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Effects of Carboxymethyl Chitosan on Yield and Whey Protein Loss in Cottage Cheese

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Abstract

A standard 1% w/v solution of CM-chitosan made from squid pen was added to milk at levels of 0.5～3% (v/v) to improve the yield and rheological properties of cottage cheese by whey protein retention. Cheese curd did not form at levels higher than 3% (v/v) CM-chitosan standard solution. Yield and total protein of cottage cheese increased up to 2% by 11 to 42% and 17 to 38% respectively, compared to control cheese. Whey protein losses were decreased by 11 to 42% and thus accounted for all of the increase in yield. Anomalous results were obtained at the 0.8% level, which neither improved yield or whey protein retention nor stabilized rheological parameters, and at the 0.5% level, which improved yield and total protein without increasing whey protein retention. Elasticity and cohesiveness of CM-chitosan-containing cheese were generally improved and stabilized during storage. Monitoring of cheese chromaticity values for four weeks revealed a delay in the onset of yellowing in cheeses with CM-chitosan compared to the controls, while the concentration of added CM-chitosan had little influence on cheese chromaticity. The addition of CM-chitosan solution could be applied directly to industrial scale cottage cheese-making without the need for any modification of the production process.

Key words: carboxymethyl chitosan, cottage cheese, whey protein, rheological properties, yield

INTRODUCTION

Studies on cheese are generally concerned with any of the four aspects: improving yield, extending shelf-life, detecting and inhibiting pathogens and controlling the evolution of sensory and rheological characteristics during ageing or storage.

Better retention of whey protein increases both protein content and nutritional value of cheese and is therefore the subject of more research. Whey proteins also have many well known functional properties. During cheese processing and storage, losses of protein reach to 20% and are greater in soft cheese than in hard cheese (1). Ultra-filtration has been used in order to increase cheese yield and whey protein recovery (2,3) while high-purity milk protein powder has been used to increase the yield of Gouda cheese (4).

When attempting to improve the protein content of cheese, rheological characteristics must also be considered at the same time. Dickinson (5) has compared the sensory properties of milk protein and polysaccharides while Beaulieu et al. (6) have studied the texture and microstructure of whey protein/low-methoxyl-pectin mixed gels with added calcium.

Even though the protein content of cheese is high and rheological properties are well characterized, it is difficult to predict the interaction between simultaneous changes in both. Rheological values and total solids in cheese are inversely proportional. If the solids ratio of a cheese increases with protein as the main solid, the rheological properties of the cheese will suffer. Excess cheese protein causes breakdown of the essential curd structure.

A new ingredient that increases the quantity of protein in cheese and maintains or improves rheological properties would be very valuable to the industry.

Chitosan has been considered for such an application. This polysaccharide is derived from chitin extracted from squid pen, shrimp, crab, shellfish and lobster carapaces. Chitosan from squid pen appears to be especially suitable because this starting material contains little ash and the extraction process is simple (7,8).

Chitosan is a cationic, deacetylated derivative of chitin from which acetyl groups have been chemically or enzymatically removed, thereby leaving glucose residues bearing one free amino group and two free hydroxyl

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groups (9,10). It is a highly versatile molecule with potential applications to fields as diverse as waste management, medicine, food processing and biotechnology. Chitosan has been used for the recovery of waste materials from food processing effluents, water purification, binding/recovery of protein and as a microbial inhibitor. Lee et al. (11) reported that chitosan is an excellent material for protein adsorption. It is generally recognized as a safe natural material and is allowed as a food additive in Japan. Its wider use in foods is expected to be approved in the near future.

Chitosan is usually insoluble at neutral pH and soluble in acidic conditions. This characteristic may be good or bad depending on the application or the research objectives (1). Even though cheese is processed at pH 4.7~6.4, it is difficult to adapt chitosan to cheese making.

During cheese curd formation, pH condition is changed from acid to neutralization. This condition causes the suspension phenomenon of chitosan. So, when cheese curd formation has begun, chitosan remains on the surface of the curd. Carboxymethyl chitosan (CM-chitosan) can solve this problem.

CM-chitosan is a carboxymethyl-group (-CH_2COO-) bearing derivative of chitosan (12-14). CM-chitosan dissolves at neutral pH and has many of the functional properties of chitosan. CM-chitosan prevents crystallization of chitosan near neutrality during cheese processing (15).

