  °SH) compared to all other samples. This discrepancy reflects the different buffering capacities of the samples. The addition of pea protein isolate in KG1 and KG2 increased protein-induced buffering capacity, reducing the titration response to the acids formed. In agreement with this, Silva et al. (2024) reported no difference in titratable acidity nor pH among control yogurt and yogurts with added pea proteins. In contrast, K1, which contains no added protein, shows weaker buffering and therefore a higher °SH value despite a comparable pH.

### Texture Properties of Kefir Samples

As shown in Table 1, the addition of PPI significantly improved all analyzed texture parameters. Furthermore, consistency, cohesiveness and the index of viscosity increased depending on the PPI level. Mixed starter cultures, composed of multiple strains, such as kefir starter, can act on various hydrolysis sites of legume proteins, producing a broader spectrum of polypeptides during fermentation. Such proteolytic activity may enhance the functional and gelling properties of legume proteins, contributing to improved yogurt texture. In that regard, it was published that fermentation by kefir starter culture degrades legume proteins and improves their viscoelasticity (Li et al., 2025). On the other hand, the increased protein content results in a denser and firmer structure of the fermented dairy products (Ünal and Akalin, 2013).

Table 1. Textural properties of kefir samples produced without PPI (K-traditional kefir and K1-lactose free kefir) and with the addition of PPI (KG1- protein increase of 25% and KG2- protein increase of 35%, both lactose-free)

Sample type	Firmness (g)	Consistency (gs)	Cohesiveness (g)	Index of Viscosity(g)
K	16.38±0.74 <sup>a</sup>	410.38±4.86 <sup>a</sup>	14.61±0.91 <sup>a</sup>	1.42±0.11 <sup>a</sup>
K1	16.40±1.18 <sup>a</sup>	409.32±4.81 <sup>a</sup>	13.49±0.65 <sup>a</sup>	1.20±0.01 <sup>a</sup>
KG1	17.60±0.65 <sup>ab</sup>	463.61±9.10 <sup>b</sup>	15.77±0.71 <sup>b</sup>	7.37±1.31 <sup>b</sup>
KG2	18.84±0.41 <sup>b</sup>	505.81±12.60 <sup>c</sup>	16.65±0.30 <sup>b</sup>	12.04±1.52 <sup>c</sup>

Different lowercase letters (<sup>a</sup>, <sup>b</sup>, <sup>c</sup>) indicate significant differences between the kefir samples,  $p < 0.05$

### Rheological Properties of Kefir Samples

Figure 1 shows a decrease in viscosity with increasing shear rate for all samples, confirming the shear-thinning behavior of kefir. Fortified kefir samples (KG1 and KG2) exhibited significantly higher viscosity than the control samples over the entire range of shear rates investigated, indicating greater stability of these samples under mechanical stresses during processing and transportation (Table 2, Figure 1). Additionally, both KG1 and KG2 had higher hysteresis, suggesting a stronger protein matrix due to higher number of bonds. An

increase in relative protein concentration has been reported as the main cause of the higher structuring rate and complex modulus values (a measure of gel firmness) observed in pea protein-based gels (Klost and Drusch, 2019).

Table 2. Apparent viscosity ( $\eta$ ) recorded at specific shear rates and hysteresis areas ( $A_{up}-A_{down}$ ) of kefir samples

Sample type	$\eta_{10}$ (Pa·s)	$\eta_{50}$ (Pa·s)	$\eta_{100}$ (Pa·s)	$\eta_{200}$ (Pa·s)	$A_{up}-A_{down}$ (Pa/s)
<b>K</b>	0.044±0.003 <sup>a</sup>	0.024±0.001 <sup>a</sup>	0.021±0.000 <sup>a</sup>	0.018±0.000 <sup>ab</sup>	115.59±7.03 <sup>a</sup>
<b>K1</b>	0.04±0.000 <sup>a</sup>	0.022±0.000 <sup>a</sup>	0.018±0.000 <sup>a</sup>	0.016±0.000 <sup>a</sup>	86.93±2.18 <sup>b</sup>
<b>KG1</b>	0.104±0.004 <sup>b</sup>	0.038±0.001 <sup>b</sup>	0.029±0.001 <sup>b</sup>	0.022±0.000 <sup>b</sup>	126.8±3.22 <sup>c</sup>
<b>KG2</b>	0.140±0.004 <sup>c</sup>	0.052±0.001 <sup>c</sup>	0.039±0.000 <sup>c</sup>	0.028±0.000 <sup>c</sup>	226.24±5.11 <sup>d</sup>

