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Development of Fermentation-induced Soymilk Gel: Effects of Different Lactic Acid Bacteria on the Physicochemical Characteristics

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Abstract

Nowadays, plant-based dairy alternatives have gained considerable attention. However, the textural and sensorial characteristics of plant-based products limit their acceptance. The exploitation of lactic acid bacteria has been proposed as a promising approach to developing plant-based dairy analogs. In this study, the performance of three proteolytic lactic acid bacteria in the induction of soymilk gelation was compared and their effects on the physicochemical properties of resulting gels were investigated. *Lactiplantibacillus plantarum MCM4*, *Streptococcus thermophilus*, and *Weissella confusa MDM8*) were inoculated to the soy milk matrix, and incubated at 37 °C until reaching pH 4.7. To understand the effects of acidifying and proteolytic activity of starter culture, syneresis, cell counts, free amino acid content (O-phthalaldehyde method), evaluation of proteolysis using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and textural parameters of soymilk gels during fermentation were investigated. There was a significant difference among the strains in terms of viable cell counts and proteolytic activity during fermentation ($p < 0.05$). The amount of syneresis was also different among the resulted gels as it was in the range from 61% (sample fermented with *S. thermophilus*) to 69.5% (fermented with *L. plantarum* MCM4). The main soy proteins were degraded to different extents as a function of fermentation time. Texture analysis showed that fermentation of soymilk with *W. confusa MDM8* resulted in soy gel with higher firmness and consistency, while the sample fermented with *L. plantarum MCM4* had higher adhesiveness and viscosity index. Overall, it can be concluded that *L. plantarum* MCM4, *W. confusa MDM8*, and *S. thermophilus* can be introduced as starter cultures for the production of novel soymilk gels with reasonable properties.

Keywords*:* Dairy alternatives, Lactic acid bacteria, Plant-based Proteins, Sustainable food production

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Introduction

Animal welfare, medical reasons such as cow's milk allergy and lactose intolerance, ethical concerns, and sustainable food production have all increased the demand for plant-based alternatives to dairy products (McCarthy *et al.*, 2017; Rastogi *et al.*, 2022). Because of their high protein content, and health-promoting effects, soy products have received much attention for the development of plant-based dairy alternatives. However, the sensory characteristics of soy-based products such as soy milk, soy gel, and soy protein isolate are not yet satisfactory and further research should be conducted to find suifie processes to mimic dairy products with better texture, aroma, taste, and shelf life (Genet *et al.*, 2023). Soymilk is a good source of protein for plant-based products and the concept of soymilk gel is significant in the food industry due to its various applications and benefits (Yang *et al.*, 2020). Soymilk gel can be used as a substitute for milk fat in cheese production, which can enhance the saturated/unsaturated fat balance in cheese without compromising nutritional value, making it beneficial for individuals with health issues related to saturated fatty acid and cholesterol content in dairy products (Rojas-Nery *et al.*, 2015). During the formation of soymilk gel, the coagulant may influence the extent of denaturation, while the electrostatic effect can disrupt the electric double layer on the protein colloid's surface, leading to increased aggregation and the development of a gel. The formation of the soymilk gel network is affected by various factors, including protein sources, the presence of stabilizers, pH, and temperature. Bacterial fermentation can be regarded as an alternative method for preparing soy gel instead of chemical coagulants (Wang *et al.*, 2022). Among the bacteria, lactic acid bacteria (LAB) have a unique place in the fermentation of plant-based products as they are recognized as safe, and have the potential to produce bioactive metabolites (such as bacteriocins and bioactive peptides) and aromatic compounds as well as reduce anti-nutritional components. Additionally, LAB increase the product shelf life because of the production of organic acids and hydrogen peroxide (Kong *et al.*, 2022; Mishra & Mishra, 2018; Somjid *et al.*, 2022). In this regard, certain LAB displayed favorable technological and functional attributes, such as protein hydrolysis, acidification, exopolysaccharide production (EPS), and aroma generation, which can improve the sensory characteristics of plant-based dairy products (Böni et *al.*, 2016; García-Cano *et al.*, 2019; Kamarinou *et al.*, 2022; Kong *et al.*, 2022).

This study analyzes the relationship between the textural and physicochemical characteristics of fermented soymilk gel and the proteolytic and acidifying activity of three LAB strains. In this regard, syneresis, cell counts, proteolysis (O-phthalaldehyde method), evaluation of proteolysis using SDS-PAGE, and textural parameters of soymilk gels during fermentation were compared. This work aims to provide a better understanding of texture modulation and physicochemical properties in plant protein substitutes by investigating the proteolytic ability of LAB in soy milk. Understanding this ability can help us introduce a starter for plant-based alternatives with desirable texture, flavor, and less allergenicity.

