

Effect of Macgel functional mixture on whey protein denaturation

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Abstract

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Introduction. The aim of the study was to investigate the effect of the functional mixture Macgel MT 100 on the thermoacid denaturation of whey proteins.

Materials and methods. Rennet whey and the functional mixture Macgel MT 100 were used in this study. The yield of protein concentrates, sensory attributes, moisture content, moisture evaporation dynamics determined by thermogravimetric analysis, and the water-holding capacity of the protein concentrates were evaluated.

Results and discussion. The use of the functional mixture Macgel MT 100 intensified the production of protein concentrate. Addition of 0.5% Macgel MT 100 to native whey reduced coagulation time by 20 ± 1 min compared with the control under identical processing conditions and increased protein concentrate yield by an average of $16.4 \pm 0.2\%$. The shortened duration of whey protein denaturation was associated with reduced energy consumption and promoted faster and more efficient unfolding of polypeptide chains, as well as the formation of new intermolecular cross-links. These effects resulted in the development of a denser and more stable protein matrix. The protein concentrate obtained with the addition of the functional mixture Macgel MT 100 exhibited greater structural homogeneity and a slightly spreadable consistency, along with lower moisture content, higher water-holding capacity ($52.1 \pm 1.3\%$), and an increased proportion of bound water compared with the control. The moisture evaporation dynamics of model protein concentrate samples were investigated. In the control sample, most of the moisture was removed within 7.0–8.0 min, whereas in the sample containing Macgel MT this process occurred within 9.0–10.0 min. The delayed moisture evaporation observed for the Macgel MT-enriched sample indicates an increased water-holding capacity and the formation of a more stable protein matrix with a higher proportion of bound water. The use of Macgel MT 100 to accelerate the process of thermoacid coagulation contributes to solving technological and raw material problems associated with the need for complete recovery of valuable whey proteins. The obtained protein concentrate is recommended for use as a milk-protein component of thermized multicomponent albumin products, semi-finished products, etc.

Conclusions. The effectiveness of the functional mixture Macgel MT 100 in intensifying the thermoacid denaturation process of whey proteins was confirmed.

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Introduction

For dairy processing enterprises, the problem of rational utilization of whey remains relevant. Standard processing approaches, including drying and membrane technologies, cannot be implemented at enterprises, including craft and artisanal ones, due to the lack of appropriate production capacities or insignificant volumes of raw material (Mirzakulova et al., 2025; Puryhin et al., 2025).

In Ukraine, due to insufficient implementation of economically feasible, low-cost, and technologically simple solutions that do not require the installation of additional equipment, whey at some enterprises is still not considered a full-fledged dairy raw material. Most often, after pasteurization, it is sent to bakery and confectionery enterprises or used for feed purposes in animal husbandry (Besediuk et al., 2024; Kochubei-Lytvynenko et al., 2022; Sychevskiy et al., 2019).

The average chemical composition of whey is (%): total solids – 4.5–7.2; proteins – 0.5–1.1; lactose – 3.9–4.9; milk fat – 0.3–0.5; minerals – 0.3–0.8 (Czarniecka-Skubina et al., 2025; Mirzakulova et al., 2025). At the same time, whey proteins are characterized by a uniquely balanced amino acid composition and are a valuable raw material for food production (Kochubei-Lytvynenko et al., 2023; Shevchenko et al., 2022; Yiğit et al., 2023). Their biological value is 104%, which exceeds the corresponding values of egg protein and casein (Escobedo-Monge et al., 2025).

Whey proteins do not form micelles, do not coagulate under the action of enzymes, and do not precipitate when acidified to pH 4.7. Conventionally, whey proteins are divided into thermolabile and thermostable fractions. Thermolabile fractions precipitate in an acidic environment during boiling for 28–32 min, whereas thermostable proteins remain in solution but undergo denaturation under the influence of acids (Minj et al., 2020).

