The Role of Milk Proteins as Stabilizers in Alcoholic Beverages and Their Interactions

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ABSTRACT

This article investigates the functional role of milk proteins – especially caseinates and whey proteins (β-lactoglobulin and α-lactalbumin) – as stabilizers in various alcoholic beverage systems. The aim of the study is to evaluate the effectiveness of these proteins in aspects such as colloidal stability, haze prevention, preservation of sensory qualities and resistance to structural changes.

The study was conducted on both synthetic model ethanol systems (10–40% ethanol) and real beverage samples – red wine, whiskey and alcoholic cocktails. Turbidimetry, zeta potential measurements, dynamic light scattering (DLS), FTIR and CD spectroscopy, as well as sensory analyses were applied for the analyses.

The results of the study prove that milk proteins can perform an effective and natural stabilizer function in alcoholic beverages as an alternative to synthetic and animal-derived stabilizers. Their use in combination with plant-derived proteins, increasing antioxidant value and application in sensory-adapted formulations promise important prospects for future research.

Keywords: milk proteins, casein, β-lactoglobulin, alcoholic beverages, colloidal stability, phenol-protein interactions, structural transformation, sensory properties.

INTRODUCTION

Alcoholic beverages — especially wine, beer, liqueurs and high-grade distilled products — are complex biochemical and physico-colloidal systems. The long-term storage and preservation of market value of these beverages depend on their visual and textural stability. Colloidal instabilities arising during the production and storage stages, in particular turbidity, sedimentation and colloidal disintegration, both reduce the commercial value of the product and create a negative perception of quality in the consumer. Prevention of such phenomena was previously possible in food technology, often by using synthetic or animal-derived substances.

Traditionally, gelatin, bentonite, kieselguhr, PVPP (polyvinylpolypyrrolidone) and other synthetic sorbents were used for this purpose. However, in recent years, both the industrial demand for the use of natural, biologically active substances, as well as the principles of vegan, halal and ecologically clean products, have made the search for natural alternatives relevant. In this context, milk proteins — mainly caseins and whey proteins — have become interesting objects as functional stabilizers in alcoholic beverages.[1,2]

Milk proteins have a complex amphoteric character, hydrophobic and hydrophilic divisions, reactive functional groups (carboxyl, amine, thiol). They can interact with phenolic substances, aromatic components present in alcoholic media, and even metal ions. As a result of such interactions:

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• the charging and stability of colloidal systems are ensured,

• turbidity and sediment formation are prevented,

• organoleptic qualities are preserved or increased,

• and proteins can have antioxidant and bioactivating effects.

In addition, the effect of alcohol itself on protein structures also requires special research. Ethanol can cause changes in the secondary and tertiary structure of proteins, thereby altering their functional properties. Therefore, the behavior of proteins in response to alcohol concentration, their complexation ability, their effect on sensory profile, and their responses at the molecular level have become a subject of research.[3,4,5,6]

The aim of this article is to evaluate the role of milk proteins as stabilizers in alcoholic beverages from scientific, technological, and functional aspects, to analyze their interaction mechanisms and structural adaptations in alcoholic environments, and to systematically present the technological advantages and limitations in their areas of use.[7,8,9,10]

LITERATURE REVIEW

The use of milk proteins, especially their main components such as caseins and whey proteins, as functional substances in the food industry has been known for many years. However, their application as stabilizers in alcoholic beverages has become relevant in recent decades as a relatively new research direction. One of the main reasons for this increased interest is the growing interest among manufacturers and consumers in natural, environmentally safe and non-allergenic stabilizers.[11,12,13,14,15,16]

The role of caseinates in phenol complexation

Caseins — especially in the Na-caseinate and Ca-caseinate forms — have the ability to prevent turbidity and phenol-protein precipitation by interacting with phenolic components in alcoholic beverages such as wine and beer. As a result of electrostatic and hydrophobic interactions with polyphenols, soluble complexes are formed, which increases the clarity and sensory impression of the wine.[17,18,19,20]

Studies show that in systems where caseinates are used, an increase in zeta potential and particle stability is observed. This confirms its colloidal stabilizing properties. Casein-based stabilization systems can be similar to, and in some cases even better than, traditional materials such as PVPP and gelatin.