Different lowercase letters (a, b, c) indicate significant differences between the kefir samples,  $p < 0.05$

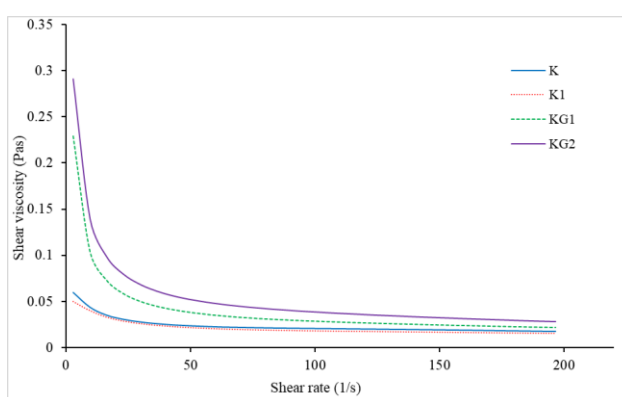


Figure 1. Change in apparent viscosity of kefir samples as a function of increasing shear rate (range 0-200  $s^{-1}$ )

## Syneresis of Kefir Samples

The addition of PPI significantly increased centrifugally induced syneresis in sample KG1 compared with the control samples; however, a higher level of PPI (sample KG2) alleviated this effect, resulting in syneresis values comparable to those of lactose free-control, K. Generally, an increase in milk nonfat solids in fermented dairy products, achieved by the addition of milk or whey protein powders, is used to reduce syneresis. However, the addition of PPI resulted in a different type of protein matrix; thus, pea protein may have disrupted the integrity of the milk protein network, leading to increased syneresis. In accordance with these findings, it has been reported that a 50% replacement of milk proteins with pea proteins in hybrid yogurt caused higher syneresis compared with the control (Silva et al., 2024). Considering that a higher amount of PPI tended to reduce syneresis, optimal amounts of PPI should be identified that do not damage the kefir protein network while still providing effective water binding, a characteristic feature of PPI.

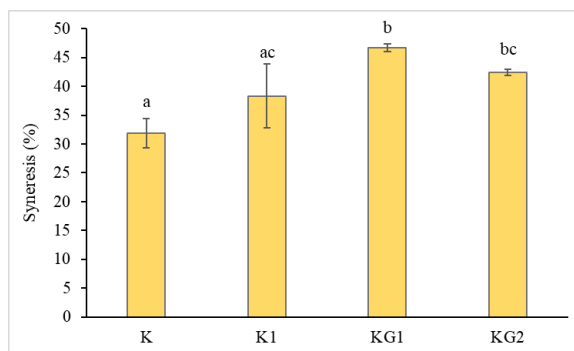


Figure 2. Centrifugally induced syneresis of kefir samples one day after production. Different lowercase letters (a, b, c) indicate significant differences between the kefir samples,  $p < 0.05$

### Protein Profile of Kefir Samples

Under reducing conditions, all kefir samples showed caseins (CN) as the dominant protein fractions, along with the main whey proteins (WP)  $\beta$ -lactoglobulin ( $\beta$ -LG) and  $\alpha$ -lactalbumin ( $\alpha$ -LA). In contrast, under non-reducing conditions,  $\beta$ -LG was not detected, and  $\alpha$ -LA appeared only in trace amounts, due to heat denaturation of whey proteins and their association with casein micelles (Dalglish et al., 1997; Guyomarc'h et al., 2003). These results indicate that the presence of pea proteins did not interfere with the formation of milk protein co-aggregates.

