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Green Technologies of Rennet Encapsulation in Biopolymeric Microparticles as Part of Cheese Production

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Abstract

One of the essential components in cheese production is rennet, which contains enzymes that participate in the milk coagulation process. Due to the high sensitivity to external conditions, it is necessary to carry out the immobilization procedure of the present enzymes. One of the simple methods used to immobilize and stabilize enzymes is encapsulation in biopolymer matrices prepared by available biopolymers, sodium alginate, and chitosan, using methods of ionic gelation and polyelectrolyte complexation. By achieving proper conditions for the concentration of biopolymers, gelling cation, and the amount of rennet used, it is possible to tailor rennet biopolymer formulation with optimal properties and enzyme release suitable for the cheese production process.

Introduction

Cheese is one of the most significant dairy products, dates back to ancient times, and has a long development history in many parts of the world. The earliest evidence of cheese production was discovered on painted cave walls dating back to 5000 BC. It marks the beginning of cheese production technology as well as the employment of enzymes in the process of converting milk into cheese. The production process of various kinds of cheese has evolved dramatically in the last few decades, which can be attributed to extensive scientific research that has enabled the development of new technologies in the preparation and production of cheeses [1]. The cheese manufacturing process is at a high level, allowing the manufacture of functional cheeses with outstanding features and characteristics, thanks to scientific study and constant improvement, as well as the application of new materials and technology.

Cheese properties are determined by the physicochemical, microbiological, sensorial, and rheological aspects of the milk, the genotype and phenotype of the animal, and the technical procedure applied during cheese production [2-5]. Milk quality and properties are directly connected with final products. Rennet and bacterial cultures are used in the technological process of most semi-hard and hard cheeses. Rennet is a complex collection of enzymes generated in ruminant stomachs. Its main component, chymosin, is a protease enzyme that curdles milk casein. Rennet also includes enzymes other than chymosin, such as pepsin and lipase [6]. Enzymes are biocatalytic proteins that can reduce chemical and biological processes' activation energy (Ea), allowing them to proceed more quickly [7]. The problems that limit their use in industrial production are sensitivity to external influences (temperature, moisture, radiation, contamination, etc.), structural and thermal instability, the possibility of contamination of the final product due to solubility, and the possibility of reaction with environmental components. Because of the preceding, it is vital to investigate the possibility of minimizing the deficiencies mentioned earlier by applying new methods, strategies, and technologies in industrial production [8].

We can enhance enzymes characteristics and stability using techniques and strategies such as enzyme immobilization, modification, and protein engineering [9]. The enzyme immobilization treatment is one of the most frequently utilized techniques. When an enzyme molecule accumulates in or onto some suitable matrix having a specific porosity, it is said to be immobilized. Immobilized enzymes are more resistant to external conditions and can be reused, reducing operation costs. Immobilization keeps the enzyme in situ, so it is simple to remove it from the final product and employ it in a new process.

This highly effective technique is frequently used in industries where enzyme-catalyzed procedures are utilized [10]. Several techniques are used for enzyme immobilization: entrapment, adsorption, covalent binding, encapsulation, and cross-linking [11]. It is always important to consider enzymes' stability/activity ratios and possible costs in industrial production, which should be minimal while applying efficient immobilization. An effective enzyme immobilization technique is encapsulation in mostly spherical microparticles of varying complexity [12]. Encapsulated enzymes are physically separated from the curd mixture during cheesemaking, thus protecting enzymes from degradation, decreasing enzyme leakage, and reducing opportunities for contamination in the processing. Enzyme released into the matrix during ripening accelerates cheese ripening [13].

Encapsulation traps active ingredients in another substance and prepares articles from nano to millimeter sizes [14]. The benefits are: (i) active ingredients are protected from the environment, (ii) their degradation during the application decreases, (iii) lower active ingredients quantity is used, and (iv) the most important fact is the possibility of active ingredients delivery at specific times to the site of action [15,16]. Successful delivery of active ingredients at the right place and time is an attainable and desirable characteristic for all bioactive ingredient delivery systems. In order to obtain effective microparticle formulation, it is crucial to optimize the parameters during their preparation (selection of the appropriate matrix for the substance to be encapsulated and the encapsulation method/procedure). Improved understanding of the structure-property relationship in biopolymer-based microparticle formulations enhances the ability to control the release properties of active ingredients and may aid in developing microparticles with precisely tailored properties [17]. Microparticles can be microspheres or microcapsules. A microsphere is a matrix microparticle, whereas a microcapsule has an inner core and outer shell. Optimizing parameters during their preparations obtain well-designed microparticles with a controlled release [18].

Biopolymer-based microparticles represent a cost-effective carrier of active ingredients due to the use of inexpensive, environmentally friendly "green" materials. Polysaccharides such as alginate (anionic biopolymer) and chitosan (cationic biopolymer) easily create microparticles in which an active ingredient can be incorporated using an aqueous system at ambient temperature. Among the many methods used for encapsulation in biopolymer matrices, ionic gelation, and polyelectrolyte complexation are two simple techniques for enzyme immobilization [19,20]. Methods rely on anionic biopolymers (sodium alginate) reacting with gelling cations (usually calcium), forming a sphere, and electrostatic interactions of alginate carboxyl functional groups with chitosan amino functional groups forming a capsule. The produced particles may readily entrap various bioactive ingredients that could be shielded from external impacts and conveniently released at certain times and locations [6].