Improvement of methods for recovering concentrates from whey and their use in various product technologies, including semi-finished products, as a base or fortifier is relevant. Thermoacid denaturation, as a classical processing method, involves treating whey at temperatures above 90–95 °C for about 88–92 min at pH 4.4–4.6, which results in high energy intensity of the process. Under such conditions, the degree of protein fraction recovery is approximately 40% (Sukmana et al., 2025).

Incomplete protein recovery is explained by the protective effect of electrolytes present in whey, as well as the dominance of the electrical charge of protein particles as a factor of their colloidal stability (Freire et al., 2022). To intensify the process of thermal denaturation, coagulant reagents are added to rennet whey, in particular whey with an acidity of 145–155 °T. The optimal pH range for the process is 4.4–4.6, which corresponds to a titratable acidity of 30–35 °T and the isoelectric point of thermolabile whey proteins, in particular the lactoalbumin fraction (Chen J. et al., 2023).

Tsygankov et al. (2018) have shown that the use of the food additive “Collagen pro 4402” in the production of albumin mass from liquid whey protein concentrate and rennet whey increases product yield and improves quality characteristics. Improvement of the thermoacid coagulation process of whey proteins requires additional scientific research to reduce denaturation time and increase protein recovery efficiency. In this context, the possibility of using the functional mixture Macgel MT 100 to intensify the denaturation process of whey proteins was considered.

Macgel MT 100 is used in the food industry to regulate consistency and increase product yield. It allows control of viscosity, texture, water retention, and product stability. In meat systems, such technological ingredients contribute to compaction of consistency, formation of gel structures, and reduction of syneresis, which increases product quality and yield (Bao

et al., 2025). In dairy products, it provides a smooth surface, increased yogurt viscosity and cheese yield, and improved textural and rheological properties of fermented dairy products (Alam et al., 2025). According to the manufacturer FOODTECH, the recommended addition level of Macgel MT 100 is 0.5–0.7 g/kg of mixture. The functional additive consists of maltodextrin and the enzyme transglutaminase, which determine its functional properties and affect the rheological characteristics and stability of food systems.

Recent scientific studies confirm that the use of transglutaminase in cheese production significantly increases overall cheese yield (El Kiyat et al., 2021; Ivanov et al., 2021). The application of transglutaminase in combination with ultrasonic treatment in the production of soft cheeses from camel milk increased product yield while maintaining appropriate physicochemical and sensory characteristics (El-Sayed et al., 2025). In addition, transglutaminase contributes to an increase in the degree of protein and milk solids utilization in soft cheese production, which reduces protein losses in whey and increases the efficiency of the technological process (Velazquez-Dominguez et al., 2023). Thus, Macgel MT 100 can be an effective technological ingredient for increasing cheese yield, improving consistency, rheological characteristics, and the nutritional value of cheese products. The aim of the present study was to investigate the effect of the functional mixture Macgel MT 100 on the process of thermoacid denaturation of whey proteins.

Materials and methods

Materials

The raw material used for protein concentrate production was rennet whey with the following physicochemical characteristics: total solids content of $6.1 \pm 0.3\%$, including protein $1.0 \pm 0.05\%$, fat $0.1 \pm 0.01\%$, milk sugar (lactose) $4.5 \pm 0.2\%$, and minerals $0.5 \pm 0.02\%$. The whey had a titratable acidity of 18 ± 2 °T and a density of 1018 kg/m^3 . The technological ingredient, the functional mixture Macgel MT 100, was a powder ranging in colour from white to light beige, with a taste and odour characteristic of the product, and a moisture content not exceeding 14%.

Method for obtaining whey protein concentrate

The study of the duration of the thermoacid coagulation process of native rennet whey was carried out as follows. From 4 l of milk whey, after reaching the optimal pH of 4.4–4.6 and a temperature of 90–95 °C, the process was conducted until protein flocs were formed. Every 10 min, the protein concentrate was removed using the same method. The duration of thermal treatment was 90 ± 2 min.

The technological ingredient (functional mixture) Macgel MT 100 was added to the milk whey at the initial stage of thermal treatment in the form of a hydromodule (additive and milk whey in a ratio of 1:12) with holding for 30 ± 2 min. Compliance with the above conditions is necessary for uniform distribution of the ingredient throughout the entire volume of whey. For research purposes, the application range of Macgel was expanded from 0.2% to 0.6%.