Materials and Methods

Chemicals and Bacterial Strains

L. plantarum MCM4, W. confusa MDM8, and *S. thermophilus* used in this study had been previously isolated from cabbage pickle (Karimian *et al.*, 2020), sourdough (Khanlari *et al.*, 2021) and yogurt (Moslemi *et al.*, 2023), respectively and stored in the microbial collection at the Department of Food Science and Technology (Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran). The frozen cultures were activated by subculturing in De Man–Rogosa–Sharpe (MRS) broth and incubating at 37 °C for 24 h. Dithiotritol (DTT) and L-Serine were purchased from Biobasic Co. (Canada). The chemicals and reagents for SDS-PAGE analysis were purchased from Sigma Aldrich (St.Louis,

Missouri, United States). Microbial culture media and other chemicals (OPA, sodium dodecyl sulfate, and di-sodium tetraborate) were purchased from Merck Co. (Darmstadt, Hesse, Germany).

Soymilk and Doy Gel Preparation

The preparation steps of soy milk gel are shown in Fig. 1 (Saraniya & Jeevaratnam, 2015). Yellow soybeans (Katul variety from the local farms, Golestan province) were soaked in water (1:3, w/v) for 10 h and ground with the blender (Philips, Netherlands,1400 W) (1:5 w/v, soy: distilled water). The mixture was then filtered and mixed with 1% glucose and soy protein isolate (SPI) until reaching 10% dry matter and heated at 95 ◦C for 15 min. After cooling to room temperature, bacterial suspensions (*L. plantarum MCM4, W. confusa MDM8, or S. thermophilus*) with optical density (OD) of 1 at 600 nm were inoculated (2% v/v). The inoculated soymilk was incubated at 37˚C until reaching pH 4.7. The fermentation was terminated by cooling the soymilk gels to 4°C.

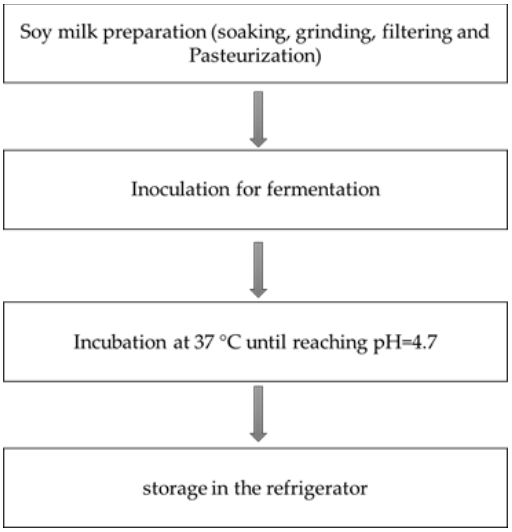


Fig. 1. Production process of soymilk gel

Determination of pH and Titratable Acidity

The pH levels of the samples were assessed with a digital pH meter throughout the fermentation process of soy milk. For titratable acidity (TA) determination, 10 grams of sample was diluted with 90 ml of distilled water and titrated with 0.1 mol/L NaOH. TA was reported as % lactic acid (Rahmani *et al.*, 2021).

Measurement of the Syneresis Value

The extent of syneresis was measured according to the method described by Rahmani et al. Tubes containing 20 grams of samples were centrifuged (10°C) at 4000 × g for 15 min. The weight percentage of released water after centrifugation was calculated and reported as syneresis extent (Rahmani *et al.*, 2021).

$$\text{Syneresis}=\frac{W1}{W2}\times 100 \quad (1)$$

where W1 is the weight of the supernatant and W2 is the weight of the sample.

Determination of Zeta-Potential

The zeta potential of soymilk gel was measured using a laser diffraction and dynamic light scattering analyzer (CORDOUAN Technologies, France). For zeta-potential measurements, the mixtures were diluted 100-fold with deionized water and placed into a specialized capillary cuvette containing two electrodes (Masiá *et al.*, 2022).

Viable Cell Counts

The changes in viable LAB cell counts (as Log CFU/mL) during fermentation were

evaluated using the pour plate technique. After preparing the serial dilutions, one mL of appropriate dilution from each sample was transferred into the plate and 15 ml of MRS agar was added. The plates were then incubated at 37 °C for 48 h, and the colonies were counted (only plates with 30-300 colonies). Two sequential dilutions for each sample (three replications) were analyzed. The following formula was used for cell count determination (Taheri *et al.*, 2019) :

$$N = \frac{\Sigma Ci}{V(n1 + 0.1n2)d} \quad (2)$$

where C represents the total number of colonies counted on all plates; V is the volume applied to each plate (in mL); n1 is the number of plates counted in the first dilution; n2 is the number of plates counted in the second dilution, and d is the dilution factor for the initial count.

Evaluation of Proteolysis Using OPA

The proteolysis in the soymilk was evaluated by measuring free amino groups (L-serine equivalent) using the OPA reagent (Shakerian *et al.*, 2015). Aliquot of 2.5 ml of fermented soymilk or soy gel was mixed with 5 ml of 5 % TCA in a test tube. The mixture was centrifuged at ۲000 × g for 1۰ min and filtered through a Wathman (0.45 µ) filter paper. Then, 200 µL of filtrate was added to 3 ml of OPA reagent and the absorbance of the mixture was read at 340 nm (UV-visible spectrophotometer, model Photonix Ar2015, IRAN) after 2 min incubation at room temperature. L-serine was used as standard amino acid for the quantification. The OPA reagent was prepared using the method outlined by Shakerian et al. (2015). In summary, 2.5 ml of 20% w/v SDS was added to a glass flask containing 25 ml of 100 mM sodium tetraborate. Then, 40 mg OPA reagent (previously dissolved in 1 ml methanol) was added to the flask. Finally, 150 µl of 20 mM dithiothreitol (DTT) was added to the flask and distilled water was then added to reach the final volume of 50 ml.