Sodium alginate is the most common encapsulation matrix for biological components such as plant cells [22], mammalian cells [23] yeasts [24], bacteria [25], insulin [26] and food items [27]. Despite being extremely fast, harmless, biodegradable, and inexpensive, encapsulation with calcium alginate into microspheres has some drawbacks, including low efficiency, polymer instability, particularly in alkaline environments, and loss of encapsulated bioactive ingredients from the pores, which may require modification of sodium alginate with cationic polymers such as chitosan forming chitosan-coated alginate microcapsules with improved properties such as enhanced morphological stability, reduced enzyme leakage, and designed physicochemical permeability and enzyme biocompatibility. The solubility of sodium alginate is affected by the presence of specific ions and the pH of the water. In water, sodium alginate produces a polymeric network structure that may be cross-linked with divalent or polyvalent cations to generate an insoluble network. There have been instances of calcium cations cross-linking the acid groups of alginate. The content and concentration of alginate and the concentration of gelling cations are critical elements in the production of alginate particles [16,28,29].

Chitosan is a naturally occurring poly (aminosaccharide) that resembles glycosaminoglycans structurally. After the acetyl moieties in the amine functional groups are taken out, chitosan is easily soluble in an aqueous acidic solution. Chitosan is soluble in acidic solutions due to the protonation of amino functional groups on the C-2 position of the d-glucosamine residues. This causes polysaccharides to be transformed into polycations. Chitosan can attach to negatively charged molecules in this situation. Because of its biological and chemical features, such as biodegradability, safety, and non-toxicity, it is frequently utilized in the food sector for food encapsulation, enzyme immobilization, and release [30].

Several studies have investigated enzyme immobilization using the ionic gelation method: immobilization of L-asparaginase [31,32] showed high encapsulation efficiency and loading capacity, and immobilization of horseradish peroxidase retained up to 64.9% of the residual activity after six consecutive cycles of dye decolorization [33]. Immobilized a purified papaya laccase [34] increased enzyme tolerance to various metal ions and organic solvents. Various studies have investigated the process and conditions of enzyme encapsulation via ionic gelation and polyelectrolyte complexation. It was discovered that the following factors influence ion gelation encapsulation processes: polymer concentration, polymer/electrolyte ratio, concentration, temperature, pH, and loading composition/polymer ratio [35]. The most important factor from all mentioned that influence ion gelation encapsulation processes and the stability of gel are polymer concentration and type of polyelectrolyte [36].

One of the most important tasks to be solved in cheese making is adequate protection and timely delivery of rennet enzymes. As previously mentioned, rennet is a complex set of enzymes, and rennet encapsulation as a novel approach for preserving

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biodiversity and delivering important ingredients into cheese is highly challenging [1]. The successful encapsulation of ingredients essential for cheese production, i.e., the combination of more than one ingredient, makes the encapsulation process even more complex [28,29].

Despite the array of methodologies for simultaneous encapsulation of more than one ingredient, there are only a few data in the literature about rennet encapsulation and delivery of present enzymes. Hosseini et al. [35] have found that factors influencing ion gelation in rennet encapsulation processes are polymer concentration, polymer/electrolyte ratio, concentration, temperature, pH, and loading composition/polymer ratio, but more studies are needed to evaluate the performance of the enzyme in the existing system and cheese production. Recently, Vinceković et al. [37] have found that encapsulation in alginate/chitosan microcapsules did not negatively impact the rennet properties keeping its activity very high.

By achieving proper encapsulation conditions, it is possible to tailor optimal microcapsule formulation with determined physicochemical properties (size, loading capacity, encapsulation efficiency, stability) and to control enzyme release in time intervals suitable for cheese production. Compared with the conventional cheese production process, encapsulation in biopolymeric microparticles offers numerous benefits, including additional desirable sensory characteristics [1]. Furthermore, the effective encapsulation of natural rennet, i.e., the use of an autochthonous breed whose milk, including rennet, is characterized by specific microbiological properties responsible for the characteristic aroma of that breed, would enable the commercialization of the production of traditional and boutique products.

Conclusion

Preserving and enhancing the biological activity and qualities of rennet over the storage and application period is one of the significant challenges in the cheesemaking process. New green innovations, technologies, and processes must be used to achieve this. One of the efficient methods is the encapsulation process. Encapsulated rennet could be released through the porous gel network. Sodium alginate and chitosan are biopolymers that have excellent properties and could be used for rennet encapsulation to improve enzyme stability and biocatalytic activity during long periods. Several aspects, including the choice of matrix material that affects the stability and effectiveness of the encapsulated rennet, the encapsulation method, and the application, should be considered before employing the microcapsule rennet formulations in the industry.

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