Methods for determining the quality parameters of protein concentrates

The yield of protein concentrates was determined by the formula:

$$B = \frac{M_{mcg}}{M_{mc}} \cdot 100$$

where B is the yield of concentrates, %;

M_{mcg} is the mass of protein concentrate with Macgel MT 100, g;

M_{mc} is the mass of protein concentrate without the functional mixture, g.

The amount of protein concentrates (g) obtained from 4 l of rennet whey under the corresponding conditions was determined by the gravimetric method.

The organoleptic properties of protein concentrates were evaluated in accordance with the requirements of regulatory and technical documentation. Appearance and color were assessed visually; taste and odor, as well as consistency, were evaluated organoleptically at a sample temperature of 18–20 °C.

The mass fraction of moisture and the dynamics of moisture evaporation of protein concentrates were measured by the thermogravimetric method using laboratory electronic moisture-analyzer scales of the ADS series manufactured by AXIS (Poland).

The water-holding capacity of protein concentrates was determined by the mass of water released from the sample under slight pressing and absorbed by filter paper (Chubenko et al., 2025).

Results and discussion

Effect of functional mixture Macgel MT 100 on yield of protein concentrates

The change in the yield of protein concentrate depending on the amount of the functional mixture Macgel MT 100 added is shown in Figure 1. The control was a protein concentrate obtained by classical technology without the use of a functional mixture. The obtained results regarding the yield of protein concentrate were adjusted depending on the amount of dry matter of the added functional mixture.

According to the results (Figure 1), the addition of the functional mixture Macgel MT 100 to rennet whey in an amount of 0.5 % increases the yield of protein concentrate by an average of $16.4 \pm 0.2\%$ compared to the control under the same conditions of thermoacid coagulation. This can be explained by the fact that transglutaminase in rennet whey forms strong chemical bonds between individual whey protein molecules, combining them into a stable network, in particular β -lactoglobulin and α -lactalbumin, both with each other and with casein residues, which results in the formation of a stable three-dimensional protein matrix (Ceren Akal, 2023; El Kiyat et al., 2021). Such enzymatic modification reduces protein losses in whey and increases the water-holding capacity of the protein concentrate, which leads to a significant increase in its yield and improvement of the rheological and structural characteristics of the product (Kim et al., 2023).

Thus, the use of the functional mixture Macgel MT 100 ensures more efficient incorporation of whey proteins into the total volume of the concentrate and increases the technological and economic efficiency of production. The addition of the functional mixture Macgel MT 100 in an amount of 0.2–0.4% increases the yield of protein concentrate to a lesser extent, while the addition of 0.6–0.7% is irrational, since only a 0.5–0.6% increase in mass yield is observed.

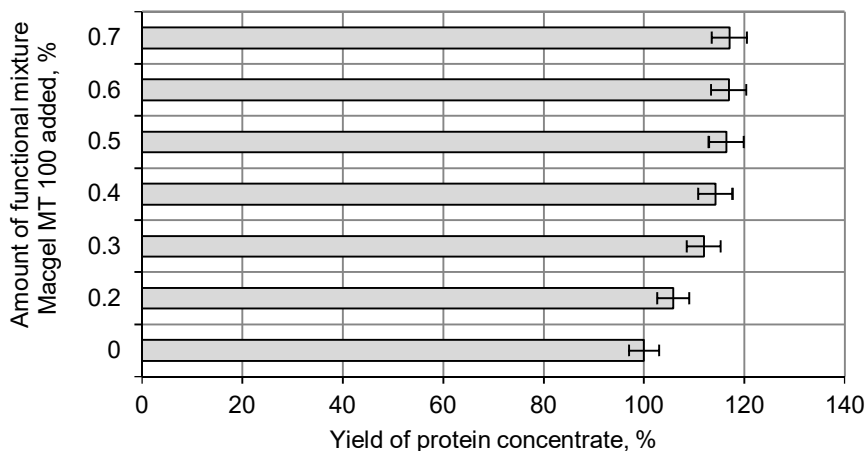


Figure 1. Effect of functional mixture Macgel MT 100 on yield of protein concentrate

Protein concentrate accumulation depending on the duration of protein denaturation

Figure 2 presents the dynamics of protein concentrate accumulation depending on process duration under constant conditions ($t = 90\text{--}95\text{ }^{\circ}\text{C}$, $\text{pH} = 4.6\text{--}4.4$). The amount of the functional mixture Macgel MT 100 added was 0.5%.