Sodium Dodecyl Sulfate-polyacrylamide Gel Electrophoresis (SDS-PAGE) Analysis

SDS-PAGE analysis was performed as described by Shirotani *et al*. using a 15% separating gel and 4% stacking gel. Samples were mixed with Tris buffer containing (10 mM Tris–HCl buffer, pH 8-9, 10% glycerol, 2.5% SDS, 5% β-mercaptoethanol, and 0.002% bromophenol blue), then heated at 95ºC for 5 min. Finally, 25 µL of the prepared sample was injected into the electrophoresis gel. The period of electrophoresis was 4 h at 130 volts, then, protein bands were stained with Coomassie Brilliant Blue followed by a destaining solution containing 10% acetic acid and 10% methanol. Standard protein markers with a molecular weight range of 11 to 180 kDa were used to determine the molecular weight range of the proteins and hydrolysates (Shirotani *et al.*, 2021).

Texture Profile Analysis (TPA)

The back-extrusion test was conducted by a Texture Analyzer instrument (Brookfield CT3-10Kg, USA). The test was conducted using a cylindrical probe (40 mm diameter) at a 1 mm/s rate to 3 cm penetration. The soymilk gels kept at 4 °C for 24 h were taken out 60 min before testing to achieve room temperature. The textural parameters including firmness, consistency, adhesiveness, and viscosity were calculated via mean values obtained from a force-time curve (Shams-Abadi & Razavi, 2021).

Statistical Analysis

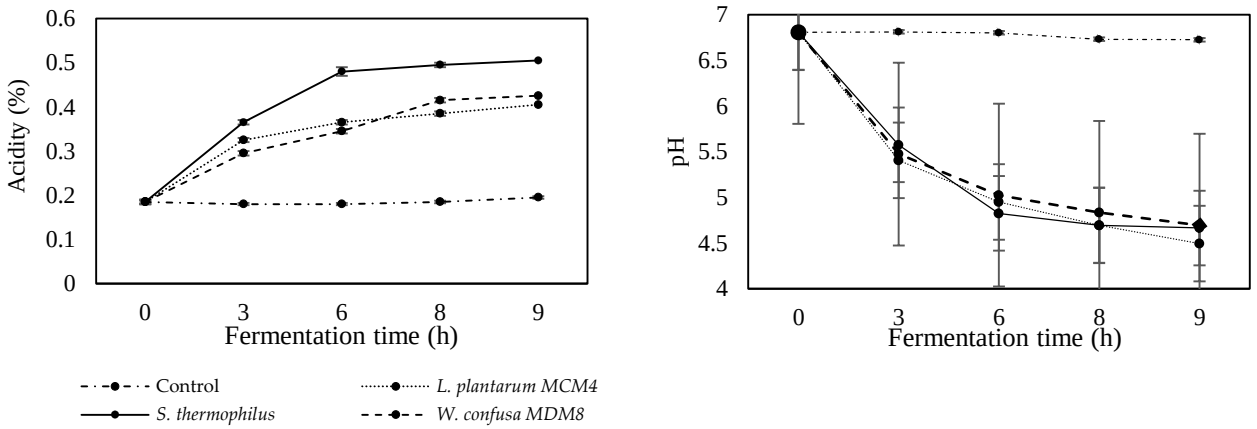
Data analysis was conducted using SPSS software (version 20). The mean values were compared to each other using the Duncan multiple range test ($p \leq 0.05$).

Results and Discussion

Titratable Acidity and pH

Changes in titratable acidity and pH during fermentation are shown in Fig. 2 (a) and (b), respectively. As shown in Fig. 2, in the control group, acidity remained stable during 9 h incubation. In soymilk fermented with *L. plantarum* MCM4 and *S. thermophilus*, acidity increased from 0.18 to 0.38% and 0.49%

respectively after 8 h of incubation. Also, the titratable acidity of soymilk fermented with *W. confusa MDM8* increased over time and reached 0.43% within 9 h. This shows the potential of tested LAB in consuming the sugars in soymilk (oligosaccharides, raffinose, stachyose, sucrose, and glucose) (Wang *et al.*, 2003). The isoelectric pH of soy proteins has been reported to be 4.5-5.0, and therefore reaching this pH is the beginning of gelation (Ningtyas *et al.*, 2021) (Fig. 3). Tang et al. reported that after pH approached the PI during fermentation, the electrostatic force between the proteins decreased and resulted in formation of SPI gel (Tang *et al.*, 2006).