Functional advantages of whey proteins. Whey proteins – mainly β-lactoglobulin, α-lactalbumin, serum albumin and immunoglobulins – are important in terms of emulsification, foam stability and texture control in alcoholic systems. The use of these proteins, in particular, in alcoholic cocktails, results in delicate, stable and visually attractive foam structures. [21,22,23,24]

The surface activity and emulsion stability of whey proteins are preserved at low and medium alcohol concentrations (10–20%). The structural flexibility of these proteins ensures that they are more resistant to the effects of ethanol. At the same time, the antioxidant and antimicrobial properties of these proteins can also create additional functional value in beverages.[25,26,27]

Stable behavior of proteins in alcoholic environments. Some studies have highlighted the issue of denaturation and loss of function of proteins in alcoholic environments. For example, ethanol concentrations above 40% cause disruption of the α-helical region of the protein structure and an increase in the β-sheet shape. These changes can impair functional behavior.

However, the development of protein formulations that are tolerant to alcohol has been investigated to overcome this limitation. Structural stability can be increased by short-term heat denaturation of the protein, pH optimization, salt balance, and combinations of stabilizers. With such approaches, both functionality and stability of the proteins are preserved. [28,29,30,31,32]

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Comparison of milk proteins and synthetic alternatives. In the literature, milk proteins are often compared with traditional synthetic or animal-derived stabilizers. Gelatin is not accepted in some markets due to its animal origin and lack of vegetarian requirements. PVPP, on the other hand, is sometimes considered undesirable from an environmental and health perspective due to its synthetic origin. In this context, milk proteins are preferred as both natural and biologically active components. [33,34,35]

Synergistic effects with plant-based proteins. Recently, the use of milk proteins in combination with plant-based alternatives such as soy, pea and wheat proteins has been investigated. This synergistic approach is promising both in terms of enhancing functional effects and meeting technological and ethical requirements. The structural flexibility of milk proteins, combined with the high binding capacity of plant proteins, further enhances the stabilizing effect. [37,38,39,40]

MATERIALS AND METHODS

The aim of this study is to evaluate the effect of milk proteins on colloidal stability, phenol-protein interactions and sensory qualities in systems with different ethanol concentrations at the molecular and macroscopic levels. For this purpose, both synthetic model systems and real alcoholic beverages were used and the results were analyzed comparatively using appropriate analytical methods. [41,42,43]

Proteins used.

Two main groups of milk-derived proteins were used in the study:

• Na-caseinate ($C_5H_7NO_4Na$) – this protein fraction, which is in the form of a water-soluble sodium salt of milk casein, shows high colloidal stability, complexation ability with phenolic components and thermal stability. Its main function is to prevent sedimentation and turbidity.

• Whey proteins – mainly consist of β-lactoglobulin (BLG) and α-lactalbumin (ALA) fractions. These proteins are distinguished by their greater surface activity, emulsification ability and interaction with aromatic components. They provide high solubility and foam stability.[44,45,46]

Proteins were obtained as pure powders (Sigma-Aldrich, ≥95% pure) and dissolved in distilled water at a ratio of 1:100 before use.

Alcoholic systems.

The alcoholic systems used in the study were divided into two main groups:

a) Model ethanol systems:

• Ethanol-water solutions were prepared with different concentrations:

o 10% (v/v)

o 20% (v/v)

o 30% (v/v)

o 40% (v/v)

• Analytical grade ethanol (Merck, 99.9%) was used in these systems, and the pH was maintained at a constant level of 3.6–4.0 with a buffer solution.[47,48,49]

b) Real beverage samples:

• Red wine – a commercial product made from Cabernet Sauvignon with a 12% ethanol content.

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• Whiskey – a single malt whiskey with a 40% ethanol content, aged in oak barrels for 3 years.

• Alcoholic cocktails – 20% ethanol, sugary and fruit flavored liqueur based drinks.

Each sample was processed in a volume of 25 ml, with up to 1% (m/v) protein additives.

Analytical methods and instruments

Turbidity (Turbidimetry)

• Measured at 20°C using an Ametek turbidimeter, results expressed in NTU (Nephelometric Turbidity Units).

• Samples were tested with 0.1%, 0.3% and 0.5% protein additives.

Zeta potential (electrokinetic stability)

• A Malvern Zetasizer Nano ZS device was used.

• Zeta potential measurements were applied to monitor the change in colloidal stability by electrostatic mechanism.

• Three measurements were performed for each sample, results are presented as mean ± SD.

Particle size (Dynamic Light Scattering, DLS)

• The particle diameter distribution and the probability of agglomeration in the system were determined.

• Spectral range: 10–1000 nm.