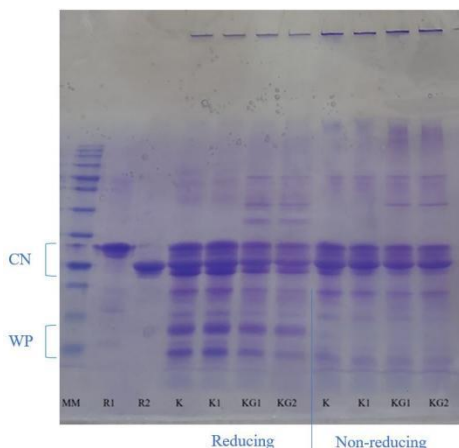


Figure 3. SDS-PAGE electrophoretogram of kefir samples in reducing and non-reducing conditions. MM - molecular weight standard; R1-  $\alpha$ -casein; R2 -  $\beta$ -casein; CN- caseins; WP- whey proteins

Heat treatment induced structural changes in pea proteins, as vicilin and legumin are known to denature in the temperature range of 75-85°C (Messin et al., 2015; Shand et al., 2007). In pea-enriched kefir samples (KG1 and KG2), a ~40 kDa pea protein fraction was observed under reducing but not non-reducing conditions, suggesting its involvement in disulfide-linked aggregates, likely corresponding to the acidic legumin ( $L\alpha$ ) subunit. Protein fractions of approximately 60 and 100 kDa were detected under both conditions, indicating non-covalent aggregation typical of vicilin and convicilin. Additionally, high-molecular-weight aggregates (>240 kDa) accumulated at the top of the gel, with greater intensity in pea-fortified samples, reflecting extensive aggregation via covalent and non-

covalent interactions. In agreement with previous studies (Akin and Ozcan, 2017; Messiou et al., 2015; Silva et al., 2024), these findings suggest that pea proteins aggregate independently and do not integrate into the casein network, potentially acting as a physical barrier to casein-casein interactions.

### Sensory Evaluation of Kefir Samples

Sensory evaluation showed that the addition of PPI did not negatively affect the overall quality of kefir, as all samples received high and comparable scores for appearance, colour, and odour, with no significant differences. The KG1 and KG2 samples, with 25 and 35% increased total protein content, respectively, achieved significantly higher consistency scores compared to the control samples, indicating a positive effect of PPI addition on kefir texture. Flavour scores were high and comparable among all samples, with slightly lower values in PPI-enriched formulations. This is commonly observed in beverages containing legume proteins - depending on species, there could be observed beany or bitter taste as well as sandy mouthfeel (Arbach et al., 2021).

Table 3. Results of the sensory evaluation of kefir samples, obtained using a five-point scale by an expert sensory panel (n = 7)

Sample type	Sensory evaluated properties				
	Overall appearance	Colour	Consistency	Odour	Flavor
<b>K</b>	4.71±0.49a	5.00±0.00a	3.71±0.57a	4.50±0.50a	4.14±0.63a
<b>K1</b>	4.64±0.48a	5.00±0.00a	3.93±0.67a	4.71±0.49a	3.71±0.70a
<b>KG1</b>	4.71±0.49a	4.86±0.38a	4.29±0.39b	4.57±0.45a	3.86±0.63a
<b>KG2</b>	4.64±0.48a	5.00±0.00a	4.19±0.38b	4.43±0.45a	4.07±0.45a

Different lowercase letters (a, b) indicate significant differences between the kefir samples (p<0.05).

### CONCLUSION

The enrichment of lactose-free goat milk kefir with pea protein isolates successfully increased the total protein content while significantly improving textural and rheological properties, particularly viscosity, consistency, and structural stability, without compromising sensory quality. The addition of PPI enhanced shear resistance and gel strength, as confirmed by viscometry, texture analysis, and hysteresis analysis. Sensory evaluation showed that PPI-enriched kefir samples were rated as highly as the control samples across all parameters, with only minor flavour differences, which is typical when plant proteins are incorporated into different food matrices. Overall, pea protein isolate represents a promising sustainable ingredient for the development of high-protein, lactose-free functional kefir products with improved technological performance and acceptable sensory quality.

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