(a) **(b)**

Fig. 2. Change in titratable acidity (a) and pH values (b) of soymilk inoculated with different LAB strains during fermentation

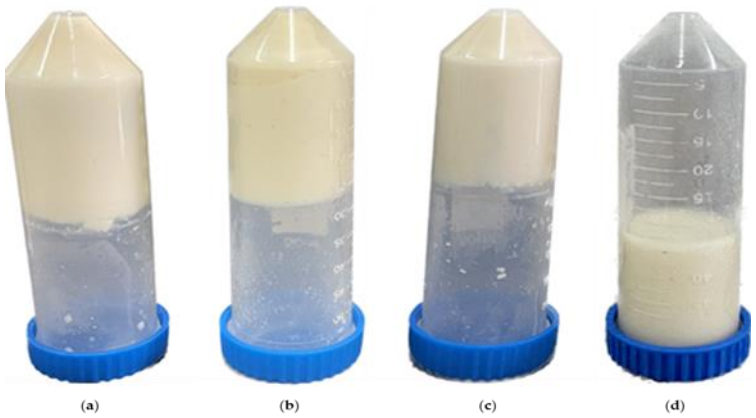


Fig. 3. Soymilk samples fermented with (a) *L. plantarum MCM4* after 8 h of fermentation; (b) *W. confusa MDM8* after 9 h of fermentation; (c) *S. thermophilus* after 8 h of fermentation; (d) unfermented (Control) after 9 h incubation

Zeta Potential

The results of measuring the zeta potential of soymilk before and after fermentation are shown in Table 1. The zeta potential, which is influenced by the pH of the dispersed phase, reflects the electrostatic interactions between protein molecules and is considered the main factor in protein aggregation (Klost *et al.*, 2020). Our results in this study show that the value of zeta potential significantly decreased from '-13.15' to ≈0. These results were similar to the results of Wang *et al.*, who showed that protein surface charges changed during fermentation and consequently the electrostatic

repulsion between them decreased. This can be mainly attributed to the neutralization of the negative charge on the original protein surface due to an increase in H^+ concentration. On the estimation of zeta-potential, it was seen that minimum surface charge was obtained at the isoelectric pH and the electrostatic repulsion is the main influence factor on aggregation behavior (Wang & Guo, 2016; Xing *et al.*, 2019).

Table 1- Zeta-potential of soymilk gel at t = 0 h and after reaching the pH= 4.7 during fermentation

Samples	Time(h)	pH	Zeta Potential/mV
*Control**	0	6.8±0.0	-13.15
Lactiplantibacillus plantarum MCM4	0	6.8±0.0	-13.15
Weissella confusa MDM8	0	6.8±0.0	-13.15
Streptococcus thermophilus	0	6.8±0.0	-13.15
Control	8	6.8±0.1	-13.15
Control	9	6.8±0.1	-13.15
Lactiplantibacillus plantarum MCM4	8	4.7±0.0	≈0
Streptococcus thermophilus	8	۴.۷±0.1	≈0
Weissella confusa MDM8	9	4.7±0.1	≈0

*Control: Unfermented soy milk

Cell Count

The cell counts of *L. plantarum MCM4*, *W. confusa MDM8*, and *S. thermophilus* in soymilk during fermentation are shown in Fig. 4. The viable cell count in samples fermented with *L. plantarum* MCM4, *S. thermophilus*, and *W. confusa MDM*8 was 6.42, 5.96, and 6.04 log CFU/ml respectively at the time of inoculation, which was increased to 7.43, 7.32, and 7.49 log CFU/ml at the end of fermentation (reaching pH=4.7). The metabolic processes occurring during the growth of lactic acid bacteria (LAB) resulted in acid production. The observed rise in titratable acidity, coupled with a reduction in pH value, signified the generation of acid during the fermentation of soy milk. All strains demonstrated a consistent increase in titratable acidity throughout the fermentation period, which was following the results of Leksono et al. (Leksono *et al.*, 2022) Considering the higher cell counts of *W. confusa MDM8* after 9h, and longer time for reaching pH 4.7, it can be concluded that it has a lower acidification activity than *L. plantarum MCM4* and *S. thermophilus* in soy milk and therefore weaker coagulation ability.

Proteolysis (FAA Content)

Free amino acid (FAA) content (mg/mL, L-Serine equivalent) in the fermented and unfermented samples is shown in Fig. 5. *L. plantarum MCM4* exhibited highest proteolytic activity (1.12 mg/mL), followed by *S. thermophilus* (1.07 mg/mL) and *W. confusa MDM8* (0.98 mg/mL) ($p< .05$). In the research conducted by Atashgahi et al., *L. plantarum MCM4* exhibited the most significant proteolytic activity in soy whey based on OPA method (Atashgahi *et al.*, 2024). The complex biosystem fermentation comprises enzymes derived from both the raw materials and microorganisms, which are responsible for hydrolysis reactions, including proteolysis (Joshi *et al.*, 2018). Microbial proteolysis can improve plant protein digestibility and bioactivity and reduce protein allergenicity (Worsztynowicz *et al.*, 2019). In this study, FAA content in all three inoculated samples increased during fermentation showing the affinity of tested LAB for soy proteins. In a study that used *Lactobacillus helveticus* for fermentation, the immunoreactivity of the soluble soy protein (β-conglycinin) decreased significantly (Meinlschmidt *et al.*, 2016). Meinlschmidt *et al*. reported that a combination of strain-specific proteolytic activity and acidic protein denaturation may cause the reduced immunoreactivity of β-conglycinin. Acidification is an aspect of LAB fermentation that may serve as a method to create a peptide

pool within a plant-based context (Gänzle *et al.*, 2008). Proteolytic system of LAB have the potential to increase protein solubility, an interesting property that could be used as a technological tool for stabilizing and producing complex plant-based products (Gänzle, 2015) leading to fermented plant-based milk a potential added value with multifunctional properties compared to nonproteolytic strains (Worsztynowicz *et al.*, 2019).