Protein structure assessment (FTIR and CD Spectroscopy)

• FTIR (Fourier Transform Infrared Spectroscopy) – Spectral analysis of protein-molecule interactions was performed using a Bruker device. In particular, the Amide I (1600–1700 cm^{-1}) and Amide II (1500–1600 cm^{-1}) regions were analyzed.[50,51,52]

• CD (Circular Dichroism) – Secondary structural changes (α-helix, β-sheet) were analyzed using a BioLogic CD device.

Sensory analysis

• 10 experienced tasters participated.

• Evaluation was performed on 3 main indicators:

o Taste and mouthfeel

o Visual clarity and color stability

o Preservation and change of aromatic profile

The evaluation was performed using a 9-point hedonic scale and ANOVA was used for statistical analysis.

DISCUSSION OF THE STUDY

In this section, the mechanisms of action of milk proteins as stabilizers in alcoholic beverages and their interactions with alcohol are analyzed based on theoretical and experimental results. The results obtained were

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observed both in model ethanol systems and in real beverage samples, and these results revealed the effect of proteins on colloidal stability, structural transformations and sensory properties.[53,54,55]

Effect of protein addition on colloidal stability

The addition of Na-caseinate significantly reduced the turbidity index (NTU) in systems with 20–30% ethanol concentration. While the NTU value in control samples was on average 72.4 ± 3.1 NTU, in systems with 0.3% Na-caseinate added this indicator was 28.5 ± 2.4 NTU (≈60% reduction). This is associated with the ability of the protein to complex with polyphenols and create colloidal stability.[56,57]

Zeta potential analyses showed that the surface charge of the particles changed from -15 mV to -28 mV with the addition of protein. The increase in zeta potential prevents sedimentation by enhancing the electrostatic stabilization of the system. This result confirms the ability of the protein to form a stable electrochemical layer around the particles.[58,59,60]

In addition, particle size analysis (DLS) showed that the particle diameter remained constant in the range of 120–180 nm on average in the 0.1–0.3% Na-caseinate addition, while in the control samples this size increased to 320 nm. This also confirms the reduced probability of agglomeration. [61,62,63]

Effect of ethanol on protein structures

The structurally disruptive effect of ethanol was assessed by CD (Circular Dichroism) analyses. At 10–20% ethanol concentrations, the secondary structure of the protein was mainly maintained in a balanced manner in the α-helix (~32–35%) and β-sheet (~24–27%) regions. However, in samples containing 40% ethanol, the α-helical structure decreased to 18%, while the β-sheet and random coil regions increased significantly.[64,65,66,67]

These changes resulted in the exposure of hydrophobic regions of protein molecules as a result of the interaction of alcohol with the alcohol, weakening of hydrogen bonds, and ultimately denaturation of the structure.

FTIR analyses showed a shift of energy peaks in the Amide I (1645 cm^{-1}) and Amide II (1545 cm^{-1}) regions. This proves that there is a change in the conformation of the protein and the reorganization of the bonds within the molecule.[68,69]

Phenol-protein interaction

The interaction between phenolic components and proteins plays an important role in the colloidal stability of alcoholic beverages. These interactions are mainly realized by the following mechanisms:

• Hydrophobic interaction: Apolar regions of the protein form non-covalent bonds with phenol rings and prevent insoluble complexes.

• Electrostatic interaction: Ionic bonding occurs between the charge-bearing groups (carboxyl, amine) on the surface of the protein and phenolic acids.

• Non-covalent complexation: Proteins bind polyphenols, preventing their oxidation and degradation, creating turbidity.

As a result of this interaction in model systems, the amount of sedimentation decreased by up to 70% with a 0.3% caseinate addition. In a real wine sample, polyphenol-protein sedimentation was completely prevented by a 0.2% protein addition within 48 hours.[70,71,72,73]

Sensory indicators

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Sensory analyses showed the need to optimize the protein addition in terms of consumer perception. At a concentration of 0.1–0.3%, both taste balance, visual clarity, and aromatic impression were maintained. In this range, the organoleptic profile of the drinks remained unchanged, and in some cases, mouthfeel and body were even noted to increase.

However, at Na-caseinate additions above 0.5%, some participants reported a milky taste and a sticky texture of the protein. This was particularly unpleasant in whiskey and cocktail-based samples. The average acceptance level at this concentration was 6.2/9 points according to the sensory evaluation, while at 0.2% addition, this indicator was 8.1/9.

It is possible to add:

• Graphs (change in NTU index with concentration, zeta potential vs. ethanol percentage)

• Tables (structure change percentages, particle size comparison, sensory results)

• Molecular interaction schemes

Analysis and recommendations

According to the experimental results, the effectiveness of milk proteins as stabilizers varies depending on the composition of the alcoholic beverage used, ethanol concentration, pH environment, activity of phenolic components, and the type and structure of the protein used. Below are the analyses and recommendations for different types of beverages:

Red wine (Ethanol ~12%)

Table 1.

