Integrating Molecular Docking and Experimental Evaluation to Assess Berberine-Loaded Double Emulsions in Dairy Systems

Ammar Kadi[1], Varisha Anjum[1], Fatimah Kadhim Ibrahim AL- Mahdawi[2] and Irina Potoroko[1]
[1]*Department of Food and Biotechnology, South Ural State University, Lenin Str. 76, 454080 Chelyabinsk, Russia*
[2]*College of Dentistry, University of Diyala, 32001 Baqubah, Iraq*
ammarka89@gmail.com ,varishaanjum786@gmail.com, irina_potoroko@mail.ru

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Abstract: The dairy industry is always looking for new and creative ways to improve the traditional dairy products' nutritional and functional value. Dairy products could benefit greatly from the addition of berberine, a naturally occurring alkaloid with antibacterial and anti-inflammatory qualities. However, adding berberine straight to milk can have a negative effect on important dairy processes like fermentation and coagulation. We investigated the use of double emulsions ($W_1/O/W_2$) as a novel encapsulation technique for berberine in order to overcome this difficulty. Additionally, we investigated the interactions of berberine with key milk proteins such as whey protein, casein-α, and κ-casein using molecular docking simulations. Our findings showed that wrapping berberine helped reduce negative effects on how milk thickens and sours, keeping the usual qualities needed for dairy processing. This study highlights how using computer models along with new encapsulation methods can help create dairy products that meet today's dietary and technological needs.

1 INTRODUCTION

Berberine is a natural stuff called an isoquinoline alkaloid. You can mostly find it in plants like Berberis vulgaris, Coptis chinensis, and Hydrastis canadensis. People have done lots of studies on it because it can kill germs, stop damage to cells, calm swelling, and help control how your body works [1].

Putting berberine into foods, like milk stuff, is hard. It does not dissolve well, sticks to proteins really strongly, and can mess with things like how cheese sets and how yogurt gets sour. This can change how milk proteins act, which messes with how cheese and yogurt are made. Therefore, it is good to know how berberine acts in milk stuff and how wrapping it up in tiny bubbles can stop it from causing problems. This could help us make healthy milk products with extra good stuff in them. [1]. At the same time, lactic acid bacteria (LAB) turn lactose into lactic acid, which lowers the pH and helps keep the product stable while also affecting its taste and smell [2].

Recently, the dairy industry has shown increasing interest in functional food products that offer health benefits beyond basic nutrition [3]. This trend has spurred efforts to incorporate bioactive compounds such as polyphenols, flavonoids, marine polysaccharides, and alkaloids into dairy matrices [4]. The promising bioactive is berberine, a plant-based alkaloid known for its antimicrobial and anti-inflammatory effects [5].

Despite their therapeutic potential, direct incorporation of such compounds into dairy systems can be problematic. Bioactives like berberine may interact unfavorably with milk proteins or enzymes, potentially impairing coagulation efficiency or fermentation dynamics [6]. Moreover, their low water solubility or instability under processing conditions may reduce their functional efficacy [7].

One emerging solution to these limitations is the use of double emulsions (DEs), particularly of the water-in-oil-in-water ($W_1/O/W_2$) type, to encapsulate bioactives [8]. DEs allow for the careful release and protection of delicate compounds while reducing their direct contact with the surrounding material [9]. Previous research has demonstrated that these systems can be designed to release the enclosed active ingredients when triggered by pH, enzyme activity, or other body signals.

In dairy systems, double emulsions can be a useful way to add functional ingredients like berberine without interfering with important processes like rennet coagulation or pH-driven fermentation [10]. However, there remains a lack of comprehensive data

on how such encapsulation affects milk coagulation time and acidification kinetics during cheese-making or fermented dairy production [11].

The goal of this study is to assess how double emulsions with berberine affect the clotting process and acidity levels of cow milk.

2 MATERIALS AND METHODS

2.1 Materials

Pasteurized full-fat cow milk (3.2% fat) was sourced from a local dairy supplier. Food-grade berberine (≥98%, plant-derived alkaloid) was obtained from Sigma-Aldrich. Emulsifiers used included polyglycerol polyricinoleate (PGPR) and soy lecithin (SL), commonly used for double emulsion stabilization [10]. Commercial rennet (strength: 1:10,000 IMCU) and a regular freeze-dried starter culture with Lactococcus lactis subsp. lactis and cremoris were bought from Chr. Hansen in Denmark.