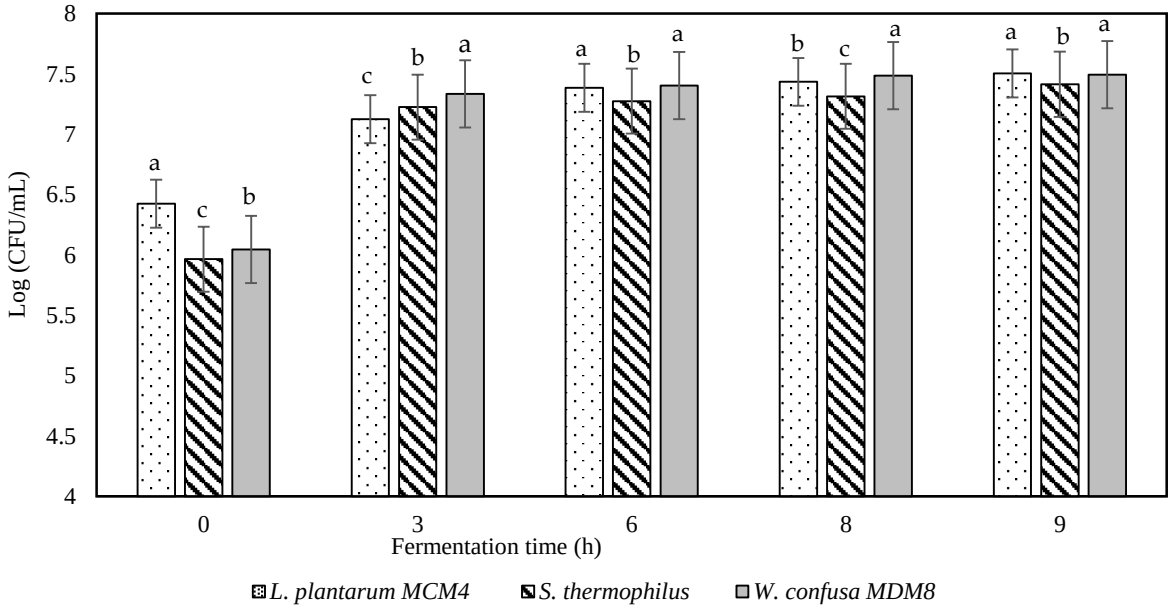


Fig. 4. Cell count (log cfu/ml) of Lactiplantibacillus plantarum MCM4, Streptococcus thermophilus, and Weissella confusa MDM8 in soymilk during fermentation

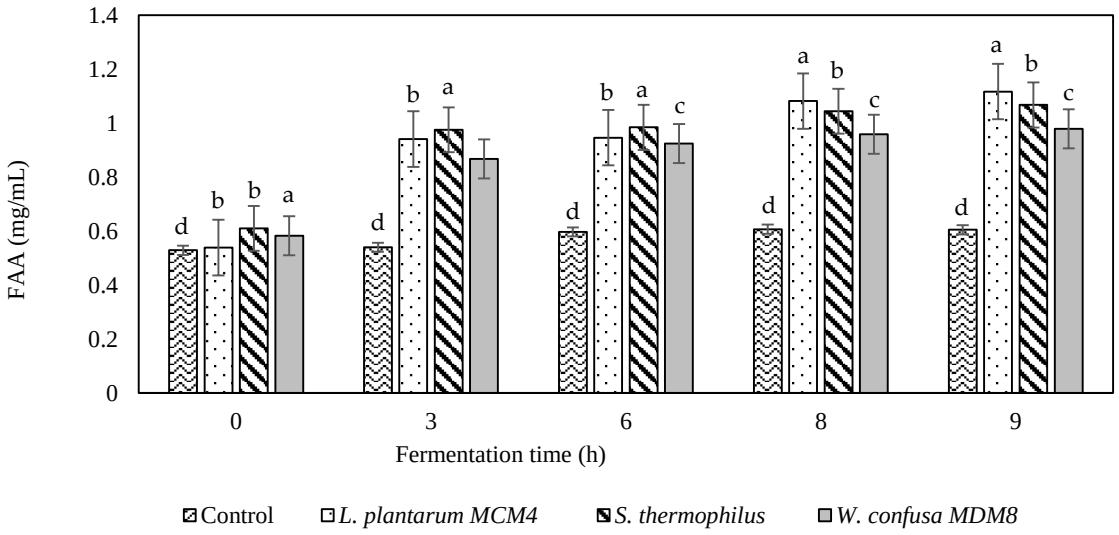


Fig. 5. Free amino acid (FAA) content (mg/ml, L-Serine equivalent) of soymilk fermented with *Lactiplantibacillus plantarum MCM4, Streptococcus thermophilus,* and *Weissella confusa MDM8* and Control sample for 0, 3, 6, 8 and 9h of fermentation

