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Short Communication

Effect of Protease Loaded Nanoliposome Produced by Heating Method on Yield and Composition of Whey and Curd During the Production of Iranian Brined Cheese

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ABSTRACT

Background and Objectives: Fast proteolysis of cheese in ripening process may lead to the premature attack of casein, release of the majority of enzymes into the whey, and loss of cheese composition from curd to whey. In this study, the effect of liposomal Flavourzyme on proteolysis of Iranian white brined cheese, as well as on the yield and composition of whey and curd was investigated.

Materials and Methods: Heating method (without using any toxic, volatile organic solvent or detergent) was used to nanoliposomal encapsulation of Flavourzyme. So, 0.15% and 0.3% w/v Flavourzyme-loaded liposome were incorporated to pasteurized cow milk. Iranian brined cheese was produced in triplicate using a complete randomized design. Then total solids were determined by drying them in an Infrared Oven. Water soluble nitrogen/total nitrogen and non-protein nitrogen/total nitrogen was determined by Kjeldahl method.

Results: Encapsulation efficiency of liposomal Flavourzyme was 25%. No significant differences between chemical components of cheese curd (total solid, protein, TCA-soluble nitrogen, water soluble nitrogen) and whey (total solid, protein) were observed between encapsulated cheese and the control. Cheese production yield in experimental cheese was not different from that in the control cheese ($P \geq 0.05$).

Conclusions: The results suggest that application of liposomal Flavourzyme for acceleration of Iranian white brined cheese inhibits premature attack of casein and the release of the majority of cheese compositions into the whey.

Keywords: Flavourzyme, Nanoliposome, Iranian brined cheese, Heating method

Introduction

Biochemical ripening reactions in Iranian white brined cheese lead to development of required texture and sensory properties (1). Proteolysis contributes to textural changes of the cheese matrix due to the breakdown of the protein network, decrease in a_w through water binding, pH increase, and finally, changes in the flavor of cheese due to the formation of peptides, free amino acids and catabolic reactions (i.e., transamination, deamination, decarboxylation, desulphuration, catabolism of aromatic amino acids, etc.) (2). In cheese production ripening is slow (45-90 days for Iranian white brined cheese) and uncontrollable, so there are economic and technological motivations to accelerate this stage (3).

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Methods for cheese ripening acceleration include elevating the ripening temperature, addition of enzymes, addition of cheese slurry, attenuated starters, adjunct cultures, genetically engineered starters, and microencapsulation of ripening enzymes (4). Addition of liposomal enzymes to milk seems to be the most promising way for this purpose (5). Liposome has generated much interest in the dairy industry for encapsulation of different cheese accelerating enzymes, e.g. lipase and protease (6). Liposomes can be made from ingredients naturally present in cheese; they protect casein from early hydrolysis during the cheese production; and well partition in curd (7, 8). Some methods are used for entrapment of enzyme in liposome for ripening acceleration (9). Heating method is a scalable and robust method, which does not require employment of high mechanical stress, any harmful chemical or extreme values of pH during preparation (7, 10). Free proteolytic enzymes are used for cheese ripening acceleration; however, most of them are water soluble, and up to 90% may be lost to whey during the cheese making (4). So yield of cheese production, casein degradation, and loss of nitrogen in whey are influenced (11). Nanoliposomal Flavourzyme was previously produced and modified for cheese acceleration in this research group (12, 13). Also kinetic characteristics of enzyme were compared before and after encapsulation in nanoliposome (14).

The purpose of the present work is to evaluate the effect of Flavourzyme-loaded nanoliposome, on cheese yield, and composition of whey and curd at the manufacture day during Iranian brined cheese production.

Materials and Methods

Preparation of Flavourzyme-loaded nanoliposome: Liposomes were prepared based on the heating method (11, 13). Lecithin 4.5% (w/v) and cholesterol solution (0.225% in 3% glycerol) were added to Flavourzyme 0.675% (w/v) (gift from Novozyme, Iran) solution in tris buffer (0.01 M, pH= 6) at 40°C. Then the mixture was stirred (900 rpm) on a hotplate stirrer (HCR2, Gerhadt, Germany) for 30 min.