For the present study, cottage cheese has been selected, since the commercialization of low-fat cheese has significantly accelerated world-wide, especially in developed countries. Cottage cheese is made from skim milk and is the simplest form and most basic type of cheese. Skim milk contains 0.1% (w/w) fat or less and its main components are protein and lactose. If CM-chitosan is effective in this cheese, it may be useful in other cheeses.

The objectives of this study were to evaluate the protein binding ability of CM-chitosan, the effectiveness of CM-chitosan in increasing whey protein retention in cottage cheese, the change in cheese yield. Some kinds of rheological properties of cheese to which were added CM-chitosan were analyzed to make sure of side effect of CM-chitosan and to confirm the CM-chitosan concentration at cheese processing as cofactor.

MATERIALS AND METHODS

Materials

Squid pen was extracted from squid processing waste (Bo-Sung Co., Korea). Skim milk was a commercial product of Seoul Milk (Seoul Milk Int. Co., Korea) purchased at a local market. The cheese starter was R-703 from Hansen Int. Co. (Denmark), which contains *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis*. Rennet was also purchased from Hansen Int. Bovine serum albumin (BSA) was purchased from Sigma (Sigma-Aldrich Co., USA). The milk was concentrated to 50% before using to find the interaction between CM-chitosan and milk protein.

Methods

Preparation of CM-chitosan: Chitosan was made by the method of Choi and Lee (14). Squid pen (from Todarodes pacificus) was washed and dried for 14 hours at 50°C. Squid pen became chitin when steeped in 1 N NaOH for 3 hours at room temperature. Chitin was treated with 40% NaOH at 110~115°C for 90 minutes to make chitosan. The characteristics of chitin and chitosan were analyzed by the methods of Sannan et al. (16) and Kurita et al. (7,8). The processing of CM-chitosan and its information were shown in Fig. 1 and Table 1. A CM-chitosan standard solution was made by dissolving 1 g of CM-chitosan in 100 mL of distilled water. This solution is used during cheese processing and the amount of addition is indicated in percentage (%).

Preparation of cottage cheese: Cottage cheese was made by the short-set cottage cheese method (17-19).

Chitosan

↓ Ground to 50 mesh

Frozen alkali chitosan

↓ 10 g of chitosan suspended in 60% NaOH with 0.2% SDS, stirred for 1 hr at 0°C, left to stand for 18 hrs at -20°C, suspended in iso-propanol at room temperature.

Alkylation

↓ Alkylation of alkali chitosan with monochloroacetic acid.

Washing and Dissolving

↓ Washed 3 times with ethyl alcohol at 20°C, dissolved in deionized water.

Precipitation

↓ Acetone added to sodium CM-chitosan solution.

Dialysis

↓ Dissolved in deionized water, dialysed for 72 hrs.

Carboxymethyl chitosan (CM-chitosan)

↓ 1 g CM-chitosan dissolved in distilled water (1 g CM-chitosan/100 mL D.W.)

CM-chitosan solution (1%)

Fig. 1. Preparation of 1% CM-chitosan standard solution.

Table 1. The information of carboxymethyl (CM) chitosan

Molecular weight	46,362 dalton
Yield (from chitosan)	73%
Intrinsic viscosity	3.67
Degree of deacetylation	92%

Starter culture was added to skim milk at a proportion of 5% (w/v) followed by $CaCl_2$ solution to 0.02% (v/v) and then rennet. The usual covering with cream (20) was omitted. CM-chitosan standard solution was added during cottage cheese making to obtain the levels indicated, expressed in percent standard solution by volume (see below). Control cheese was made without CM-chitosan. The cheeses were kept at 4°C.

Analyses

Characteristics of the CM-chitosan solution : Protein binding ability and specific viscosity of the CM-chitosan standard solution (CM-chitosan 1.0 g/100 mL D.W) were measured to evaluate its impact on cheese manufacture. BSA was used to measure protein binding ability. Standard solution was added to 100 mL of BSA solution (1%, v/v) to levels of 0.1 ~ 1.0% (v/v) and the mixture was then stirred for 1 h. The solution was filtered by gravity through filter paper (Whatman #1) and its protein concentration was assayed by the Lowry method (21), measuring absorbance at 500 nm using a UV spectrophotometer (Shimadzu Co., Kyoto, Japan). Specific viscosity was measured to determine flow and diffusion properties. The specific viscosity of solutions containing CM-chitosan at concentrations of 0.1 ~ 1.3 (w/v %) was measured using the Ubbelohde capillary viscometer (Cornig Co., NY, USA).