SDS-PAGE Analysis

Changes in soymilk protein profile during the fermentation process are shown in SDS-PAGE pattern (Fig. 6). In all fermented samples the band density of most of the proteins reduced after 3 h of fermentation and disappeared after the end of fermentation. Also, the SDS-PAGE pattern shows that new bands were derived from the acidic subunit of glycinin and the glycinin A5 subunit. The ability of proteolysis may vary due to differences in microbial species, the secreted protease as well as its ability to degrade protein (Yang *et al.*, 2020). *W. confusa MDM8* showed the highest degradation of β-subunits and acidic subunits of glycinin at the end of fermentation. It is essential to note that β-conglycinin was broken down more easily than glycinin in all strains. The phenomenon was probably due to the difference in structural characteristics between β-conglycinin and glycinin. Glycinin has a compact structure stabilized by disulfide bonds which makes it more difficult for the microorganism to access the cleavage sites. Glycinin and β-conglycinin are the two major proteins of soybean which make up approximately 40% and 30% of the total soybean proteins, respectively. Among the legume proteins, soy proteins have particular interest as they are connected to allergenicity (Meinlschmidt *et al.*, 2016). Many studies proved that the hydrolysis of allergenic proteins by enzymes secreted from LAB is an effective method to reduce soy allergenicity and easy digestion of the final product (Aguirre *et al.*, 2014; Holzhauser *et al.*, 2009; Tavano, 2013). In addition, soy protein is a protein of large molecular size, which is why products made from soybeans usually have a grainy texture (Shewry *et al.*, 1995). The results of electrophoresis showed that microbial proteolysis can break down large soy protein molecules into smaller peptide fragments and may solve the problem of granular texture (Li *et al.*, 2013).

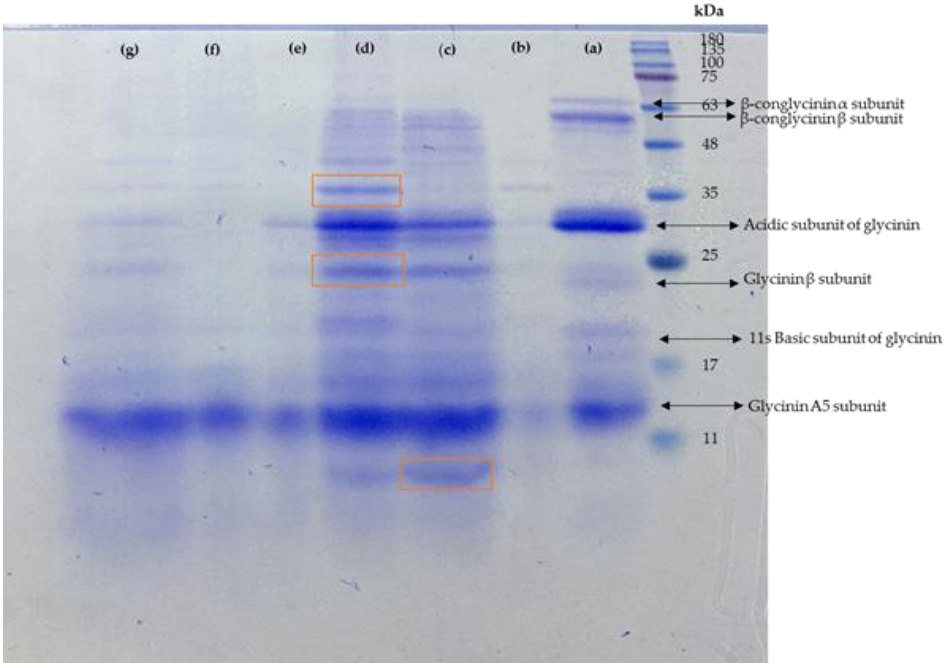


Fig. 6. SDS-PAGE profiles of soymilk fermented by three LAB strains and control sample during fermentation. (Lane a) control, unfermented soymilk - incubated for 9h; (Lane b) fermented soymilk for 9h with *Weissella confusa MDM8* ;(Lane c) fermented soymilk for 3h with *Weissella confusa MDM8* ;(Lane d) fermented soymilk for 3h with *Lactiplantibacillus plantarum MCM4*;(Lane e) fermented soymilk for 8h with *Lactiplantibacillus plantarum MCM4;*(Lane f) fermented soymilk for 3h with *Streptococcus thermophilus*;(Lane g) fermented soymilk for 8h with *Streptococcus thermophiles*