2.2 Molecular Docking Simulations

The first step in the in-silico drug design process involves identifying and selecting the suitable drug target or receptor. Docking was performed using Autodock 4.2 to investigate the interaction, binding modes, and selectivity of milk protein with the quantified secondary metabolite berberine. The crystal structures of the target proteins, specifically whey protein (7ER3), casein-α (4AA8), and kappa-casein (P02668), were sourced from the Research Collaboratory for Structural Bioinformatics (RCSB) protein data bank. Berberine, the ligand, was obtained as 3D-structured SDF files from PubChem and subsequently optimized using Ligands Input in AD 4.2. The preparation of the target enzyme with AutoDock Tools (ADT) included the addition of all polar hydrogen atoms, the merging of nonpolar hydrogen atoms, and the removal of water molecules and other heteroatoms, which is essential for calculating partial atomic charges. Gasteiger charges were assigned to the ligands, and all rotatable bonds were designated as rotatable. The protein-ligand docking was performed utilizing the Lamarckian Genetic Algorithm (LGA) method. Three-dimensional affinity grids measuring 126 × 126 × 126 Å were generated for each atom type: HD, C, A, N, OA, and SA, encompassing all probable atom types in the target enzyme. The ADT offers multiple methods for analyzing docking simulation results, including conformational similarity assessment, visualization of the binding site and its energy, and parameters such as intermolecular energy and inhibition constant [12]. Ligand molecules were optimized and subsequently docked with refined milk protein utilizing Auto Dock 4.2. The docking results were evaluated utilizing Biovia Discovery Studio 2024. A PDBQT file, an extended PDB format, was utilized for coordinating files and incorporates atomic partial charges. The ADT facilitated the conversion of PDB files into PDBQT format (Anjum et al., 2023). The ligand was docked into the active site of the Wilk protein using AD 4.2. Upon concluding the docking searches, the optimal conformation was selected from the most populated cluster exhibiting the lowest binding energy [13].

To ensure the reliability of the docking protocol, a re-docking validation was performed using the native ligands of reference proteins retrieved from the Protein Data Bank. The Root Mean Square Deviation (RMSD) between the experimental and predicted poses was calculated, with values below 2.0 Å considered acceptable. This confirmed the accuracy of the docking parameters used for berberine–protein interactions.

2.3 Preparation of Double Emulsions ($W_1/O/W_2$)

Double emulsions were prepared using a two-step emulsification method adapted from Garti & Benichou [11]:

Step 1: W_1/O primary emulsion, the internal aqueous phase (W_1) consisted of either fucoidan or berberine dissolved in distilled water. This mixture was blended into the oil phase (sunflower oil + PGPR) using a high-speed mixer (Ultra-Turrax T25, IKA, Germany) at 12,000 rpm for 3 minutes to create a stable primary W_1/O emulsion. Step 2: $W_1/O/W_2$ emulsion: the primary emulsion was then emulsified into the external aqueous phase (W_2), which contained 2% SL. The final double emulsion was homogenized again at Ultrasound. All emulsions were freshly prepared and used immediately for milk fortification.

2.4 Experimental Design and Sample Groups

Milk was divided into three treatment groups:

1) Control (no bioactives);
2) 5% Berberine without DE;
3) 5% Berberine in DE.

Each sample was prepared in triplicate. Emulsions were gently mixed into 100 mL of milk at 35°C. The

starter culture was added at 1% (v/v), followed by 1 mL/L rennet. The samples were incubated at 35°C under static conditions.

2.5 Rennet Coagulation Time Measurement

Coagulation time was determined using the visual clean break method, with observation every 5 minutes. The time point at which a firm gel was formed and showed a clean break upon probing was recorded. This method reflects enzymatic activity on κ-casein and aggregation kinetics [14].

2.6 Acidification Profile Monitoring

pH measurements were taken at 0, 30, 60, 90, 120, 180, 240, 300, and 360 minutes using a calibrated pH meter (Hanna Instruments, USA). All measurements were performed at 35°C to reflect fermentation conditions. The acidification curves were used to assess the dynamics of lactic acid production [15].