Cheese-curd setting time and total protein concentration in cottage cheese : The time required to adjust the pH of milk to reach at 4.7, after adding the starter culture, was measured by checking the pH every half-hour. The micro-Kjeldahl method (22) was used to determine total protein. Cheese samples were analyzed every 7 days.

Whey protein concentration in cottage cheese whey : 1 mL of cottage cheese whey was filtered and assayed by the Lowry method (21), with absorbance measured at 500 nm using a UV spectrophotometer (Bio-spec 1601, Shimazu Co., Kyoto, Japan).

Cheese chromaticity : A sample of cottage cheese was withdrawn inside a 13 mm cylinder. The chromaticity was measured after cheese-making and every week for a month using a colorimeter (JC801, Color Technosystem Co., Tokyo, Japan). The measured parameters were L- (brightness), a- (red to green), and b- (yellow to gray) values.

Effect of CM-chitosan solution on cheese yield : Cheese yield was calculated as cheese weight divided by milk weight before the addition of starter culture. Reported values are the ratios of the yield obtained for CM-chitosan-containing cheeses to the yield obtained for the control cheese.

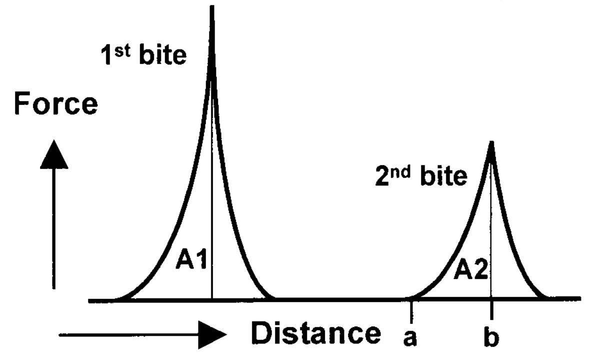

Fig. 2. Instron texturometer profile of cube of cottage cheese. A1: Area of 1st bite, A2: Area of 2nd bite, A2/A1: Cohesiveness, distance from a to b: Elasticity.

Rheological properties of cottage cheese : There is no standard method confirmed for measuring the rheological property of cottage cheese. So, its methods were referred to the rheological property measurement method of cheddar. Cottage cheese withdrawn inside a 13 mm cylinder was pressed at 3 bars of pressure for 24 h at 4°C for rheological analysis. Elasticity and cohesiveness were measured (Fig. 2) using a universal testing instrument (Instron 1011, Instron Co., Norwood, MA, USA). The measurement condition were 5 kg load-cell, 1 kg load-range, 13 mm cross head diameter, room temperature and 20% compression of sample height.

Statistical analysis : Each condition of experiments was performed in 11 times. 7 to 9 samples of one concentration of CM-chitosan were selected as a result. Their difference was within 5%.

RESULTS AND DISCUSSION

Characteristics of CM-chitosan

Protein binding ability : The standard CM-chitosan solution at 0.1% by volume reduced the protein concentration of 1% BSA solution by a factor of 4.5 (Fig. 3). An additional almost two-fold decrease in dissolved BSA was obtained at CM-chitosan solution level of 1.0% (v/v). Therefore, we supposed that it should be effective at levels of 0.5 ~ 1.0% (v/v) in reducing whey protein loss of liquid exuded during cottage cheese processing. This result is caused by the structure of CM-chitosan. CM-chitosan has an anionic of carboxymethyl group ($-CH_2COO^-$) and an amide group (NH^{2+}). So, it guesses that CM-chitosan combined with water as hydrogen bond and with proteins as ionic bond. This reaction makes