Syneresis in Soymilk Gels

The syneresis value of different soymilk gels was quantified at the end of fermentation and the results are presented in Fig. 7. There was a significant difference in the syneresis value among the samples. In soymilk gels fermented with *L. plantarum MCM4*, *S. thermophilus*, and *W. confusa MDM8*, the syneresis values were 69.5, 61 and 66%, respectively. Kuipers et al. reported that soy protein gels with elevated degree of hydrolysis showed higher syneresis (Kuipers *et al.*, 2005) which is in line with our results indicating higher syneresis in the gels fermented with *L. plantarum MCM4* with higher proteolysis, which could be due to greater contraction of the protein matrix. It is essential to note that β-conglycinin was broken down more easily than glycinin in all strains (Beal *et al.*, 1999). Such results were not in agreement with the results of our study, as soymilk fermented with *L. plantarum MCM4* had more syneresis than soymilk fermented with *W. confusa MDM8*, while its fermentation time was less. The length of fermentation is another factor that could affect the degree of syneresis and the texture of the gel (Purohit *et al.*, 2009). Furthermore, the effect of EPS on gel texture and syneresis is very important. EPS can bind water and enhance the viscosity and firmness of the gels (Folkenberg *et al.*, 2006). The functionality of EPS depends on their structural characteristics such as molar mass, degree of branching, and charge (De Vuyst & Degeest, 1999) and also the concentration of EPS (Mende *et al.*, 2013). Considering that the effect of EPS on the properties of fermented soymilk gel was not investigated in the present study, this can be a suggestion for future studies.

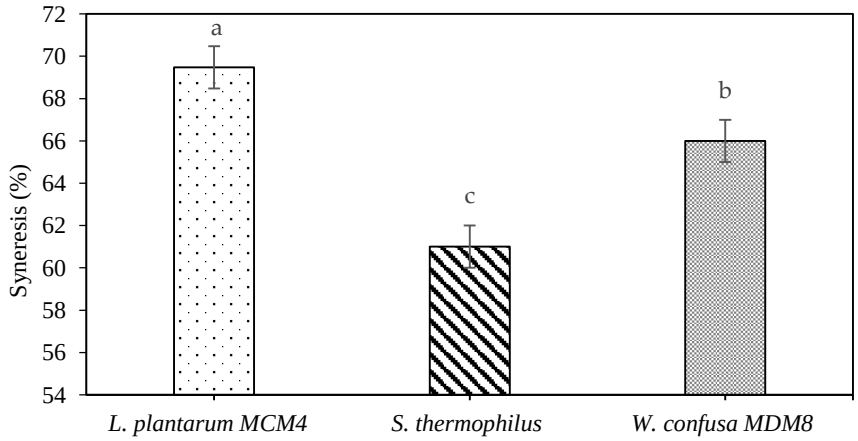


Fig. 7. Comparison of Syneresis of soymilk gel by inoculation of three LAB (*Lactiplantibacillus plantarum MCM4, Streptococcus thermophilus,* and *Weissella confusa MDM8*) at the end of fermentation

Texture

Texture parameters of the soymilk gels were assessed using back extrusion testing after 24h storage at 4 ˚C (Table 2). The adhesiveness of soy milk gels can be understood in terms of the adhesive force required to surpass the surface adsorption forces acting between the particles. Consequently, it can be inferred that a more robust gel structure and a denser protein network will result in a greater adhesive force (Shahbandari *et al.*, 2016), and the maximum force applied during compression is called firmness, while consistency refers to the thickness of the sample (Ciron *et al.*, 2010). The viscosity index is also the extrusion energy of adhesion when it increases, more resistance is required when pulling out the sample (Nasaruddin *et al.*, 2012). Generally, sample fermented with *S. thermophilus* showed the lowest values of textural characteristics and sample fermented with *L. plantarum MCM4* had higher consistency, adhesiveness, and

viscosity index. Moreover, sample fermented with *W. confusa MDM8* had the highest hardness. The final pH values of the gels were similar, but the difference in acidification rate could affect subtle differences in the microstructure (Liu *et al.*, 2023). In Cavallieri *et al.*'s study on the acidic cold set gelation of whey proteins, it was reported that at lower acidification rates the structure was stronger, probably due to a more organized formation during the slower acidification process. During slow acidification conditions, the molecular rearrangements occur in parallel with pH reduction and have enough time to interact and consequently organize the structure (Cavallieri & Da Cunha, 2008). In general, our results show that the use of LAB isolated from plant sources may be a useful strategy for improving the textural characteristics of plant-based suspensions because they can be better adapted to plant proteins. Korcz and Varga stated that EPS-producing LAB can induce textural properties in plant-based products because added hydrocolloids led to improve the clean products (Korcz & Varga, 2021). In another study, it was reported that fermentation with an EPS-producing strain of *Weissella confusa* increased the viscosity and water-holding capacity of the product compared to fermentation with non-EPS-producing LAB strains (Lorusso *et al.*, 2018). Also, in the study of Zannini *et al*., fermentation by *Weissella cibaria* resulted in the production of a quinoa-based yogurt product as viscous as dairy yogurt. They attributed the textural properties to extensive proteolysis of the protein matrix and increased viscosity to the production of high molecular-weight EPS (Zannini *et al.*, 2018).