2.7 Statistical Analysis

All measurements were performed in triplicate (n = 3). Results are presented as mean ± standard deviation (SD). Data were statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to assess significant differences between groups. A p-value of < 0.05 was considered statistically significant. Statistical analyses were conducted using SPSS software (version 22.0, IBM, USA) [16].

3 RESULTS AND DISCUSSIONS

3.1 Molecular Docking Simulations

The target milk proteins, namely whey protein (PDB ID: 7ER3), casein-α (PDB ID: 4AA8), and kappa-casein (PDB ID: P02668), were virtually screened against berberine. The outcomes demonstrated that the berberine molecule was attached to the inhibitor-binding site of the enzyme. The c-helix region, recognized for phosphorylation (catalytic activity), was discovered to connect with them. Binding in this region would inhibit the key residues from being activated. Consequently, the phytocompounds' binding in that region may cause the reduction in enzyme activity. The number of intermolecular interactions, including Hb, however, was anticipated to be such that berberine would have the greatest affinity for binding whey protein, followed by casein-α and lastly κ-casein. The protein in question was chosen for more in silico research because, in contrast to berberine, it meets the prerequisites for a pharmaceutical target. The results of the virtual screening of berberine against the target protein are shown in Table 1. Inhibitory binding sites of whey protein (7ER3), casein-α (4AA8), and kappa-casein (P02668) were docked, and the binding affinity was measured in kcal/mol (Fig. 1-3). We hypothesize that the chosen proteins may be suitable targets and therapeutic candidates, given their likely interactions with the medicine to lessen the harmful effects of gastric disorders and pathologies.

The study conducted using berberine (Berb; PubChem CID: 2353) found it to have the lowest binding affinity (-7.8 kcal/mol) with whey protein (7ER3), followed by casein-α (4AA8; -6.5 kcal/mol) and κ-casein (P02668; -6.1 kcal/mol), which were considered for analyses. Berberine is a quaternary ammonium salt from the protoberberine group of benzylisoquinoline alkaloids that shows strong hydrogen bonding with Met 145 at a bond length of 2.55, 3.20, and 3.58 Å, respectively, with whey protein (Fig. 1). 5 weaker hydrogen bonds were formed with Ala 274, Thr 276, Ser 273, and Gln 278 by Casein-α (Fig. 2), whereas κ-Casein formed one strong hydrogen bond with Tyr 195 (Fig. 1-3; Table 1).

Table 1: Molecular interactions of ligands with amino acids of proteins.

Protein	Binding energy (Kcal/mol)	Interacting residue with bond length (Å)
	Berberine	
Whey Protein (7ER3)	-7.8	Met (C:145; 2.55, 3.20, 3.58); Lys (C:141; 3.70); Pro (C:144; 2.78)
Casein-α (4AA8)	-6.5	Ala (A:274; 3.44); Thr (A:276; 3.39, 3.77); Ser (A:273, 3.73); Gln (A:278; 3.93)
κ-Casein (P02668)	-6.1	Tyr (A:195; 3.03)