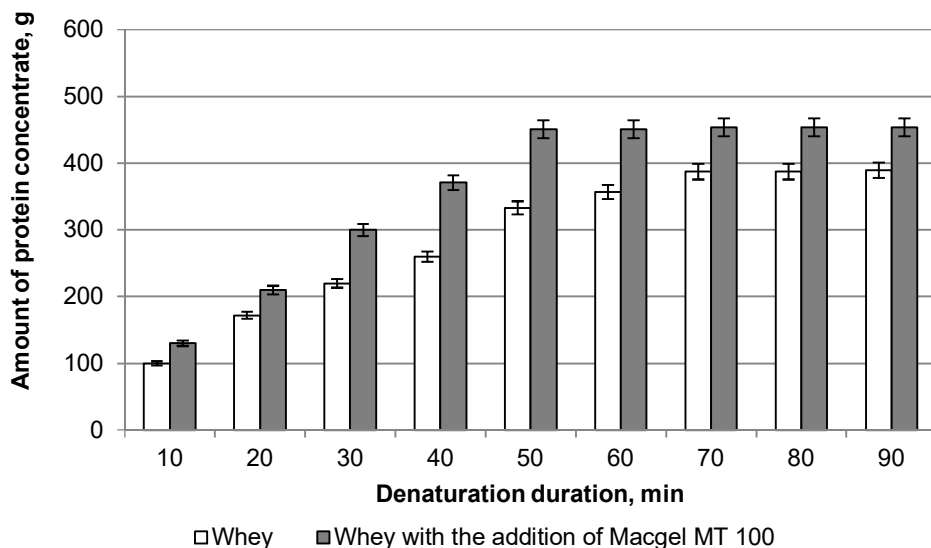


Figure 2. Protein concentrate accumulation depending on the duration of protein denaturation

An increase in denaturation time led to a marked rise in protein concentrate yield for both samples; however, the rate of product accumulation was significantly higher in whey supplemented with Macgel MT 100. Thus, after 50 ± 2 min of thermal treatment, the yield of concentrate in the sample with the additive reached 450.7 ± 1.1 g, after which a further increase in heating duration was not accompanied by a significant increase in product mass.

For the control sample, the maximum effective increase in concentrate yield was observed up to 70 ± 2 min of denaturation, while the absolute yield was 387.2 ± 1.1 g. Further thermal treatment was inefficient and resulted only in a slight increase in concentrate mass.

The effective denaturation time for whey with Macgel MT 100 is 50 ± 2 min, whereas for the control it is (70 ± 2) min. Further thermal treatment up to 90 ± 1 min is impractical, since the additional product yield is insignificant and amounts to only 453.8 ± 1.1 g for the sample with Macgel and 389.6 ± 1.0 g for the control.

The obtained data are consistent with scientific studies indicating a significant effect of time and thermal treatment conditions on whey protein denaturation. Modification of protein systems through the addition of specific protein or functional components can change the kinetics of denaturation and aggregation, affecting the technological parameters of the final product (Pu et al., 2024; Wang et al., 2024). This is consistent with the experimental values obtained, where the introduction of Macgel MT 100 leads to faster denaturation and higher product yield at a shorter heating time.

Therefore, the use of the functional mixture Macgel MT 100 makes it possible to reduce the duration of effective thermal treatment by 20 ± 1 min compared to the control, while simultaneously ensuring a significantly higher absolute yield of protein concentrate.