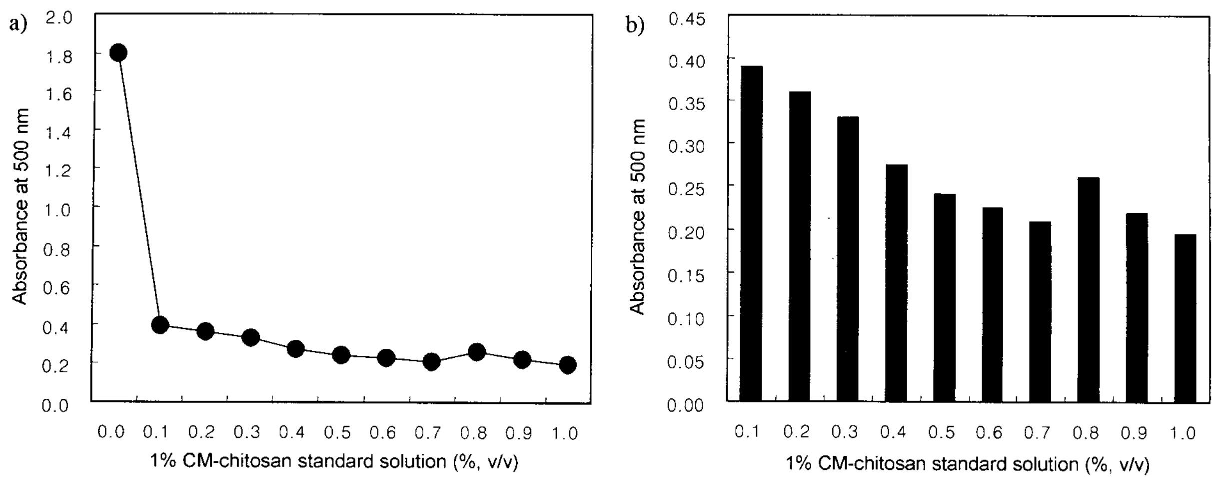

Fig. 3. Residual dissolved BSA (by Lowry assay) after precipitation with 1% CM-chitosan standard solution. Initial BSA concentration: 1% (w/v), Reaction time: 1 hr. b) is redrawn up the range of 0.1~1.0% CM-chitosan standard solution in a).

a network between CM-chitosan, water and proteins. These networks protect the protein exhaustive. However, 0.8% standard CM-chitosan solution showed the slight decrease in BSA protein binding ability.

Flow behavior of CM-chitosan solution : Specific viscosity was measured to evaluate the flow behavior of CM-chitosan solution. A conspicuous increase in the rate of viscosity change as a function of concentration occurred at 0.8% (w/v) (Fig. 4). Park et al. (23) reported that the point of the angle corresponding to this increase is the critical concentration of the material. Molecular interaction is the greatest and diffusion velocity is the lowest at this critical concentration and the ability of CM-chitosan molecule to bind or to adsorb to other molecules is weak. In the present study, 0.8% (v/v) of CM-chitosan standard solution is the level at which a noticeable decrease in BSA binding was observed (Fig. 3).

Influence of CM-chitosan on curd formation time

Curd formation is the most important step in the cottage cheese-making process and requires a variable amount of time. During the initial step of cheese-making, pH decreases due to the action of the starter culture and the point of curd setting is determined by a specific pH. This pH has been the subject of much study and different values of pH have been recommended. Yoon et al. (17) suggested pH 4.6 as a setting point of cheese curd. Baek (19) reported that an optimum pH was 4.6 at 9.7% milk solids and Kosikowski (24) also reported the best setting pH to be 4.7. We chose pH 4.7 for this study.

Cheese curd was formed at all concentrations of CM-chitosan standard solution levels up to 3% (v/v) while setting times increased by at least 1 hour at levels of 0.8~3% (v/v) (Fig. 5). Although CM-chitosan has a visible impact on curd formation, this effect may be negligible in cheese-making compared with total processing time. It appears that total curd formation time may not be significantly changed by CM-chitosan. Curd was not formed at CM-chitosan standard solution levels higher than 3% (v/v) and therefore this level may be considered as an upper limit for industrial purposes (Table 2).