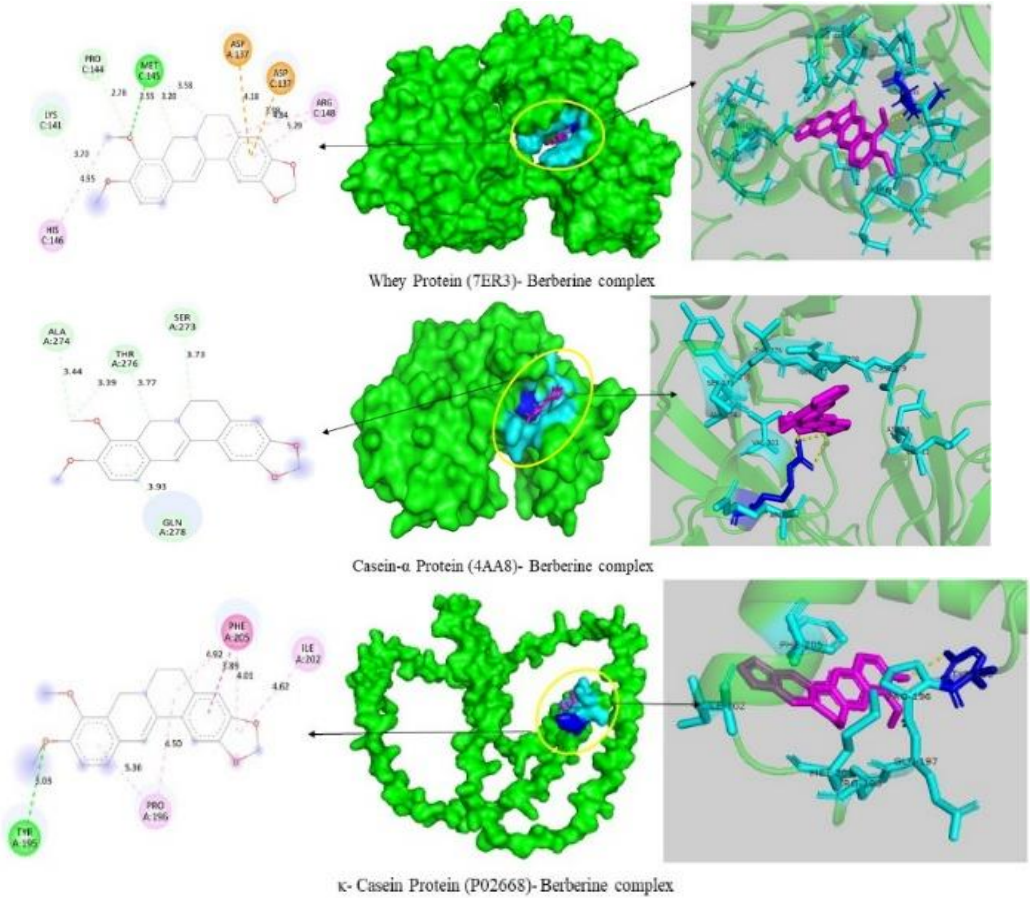

Figure 1: Predicted molecular interactions of berberine with: a) whey protein (7ER3), b) α-casein (4AA8), c) κ-casein (P02668), showing hydrogen bond distances and key amino acid residues involved in binding.

The molecular docking findings help explain the experimental trends observed in milk coagulation and acidification. The strong hydrogen bonding of berberine with κ-casein and whey protein suggests possible interference with the enzyme chymosin's cleavage sites during renneting, leading to delayed gel formation. However, encapsulation within the double emulsion likely prevented this interaction by spatially isolating berberine, allowing normal coagulation kinetics. These molecular-level interactions thus provide a mechanistic basis for the observed restoration of milk clotting behavior in the DE-treated samples.

3.2 Rennet Coagulation Time

The incorporation of bioactive compounds, both in their free and encapsulated forms, significantly affected the coagulation time of milk. As illustrated in Figure 2, the control group exhibited an average coagulation time of 12.0 minutes, consistent with expectations under standard rennet activity and milk composition [17].

The direct addition of berberine showed the most pronounced delay, with a coagulation time of 14.5 minutes, possibly due to its strong protein-binding and mild antimicrobial properties, which may slow enzymatic curd formation [18].

Berberine DE restored coagulation time to 12.0 minutes, nearly matching the control, confirming that encapsulation helped prevent premature interactions between berberine and milk proteins., Gly111(H), Ser63(M), Arg61(M), Asp208(H), Ile195(H), Gln79(M), Ala60(M), Ser76(M) as shown in Table 1.

These findings show how double emulsions help control the release of bioactive substances and keep enzymes working properly during the important coagulation phase.

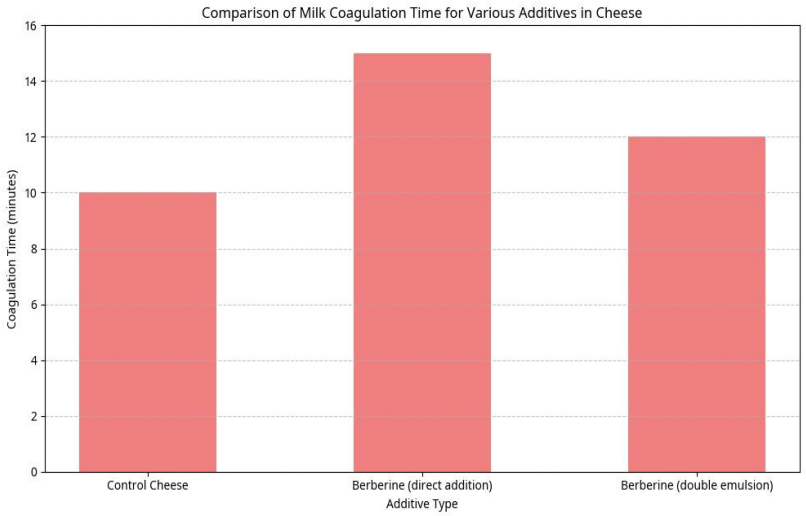

Figure 2: Rennet coagulation time (minutes) in control and treated milk samples with fucoidan and berberine (direct and DE forms).