Quality parameters of protein concentrates

Table 1 presents the results of the effect of the functional mixture Macgel MT 100 on the quality parameters of the protein concentrate.

Table 1

Quality parameters of protein concentrate with the functional mixture Macgel MT 100

Parameters	Characteristics of protein concentrate	
	Control	With the addition of Macgel MT 100
Organoleptic parameters		
Consistency	Homogeneous, delicate	Homogeneous, delicate, slightly buttery
Taste and odor	Clean, fresh with an albumin aftertaste	
Color	White	
Physicochemical parameters		
Titrateable acidity, °T	93.0±1.7	81.0±1.1
Mass fraction of moisture, %	77.4±1.5	74.8±0.8
Water-holding capacity, %	48.8±1.2	52.1±1.3

The control sample of the concentrate was characterized by a homogeneous, delicate consistency, a clean and fresh taste with a characteristic albumin aftertaste, and a white color. The taste, odor, and color of the product remained appropriate for a protein concentrate, without foreign flavors.

The introduction of Macgel MT 100 contributed to the formation of a more homogeneous structure and a slightly buttery consistency. These results are likely related to the fact that the enzyme transglutaminase, which catalyzes the formation of cross-links between amino acids of proteins, is able to modify the structure of whey proteins, improving their physicochemical properties such as gel formation, emulsion stability, and microstructure of products after thermal treatment (Chen J. et al., 2025). Similarly, enzymatic modification of whey proteins using transglutaminase improves the mechanical properties and microstructure of protein gels, indicating strengthening of intermolecular bonds in protein aggregates (Zhang et al., 2023).

Analysis of physicochemical indicators showed that the use of the functional mixture Macgel MT 100 significantly affects the acidity and moisture content of the concentrate. The mass fraction of moisture in the concentrate with Macgel MT 100 decreased by $2.6 \pm 0.3\%$ compared to the control sample. Titratable acidity in the sample with the additive was 81 ± 1.1 °T, which is lower by 12 ± 1.1 °T compared to the control.

The decrease in acidity may be associated with stabilization of the protein matrix and changes in the course of denaturation–aggregation processes during thermal treatment of proteins in the presence of modifying agents, which corresponds to general patterns of changes in protein properties under the influence of enzymatic modifications (Ma et al., 2025).

It was found that the water-holding capacity of the protein concentrate sample in the presence of Macgel MT 100 increased by $3.3 \pm 0.7\%$ compared to the control. This is likely related to the mechanism of action of transglutaminase, which is a component of Macgel MT 100 and catalyzes the formation of covalent ϵ -(γ -glutamyl)-lysine bonds between protein chains, strengthening the three-dimensional gel structure and limiting water mobility within the protein matrix (Chen X. et al., 2025).

Modern studies confirm that the use of transglutaminase increases the density of protein gels and improves water retention capacity in protein systems, in particular in starch–meat composite gels (Wu et al., 2023) and in plant protein isolates (Min et al., 2025), as well as forms a homogeneous and compact network that promotes molecular water retention (Zhan et al., 2022).

The sample with Macgel MT 100 is characterized by increased water-holding capacity. This is explained by the fact that the protein matrix effectively retains water inside the gel, which potentially improves the structural properties of the product.

The use of the functional mixture Macgel MT 100 makes it possible not only to preserve high sensory parameters of the protein concentrate but also to improve its structural characteristics, reduce acidity, and optimize moisture content, which is important from the standpoint of technological stability and quality of the final product.

Effect of the functional mixture Macgel MT 100 on the dynamics of moisture evaporation from protein concentrates

The dynamics of moisture evaporation from model samples of protein concentrates are shown in Figure 3.

Measurements showed that the main portion of moisture (free water) was removed faster from the protein concentrate without the functional mixture 7.0–8.0 min than from the sample with Macgel MT 100 (9.0–10.0 min). This slower moisture evaporation in the Macgel-containing sample indicates a higher water-holding capacity, likely due to the formation of more stable gel-forming protein structures that bind water within the matrix (Liu et al., 2025).