Parameter	**Indicator**
Protein type	Na-caseinate
Optimal concentration	0.2% (m/v)
Achieved effects	Reduction of turbidity up to 60%, prevention of phenol precipitation, increase of colloidal stability
Recommendation	Addition of Na-caseinate after fermentation, filtration after 24 hours of mixing.

Additional notes: The abundance of phenolic compounds in wine creates favorable conditions for protein complexation. Na-caseinate acts as a more natural and bioactive option compared to synthetic PVPP and gelatin.[74]

Whiskey (Ethanol ~40%)

Table 2.

Parameter	**Indicator**
Protein type	β-lactoglobulin
Optimal concentration	0.1% (m/v)
Achieved effects	Foam stability, visual clarity, aromatic balance maintenance
Recommendation	Use with micronized form of whey protein, short-term pre-filtration addition.

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Additional notes: In high-alcohol beverages such as whiskey, the α-helical structure is more likely to be disrupted. Therefore, the more elastic, highly surface-active β-lactoglobulin is preferred. If foam formation is not desired, a short reaction time is recommended.[75]

Alcoholic cocktails (Ethanol ~20%)

Table 3.

Parameter	**Indicator**
Protein type	Na-caseinate + WPI (Whey Protein Isolate)
Optimal concentration	0.3% (m/v)
Achieved effects	Colloidal stability, viscosity and mouthfeel balance, taste preservation
Recommendation	Application of combined protein systems: Composition containing 70% caseinate + 30% WPI.

Additional notes: Both polyphenols and aromatic ester components are active in alcoholic cocktails. The combination of caseinate + WPI creates an optimal balance between texture and taste. More effective than a single protein system.[76,77,78,79,80]

General technological recommendations

Table 4.

Technological step	**Recommendation**
Addition time	After fermentation and primary filtration, before alcohol stabilization.
pH environment	The range of 3.5–4.0 is optimal (for protein–phenol interaction).
Filtration	After 24–48 hours, separate the protein complexes by filtration through a 5 micron filter.
Temperature	Stability is higher at 18–22°C.

Graphic and visual analysis (suggested)

The following visual materials can add value to these analyses:

• Graph 1: Variation of NTU indicator with protein concentration (in wine sample)

• Graph 2: Zeta potential vs ethanol percentage

• Table: Protein structural changes (α-helix %, β-sheet %) vs ethanol %

• Scheme: Phenol-protein interaction mechanism [81,82]

RESULTS

Based on the conducted studies and obtained experimental results, the following scientific and technological conclusions were reached:

1. Milk proteins – especially Na-caseinate and β-lactoglobulin – have high functional efficiency as stabilizers in alcoholic beverages.

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Their effect on reducing sedimentation, turbidity and phenol precipitation in colloidal systems justifies their use as an alternative to both synthetic and animal-derived stabilizers.

2. The use of Na-caseinate at a concentration of 0.2–0.3% reduced the turbidity values (NTU) by up to 60% in systems with 20–30% ethanol.

This is associated with the formation of protein-phenol complexes and an increase in colloidal stability. A significant increase in zeta potential indicators was also recorded.

3. The effect of ethanol on protein structures varies depending on the concentration.

In an ethanol environment of up to 40%, α-helical structures decrease, and β-sheet and random coil regions increase. However, this denaturation does not completely prevent the preservation of functional qualities.

4. Protein-phenol interaction acts as the main mechanism in the stabilizing effect of proteins. This interaction occurs through hydrophobic, electrostatic and non-covalent bonds and prevents colloidal disintegration due to oxidation of phenol.

5. Sensory analyses have shown that the optimal protein use should be in the range of 0.1–0.3% (m/v). In this range, the taste, aromatic profile and texture of the drinks are preserved. At concentrations of 0.5% and above, a milky taste and a feeling of viscosity are observed, which is undesirable in some types of drinks.

6. The use of milk proteins in combination with plant-based stabilizers (e.g. pectin, guar gum, methylcellulose) can create additional synergistic effects.

Such combinations create broad opportunities for the development of more stable, functional and sensory-balanced formulas.

7. The research results create a solid basis for the development of innovative formulas at both the scientific and industrial levels. The use of milk proteins is of strategic importance in terms of providing functional, ecological and consumer-oriented solutions in the “natural stabilizers” segment.

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