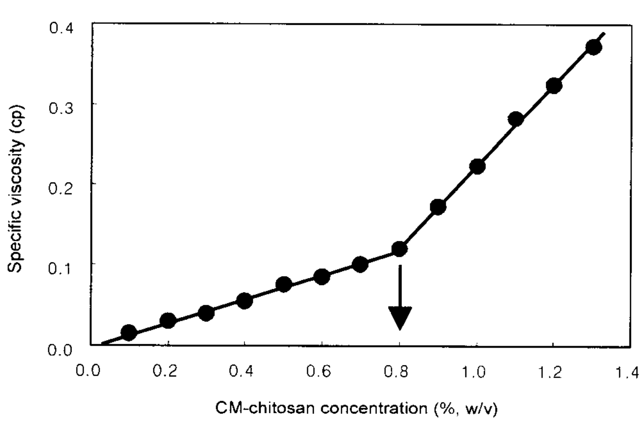

Fig. 4. Specific viscosity of CM-chitosan solution.

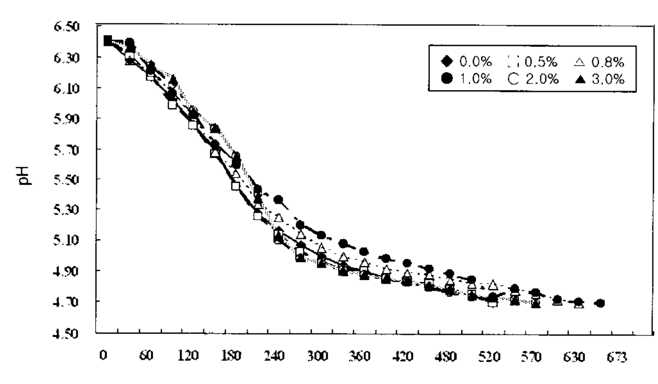

Fig. 5. Influence of CM-chitosan on cottage cheese setting time. % at legend means 1% (w/v) CM-chitosan standard solution to milk ratio. The time of x-axis means required time to reach pH 4.7.

Table 2. Cheese curd formation in the presence of CM-chitosan

% (v/v) addition* to milk	Curd formation
0.0	+
0.5	+
0.8	+
1.0	+
2.0	+
3.0	+
3.3	-
3.5	-
4.0	-
5.0	-

* of 1% (w/v) CM-chitosan solution.
+: Curd formed, -: No curd formed.

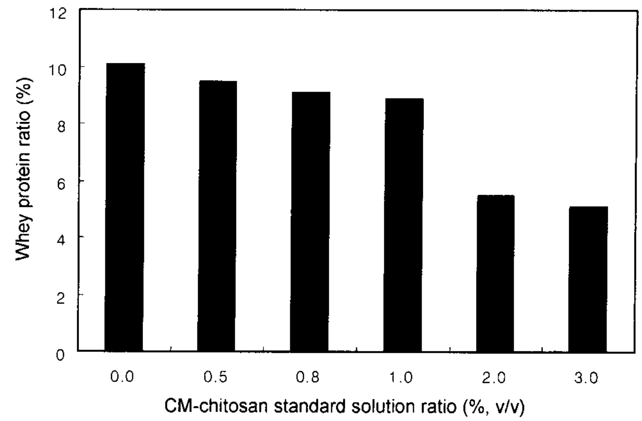

Fig. 7. Whey protein loss from CM-chitosan-containing cottage cheese after storage for 4 weeks at 4°C.

Changes in the protein content of cottage cheese

Total protein concentration : Protein concentration was measured over a four-week period and averaged. The amount of protein in the cheeses was increased by the presence of CM-chitosan (Fig. 6). These results are consistent with the protein-binding property of CM-chitosan in solution. The binding kinetics of CM-chitosan may depend on its concentration. The reaction of CM-chitosan and milk protein is similar to the reaction of most polysaccharides and milk protein, since CM-chitosan is a polysaccharide (5). The overall interaction between two biopolymers is the result of an averaging over the large number of different intermolecular forces arising between the various segments and side-chains on the two macromolecules.

Whey protein concentration : The property of CM-chitosan to bind whey protein (Fig. 7) was different from the BSA binding ability of CM-chitosan and its effect on total protein concentration of cheese. The whey protein binding efficiency of CM-chitosan solution was low at 0.5 ~ 1.0% (v/v) but then improved at 2% (v/v) and 3% (v/v) CM-chitosan standard solution. The result obtained at the "critical" concentration (0.8%) was similar to those at 0.5% (v/v) and 1.0% (v/v) CM-chitosan solution. It thus appears that the standard CM-chitosan solution used at 2% (v/v) may reduce whey protein loss and hence water pollution.