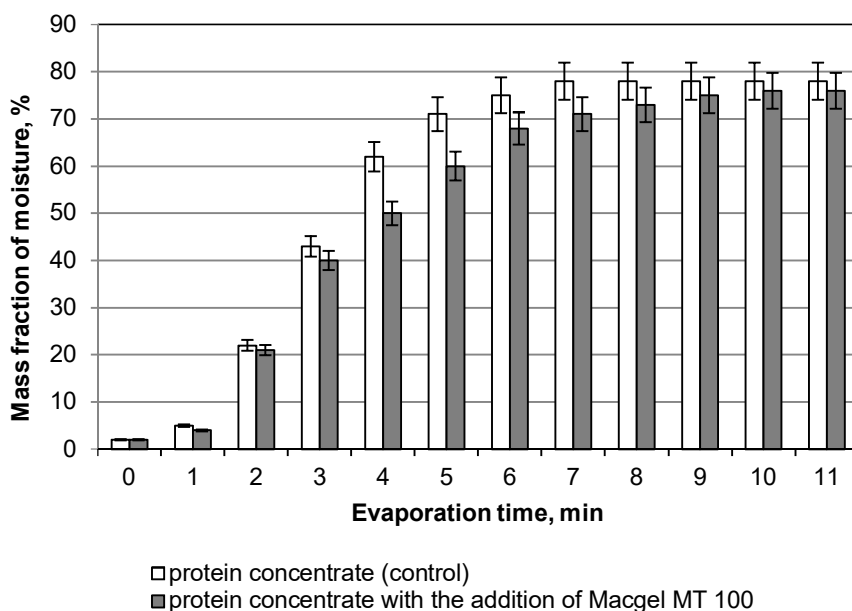


Figure 3. Moisture evaporation dynamics of model protein concentrate samples with and without Macgel MT 100

At the molecular level, thermoacid treatment of whey protein causes unfolding of polypeptide chains that were folded in the native molecule, resulting in the exposure of hydrophobic and charged polar groups on the surface (Freire et al., 2022). This allows the formation of new cross-links between protein molecules and with water, creating a dense, partially gel-forming matrix (Seddiek et al., 2025). As a result of these structural changes, free water partially transitions into a bound form, being firmly retained in the protein matrix, which slows its evaporation (Ozel et al., 2021).

In the initial evaporation phase (up to 2 min), removal of free water is observed, with the difference between samples being about 1–2 min. In the middle phase 3–7 min, the sample with Macgel MT 100 shows a more pronounced lag compared to the control (difference up to 10 min). This indicates delayed diffusion of water within the protein structure and a higher proportion of bound water. By the end of the experiment 8–11 min, the mass fraction of moisture stabilizes at the level of 76–78 % for both samples, indicating the achievement of equilibrium moisture content.

Thus, the addition of Macgel MT 100 promotes the formation of a more stable and dense protein matrix that retains moisture and slows the evaporation process. This confirms the prospects of using the functional additive to enhance the water-holding and textural properties of protein concentrate under thermoacid treatment conditions.

Conclusions

It was established that the addition of 0.5% of the functional mixture Macgel MT 100 to milk whey increased the yield of protein concentrate by an average of $16.4 \pm 0.2\%$ compared to the control under the same thermoacid coagulation conditions and shortened the

process duration. The effective coagulation time for whey with Macgel MT 100 was 50 ± 2 min, whereas for the control it was 70 ± 2 min. Quality indicators of protein concentrates were also investigated, showing that the addition of Macgel MT 100 contributed to more pronounced structural homogeneity and a slightly buttery consistency of the product. The mass fraction of moisture and titratable acidity of the experimental sample decreased by $2.6 \pm 0.3\%$ and $12 \pm 1.1^\circ\text{T}$, respectively, compared to the control, while water-holding capacity increased by $3.3 \pm 0.7\%$. Thermogravimetric analysis revealed that during the middle phase of evaporation, the difference between the experimental and control samples reached 10 min, and at the end of the process, the moisture content of the concentrates remained at 76–78%. These results confirm the formation of a denser and more stable protein matrix in the protein concentrate.

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