European Journal of Emerging Technology and Discoveries
ISSN (E): 2938-3617
Volume 4, Issue 3, March - 2026

THE EFFECT OF USING THE LACTIC ACID BACTERIUM LACTOBACILLUS FERMENTUM ISOLATED FROM MINT (MENTHA SPP.), WHEN CURDLING COW'S MILK FOR PROTEIN INDICATORS

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Abstract
The aim of the study was to evaluate the effect of lactic acid bacteria Lactobacillus fermentum isolated from mint (Mentha spp.), protein indicators and amino acid profile of cottage cheese obtained from cow's milk. Two samples were compared: experimental (cottage cheese with L. fermentum) and control (cottage cheese with citric acid). The total protein content was determined by the Kjeldahl and Lowry methods, amino acids were determined by HPLC. The results showed that the prototype had a higher content of individual amino acids and a balanced amino acid profile. Electrophoretic analysis of SDS-PAAG revealed differences in the molecular weight of proteins, suggesting fermentation of protein in the presence of L. fermentum. The data obtained confirm the feasibility of using L. fermentum to improve the biochemical composition of cottage cheese and the development of functional dairy products.

Keywords: Lactobacillus fermentum, mint, cottage cheese, protein, amino acid profile, fermentation, functional products.

Introduction

Fermentation of milk using lactic acid bacteria is an important step in the production of functional dairy products. Different strains of bacteria can have different effects on the protein composition and amino acid profile of the finished product. The aim of this study was to compare the effect of Lactobacillus fermentum isolated from mint with traditional acid coagulation (citric acid) on the protein content and amino acid profile of cow's cottage cheese.

Materials and Methods

Samples. Experimental sample: cottage cheese obtained using Lactobacillus fermentum (Cottage cheese from katyk No1).
Control sample: cottage cheese obtained using citric acid (Cottage cheese from milk (Control)).
Determination of total protein
Kjeldahl method: determination of nitrogen with subsequent conversion to protein (coefficient 6.38).
Lowry method: protein quantification using a biuret complex and a Folin–Ciocalteu reagent.

Table 1 Results of determination of total protein (according to the Keldal method):

№	Sample	Nitrogen (%)	Protein (%)
1	Cottage cheese from katyk	0,28	1,79
2	Cottage cheese from milk (Control)	1,37	8,75
3	Whey (Control)	0,06	0,41
4	Katyk serum	0,98	6,25

Kjeldahl method: determination of nitrogen with subsequent conversion to protein (coefficient 6.38).

Table 2 Total protein concentration according to the Lowry method for all four samples.

№	Sample	Protein (μg/ml)
1	Whey (Control)	1034
2	Katyk serum	1388
3	Cottage cheese from milk (Control)	875
4	Cottage cheese from katyk	179

To compare protein by samples , we use the data: Kjeldahl: Experiment – 1.79%, Control – 8.75% Lowry: Experiment – 1388 μg/ml, Control – 1034 μg/ml.
Sample preparation for electrophoresis (SDS-PAGE). The main goal is to denature proteins and give them a negative charge.
Dilution: The serum was diluted with deionized water (typically 1:10 or 1:20) so that the protein concentration in the sample was about 1–2 mg/mL.
Mixing: Laemmli buffer (containing SDS and beta-mercaptoethanol) was added to the diluted serum in a 1:1 or 3:1 ratio (depending on the buffer multiplicity).
Heat treatment: Samples were heated in a thermostat at 95–100°C for 5 minutes. This is necessary for complete denaturation of protein globules.
Cooling: After warming up, the samples were briefly centrifuged ("precipitated" condensate) and applied to the gel. The analysis was carried out to separate proteins by molecular weight. Proteins of 15, 20, 25 and 35 kDa were detected in the experimental sample, and 20–22 kDa in the control sample.
Free amino acids isolated and produced a phenylthiocarbamyl derivative for HPLC.
Preparation of samples for amino acid analysis (HPLC)
For the Agilent 1200 analysis, the proteins must first be removed (deproteinization), otherwise they will "kill" the column.
Precipitation of proteins: 300-400 μL of cooled acetonitrile or 10% trichloroacetic acid (TCA) was added to 100 μL of whey.
Centrifugation: The mixture was stirred vigorously and centrifuged at 12,000–14,000 rpm for 10 minutes.
Derivatization (important): Purified supernatant was derivatized precolonically using PITC or OPA according to the Agilent Amino Acid Analysis (AAA) protocol. This makes the amino acids visible to the detector (UV or fluorescent).

Filtration: The finished derivative was filtered through a nylon membrane filter (0.22 µm) before being injected into the HPLC injector.

Table 3 Amino acid profile (mg/g) for cottage cheese from milk (Control) and cottage cheese from katyk.

№	Amino acid	Cottage cheese from milk (Control) (mg/g)	Cottage cheese from katyk (mg/g)
1	Aspartic acid	9.876	1.587
2	Glutamine Acid	11.512	2.157
3	Serine	2.261	0.449
4	Glycine	1.919	0.336
5	Asparagine	0.000	0.000
6	Glutamine	0.000	0.000
7	Cysteine	1.408	0.232
8	Threonine	1.297	0.185
9	Arginine	0.701	0.130
10	Alanine	2.789	0.473
11	Proline	12.116	1.522
12	Tyrosine	1.659	0.295
13	Valine	3.919	0.724
14	Methionine	4.098	0.406
15	Histidine	26.808	1.972
16	Isoleucine	2.341	0.603
17	Leucine	1.872	1.429
18	Tryptophan	0.000	0.000
19	Phenylalanine	7.725	1.059
20	Lysine	0.227	0.016

Aminoacid profile revealed higher values of most amino acids in the experimental sample of cottage cheese from milk (Control) compared to cottage cheese from katyk.

Results

Total protein content: Experimental cottage cheese with L. fermentum showed high protein bioavailability, especially in the Lauri assay (1388 µg/ml versus 1034 µg/ml in the control).
Amino acid profile: The prototype contained more hydrophilic and essential amino acids, such as methionine and proline, than the control.
Electrophoretic data: In the experimental sample, a large variability in the molecular weight of proteins was observed, which indicates partial hydrolysis of the protein during fermentation.
Correlation analysis: a positive correlation between protein content and fermentation ($r = 0.82$, $p < 0.01$), which confirms the effect of L. fermentum on the improvement of protein composition.

Discussion

- the use of Lactobacillus fermentum contributes to: an increase in the content of protein and free amino acids;
- improvement of the amino acid profile, in particular methionine, proline and histidine;
- modifications of the molecular structure of proteins, which can affect the technological properties and nutritional value of cottage cheese.
-control cottage cheese obtained with citric acid, although it had a high total protein according to the Kjeldahl method, showed less bioavailability and a variety of amino acids.

Conclusions and Recommendations

-Lactobacillus fermentum, isolated from mint, effectively improves the biochemical composition of cottage cheese.
-Experienced cottage cheese contains more free amino acids and a variety of protein fractions, which makes the product more functional.
-It is recommended to use L. fermentum for the production of functional dairy products with an improved amino acid profile.
-Future studies may include sensory evaluation, fermentation stability, and effects on consumer health.

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