Table 2- Texture characteristics of fermented soy milk gels**

Samples	Firmness (N)	Consistency (N*s)	Adhesiveness (N)	Viscosity index (N*s)
Fermented soy milk with *Lactiplantibacillus plantarum MCM4*	0.7755 ± 0.02^{b}	24.4665 ± 0.14^{a}	-0.503 ± 0.02^{b}	-1.21 ± 0.14^{b}
Fermented soy milk with *S. thermophilus*	0.5425 ± 0.04^{c}	11.4345 ± 0.20^{b}	-0.2785 ± 0.03^{a}	-0.79 ± 0.08^{a}
Fermented soy milk with *Weissella confusa MDM8*	1.132 ± 0.04^{a}	24.408 ± 0.04^{a}	-0.479 ± 0.02^{b}	-1.05 ± 0.04^{ab}

**Mean values ± SD. Samples with different superscripted letters in the same column are significantly different ($P < 0.05$).

Conclusion

The shift towards healthier and sustainable food consumption requires more studies on plant-based proteins. Plant-based gel has gained growing attention from the food industry, because many food systems, such as processed meats and cheeses, are plant-based gels. This work aims to evaluate the possibility of using LAB as a starter culture in the production of soy protein-based gels as a novel approach for healthier foods. The results of the present study clearly showed that the choice of applied microorganisms has a decisive role in the textural and physical characteristics of soymilk gels. In comparison to other samples, soymilk gel induced by fermentation with *Weissella confusa MDM8* was found to be more favorable in terms of physical and proteolytic properties. Although it took longer to reach the desired pH, SDS-PAGE analysis showed higher proteolysis and the resulting gel had better textural characteristics. Analysis of sensory properties, flavor, and shelf life of soy milk gel produced by such LAB strains can be the subject of future research.

Author Contributions

F. Rahmani: Funding acquisition, investigation, writing-original draft; **A. Moayedi:** Project administration, supervision, conceptualization, writing–review and editing; **M. Khomeiri:** Data curation, methodology; **M. Kashiri:** Formal analysis, software.

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Conflict of Interest

The authors declare no conflicts of interest.

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تولید ژل شیرسویا تخمیری: تأثیر لاکتیک اسید باکتری‌های مختلف بر ویژگی‌های فیزیکوشیمیایی

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چکیده

امروزه فرآورده‌های گیاهی جایگزین لبنی توجه زیادی را به خود جلب نموده است. با این حال، ویژگی‌های بافتی و حسی نامطلوب این محصولات پذیرش آن‌ها را محدود کرده است. بطور کلی استفاده از باکتری‌های لاکتیک اسید، به‌عنوان رویکردی نویدبخش برای توسعه آنالوگ‌های لبنی بر پایه گیاه پیشنهاد شده است. در این مطالعه، پتانسیل سه باکتری اسید لاکتیک پروتئولیتیک در ایجاد ژل شیرسویا مورد مقایسه قرار گرفت و تأثیر آن‌ها بر ویژگی‌های فیزیکو-شیمیایی ژل حاصله بررسی شد. *Lactiplantibacillus plantarum MCM4*، *Streptococcus thermophilus* و *Weissella confusa MDM8* به ماتریس شیر سویا تلقیح و تا رسیدن به pH حدود ۴/۷ گرمخانه‌گذاری شد. به‌منظور درک تأثیر سرعت اسیدیفیکاسیون و فعالیت پروتئولیتیک کشت‌های آغازگر مورد استفاده، سینرزیس، شمارش سلولی، محتوای آمینواسید آزاد (به روش اورتوفتال آلدهید)، الگوی SDS-PAGE و پارامترهای بافتی ژل حاصله ارزیابی شد. هر سه سویه باکتریایی، توانستند شیر سویا را منعقد کنند، با این حال *L. plantarum MCM4* فعالیت تولید اسید سریع‌تری در دمای ۳۷ درجه سانتی‌گراد نشان داد. بین سه سویه مورد مطالعه، از نظر شمارش سلولی و فعالیت پروتئولیتیک در طی تخمیر تفاوت معنی‌داری وجود داشت ($p<۰,۰۵$). همچنین مقدار سینرزیس نیز در بین ژل‌های به‌دست آمده متفاوت بود و در محدوده ۶۱٪ (نمونه تخمیر شده با *S. thermophilus*) تا ۶۹,۵٪ (نمونه تخمیر شده با *L. plantarum MCM4*) قرار داشت. علاوه بر این، سه سویه مورد مطالعه توانستند پروتئین‌های اصلی سویا را در طول تخمیر، هیدرولیز کنند. تجزیه و تحلیل بافت نشان داد که تخمیر شیر سویا با *W. confusa MDM8* منجر به ژل سویا با سفتی و قوام بالاتر شده است، در حالی‌که نمونه تخمیر شده با *L. plantarum MCM4* دارای چسبندگی و شاخص ویسکوزیته بالاتری بود. به‌طور کلی می‌توان نتیجه گرفت که *L. plantarum MCM4*، *W. confusa MDM8* و *S. thermophilus* را می‌توان به‌عنوان کشت‌های آغازگر برای تولید ژل‌های جدید شیر سویا با ویژگی‌های مناسب معرفی کرد.

واژه‌های کلیدی: باکتری‌های لاکتیک اسید، پروتئین‌های گیاهی، تولید غذا پایدار، جایگزین‌های لبنی

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