Iranian brined cheese manufacture: The milk was standardized to a fat content of 2.5%, pasteurized at 65°C for 30 min, and cooled down to 32-35°C. $CaCl_2$ was added at a level of 1.5g 10 kg^{-1} of milk, followed by the addition of starter culture R704 according to Chr. Hansen co. (Denmark) recommendation. After decrease of pH to 6.2, commercial powdered microbial rennet Rennilase ®, (DSM, French) (clotting activity of 1 g/100 kg) was added at a level of 2.5 g 100 kg^{-1} of milk to coagulate it, which usually requires about l h. Following coagulation, the curd was cut, and stirred. After the whey drained off, the curd was pressed in the vats by using weights for l h (20 kg weights 30 kg^{-1} final curd). The curd weight was measured, and then it was cut into a suitable shape and size (3). Liposomal Flavourzyme was added to the milk at the renneting stage. Three different cheese batches containing (A) 0.15%, (B) 0.3%, and (control) 0.0% Flavourzyme-loaded liposome were prepared in triplicate. The drained off whey and unsalted curd were collected for further analysis.

Evaluation of Flavourzyme loaded nanoliposome characterization: Activity of Flavourzyme was measured by the procedure of Kailasapathy *et al.* and Anjani *et al.* using L-leucine-p-nitroanilide (leu-p-Na) as a substrate based on the reaction rate and introduced as leucine aminopeptidaes units per milliliter (LAPU ml^{-1}) (15, 16). The liposomes were separated from the unencapsulated enzymes by centrifugation at 45000×*g* for 1.5 h at 4°C (17). The encapsulation efficiency was determined as described earlier (12, 13).

Chemical analysis: Curd pH was measured by a digital pH meter (Ino Lab WTW, American). Total solids and nitrogen were determined by drying in an Infrared Oven (Memmert laboratory oven; South Africa) and Kjeldahl method (Gerhard, Germany), respectively (18). Water soluble nitrogen (WSN) of the cheese was determined using addition of 1 mL of 10% acetic acid (V/V) and 1ml of 1M sodium acetate for pH adjustment. 10 g of the cheese was homogenized with 100 mL of distilled water after pH adjustment at 40 °C for 1 h. After precipitating, it was filtered through filter paper (Whatman No.1, International Ltd., Maidstone, England), and the result was expressed as total nitrogen (WSN/TN). Non-protein nitrogen (NPN) was determined in the 12% TCA-soluble fraction, and expressed as a percentage of total nitrogen (TCA-SN/TN) (18).

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Statistical analysis: Cheese making was carried out in triplicate using a complete randomized design. Data were analyzed variance analysis (one-way ANOVA) (SAS 9). Statistically significant differences between different treatments were determined by Fisher's least significant difference (LSD) among the chemical contents of cheese ($P<0.05$).

Results

Nanoliposome characterization: The encapsulation efficiency and average diameter of liposomal Flavourzyme (produced by modified heating method without solvent and toxic detergent) were 25% and 179 nm, respectively.

Compositional analysis: In order to study the effect of addition of nanoliposomal Flavourzyme to milk on pre-maturation of milk protein during production of Iranian brined cheese (Renneting stage and whey drain off), cheese yield, as well as curd and whey composition were studied. The yield of cheese production in the samples and control at the manufacture day (after pressing stage) is shown in Figure 1.

The addition of Flaourzyme-loaded nanoliposme to milk for ripening acceleration of Iranian brined cheese did not significantly influence the cheese yield. The protein content of Iranian brined cheese supplemented with liposomal Flavourzyme is shown in (Table 1). Protein breakdown influenced the amount of water soluble nitrogen (WSN/TN%), variety of peptides, and tricholoroacetic acide soluble nitrogen (TCA-SN/TN%) (1). By addition of liposomal Flavourzyme, the pH, WSN/TN (%) and TCA-SN/TN (%) of curd in the control and experimental cheeses were not significantly influenced (Table 1) ($P< 0.05$) (Table 1); however, the TCA-SN of whey was increased significantly (Table 2).