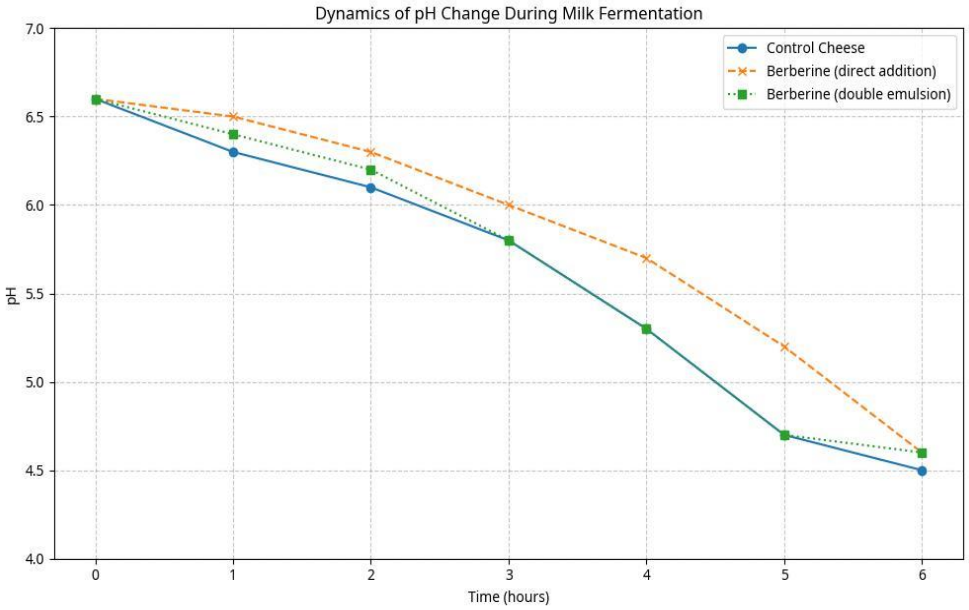

Figure 3: pH dynamics during 6-hour fermentation of milk fortified with bioactives: control (no bioactives), berberine in DE, and berberine without DE.

3.3 Acidification Profile

The acidification behavior of milk samples during fermentation is shown in Figure 3, which compares the pH decline across the control, berberine without DE, and berberine–DE treatments. Encapsulated berberine supported a faster reduction in pH compared with the direct addition group, indicating that the double emulsion helped maintain normal starter culture activity by preventing premature interactions between berberine and microbial enzymes. The direct berberine treatment, in contrast, showed a slower acidification rate, suggesting a mild inhibitory effect on lactic acid bacteria activity.

These results support the notion that double emulsions not only serve as protective carriers but also actively modulate the kinetics of acid production, crucial for controlling fermentation time, gelation, and product safety in functional dairy systems.

4 CONCLUSIONS

We found that putting berberine in a double emulsion helps stop it from messing up how milk clots and ferments. Molecular docking showed that certain parts (Met145 in whey protein and Tyr195 in κ-casein) are important for how berberine interacts with milk, and experiments proved that encapsulation fixes normal dairy functions. Next steps involve making sure the emulsion stays stable during normal factory processes and checking how berberine-boosted dairy products taste and what nutrients they provide. This gives us a way to create better dairy products with added good stuff, using computers and encapsulation.

The research demonstrates how computational modeling with food processing technologies enables developers to create advanced functional dairy products. The combination of in silico prediction with experimental validation creates an effective method to identify bioactive compounds and develop optimal encapsulation methods and predict their behavior in complex food systems. The method can be expanded to analyze different plant-based compounds, which will lead to the creation of personalized functional foods that preserve manufacturing stability and provide specific health advantages.

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