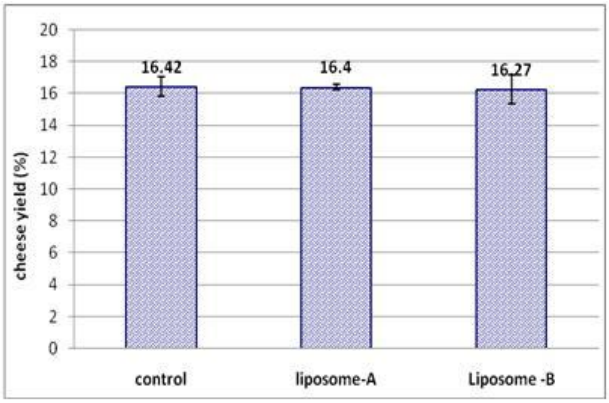

Figure 1. Iranian brined cheese yield of control cheese (C) and nanoliposome treated cheese: A) addition of 0.15% nanoliposomal Flavourzyme to milk for production of cheese, and B) addition of 0.3% nanoliposomal Flavourzyme to milk for production of cheese

Table 1. Composition of Iranian brined cheese in the control cheese (C) and nanoliposome treated cheese (A: addition of 0.15% nanoliposomal Flavourzyme and B: addition of 0.3% nanoliposomal Flavourzyme)

Chemical parameters	A	B	C	P-value
Total solid (%)	39.80±1.13	37.92±1.38	40.15±0.8	0.186
Total protein (%)	17.24±1.01	16.32±0.4	17.13±0.281	0.257
pH	5.33±0.07	5.34±0.06	5.39±0.12	0.49
Water soluble nitrogen/TN (%)	8.35±0.13	8.33±0.10	8.15±0.14	0.176
Tricholoroacetic acid soluble nitrogen/TN (%)	3.19±0.35	2.94±0.3	2.8±0.29	0.368

mean±SD

Table 2. The whey composition of Iranian brined cheese in the control cheese (C) and nanoliposome treated cheese (A: addition of 0.15% nanoliposomal Flavourzyme and B: addition of 0.3% nanoliposomal Flavourzyme)

Chemical parameters	A	B	C	P-value
Total solid (%)	6.48±0.28	6.3±0.107	6.52±0.24	0.26
Total protein (%)	0.77±0.06	0.72±0.03	0.71±0.04	0.06
Tricholoroacetic acid soluble nitrogen (%)	0.36^{a}±0.011	0.35^{a}±0.02	0.27^{b}±0.037	0.015

mean±SD

[a, b] Mean with superscript differs ($P<0.05$) from the control.

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Discussion

In this research, the encapsulation efficiency was similar to nanoliposomal Flavourzyme produced by proliposome procedure (20.2% based on enzyme activity and 23% based on protein) (17). Encapsulation efficiency and mean diameter of different types of Trypsine liposmes showed different values of 10-14% and 110-1540 nm, respectively (19).

Addition of encapsulated enzyme does not lead to significant difference in the total solid of the produced cheese compared with the control. While increasing of moisture content in the nanoliposome treated cheese has been previously reported (20), which may be due to the water binding of liposome phospholipids (17).

In order to study of the effect of liposomal Flavourzyme on proteolysis of milk total protein (%), WSN/TN (%) and TCA-SN/TN (%) of the curd were studied. Lower protein content of the liposome-treated cheese was reported (17, 19, 20). Similar result has been reported in (21, 22) with a different encapsulated enzyme in cheddar cheese. Mohedano *et al*. showed that using different concentrations of cystein protease did not effect on the protein content of Manchego cheese (11).

It has been reported that unencapsulated cystein protease affected the breakdown of α_{S1}-casein and β-casein in the 1st day during the ripening of Machego cheese (11). Addion of Neutrase also increased WSN, NCN, NPN/TN and NH_2N/TN in cheese (23). Protein proteolysis indexes (WSN/TN% and TCA-SN/TN%) in the control and samples suggest that liposomal Flavourzyme inhibits the early proteolysis of casein by Flavourzyme activity during Iranian brined cheese production. In production of Manchego cheese, addition of unencapsulated protease to ovine and bovine milk caused increasing loss of soluble nitrogen in the whey, and decreased the cheese yield (24).

Use of heating method for preparation of encapsulated Flavourzyme (a fungal compelex exopeptidase and endoprotease) in nanoliposome was reported for the ripening acceleration of Iranian brined cheese. Incorporation of liposomal Flavourzyme did not affect cheese yield, as well as curd and whey composition significantly. The encapsulation protected casein from early proteolysis and inhibited pre-maturation of protein curd. So, proteolysis indexes (WSN/TN% and TCA-SN/TN %) of cheese in the manufacture day were not influenced by Flavourzyme-loaded liposome. Investigation of liposomal Flavourzyme on the ripening of Iranian brined cheese is the future aim of this study.

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