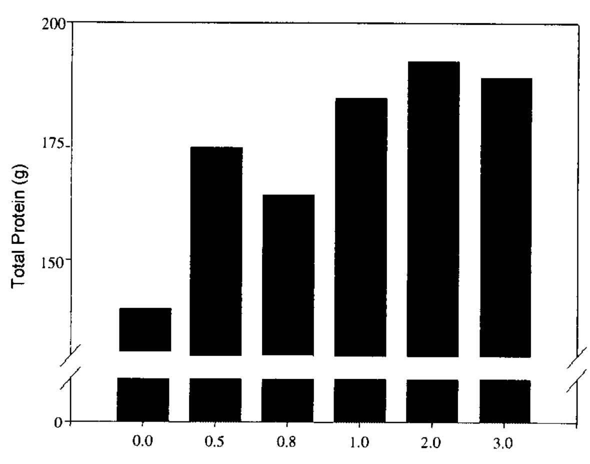

Fig. 6. Average total protein content of CM-chitosan-containing cottage cheeses after storage for four weeks at 4°C. Initial quantity of skim milk is 3 L.

The reduced binding ability of CM-chitosan solution at 0.8% (w/v) needs to be explained. We believe that the solution flow properties provide the explanation. Park et al. (23) reported that homo-molecular interaction increases and binding ability with other molecules decreases at a critical concentration, which they held to correspond to 0.8% (w/v) and which is the value we determined, based on the break point in the viscosity graph (Fig. 4). It was thus expected that protein binding ability, yield and rheological properties of CM-chitosan cheese might be inferior at 0.8% (w/v) CM-chitosan solution. This was confirmed by examination of the cheeses.

Cheese chromaticity

Chromaticity was measured to determine the possible effects of CM-chitosan on product appearance during storage (Table 3). Cheese color was shown as L-, a- and b-values by colorimeter. L- and a-values of CM-chitosan cheeses were not different from control cheeses, but the b-value of control cheese was higher than for CM-chitosan cheese after two weeks and the range was wide. After four weeks, the control cheeses were changed to yellow while CM-chitosan cheeses did so, only slightly. The b-values of CM-chitosan cheeses at four weeks were lower than that of control cheese at one week. The color change of cottage cheese was thus delayed by CM-chitosan solution.

Chromaticity is an important aspect of the visual appeal of cottage cheese. All cheeses have natural color,

Table 3. Effect of CM-chitosan on cottage cheese color during storage at 4°C

Weeks of storage	Color score	% (v/v) added CM-chitosan solution					
		0.0	0.5	0.8	1.0	2.0	3.0
0	L[1)]	94.72	94.02	94.56	94.79	93.89	93.87
	a[2)]	-2.58	-1.98	-1.88	-1.56	-1.02	-1.76
	b[3)]	8.56	8.45	8.36	8.25	8.55	8.62
1	L	93.42	94.02	93.86	94.32	93.54	94.01
	a	-2.42	-1.50	-1.84	-1.04	-0.80	-1.46
	b	9.85	9.23	8.91	9.01	8.99	8.83
2	L	92.91	92.44	94.47	94.24	93.19	93.77
	a	-2.20	-1.37	-15.1	-0.91	-0.72	-1.34
	b	11.09	9.40	9.45	9.51	9.62	9.16
3	L	94.27	92.23	93.84	90.02	91.28	90.31
	a	-1.20	-0.95	-1.01	-0.89	-0.78	-0.89
	b	11.57	9.49	9.22	8.84	9.14	9.22
4	L	95.05	93.89	92.75	92.96	93.19	83.20
	a	-2.02	-1.76	-0.31	-1.32	-0.75	-1.02
	b	11.86	9.59	8.84	9.49	9.26	9.44

1)Brightness. 2)Redness (+) or Greeness (-). 3)Yellowness (+) or Grayness (-).

which differs depending on the type of cheese. Cheese color is often used as a market standard, since its maintenance affects consumer preference. The natural color of cottage cheese is generally bright white but it is known that this color changes towards yellow during storage. In the present study, the yellowing of CM-chitosan cheeses was delayed more than three weeks. This protection against yellowing was not dependent on the concentration of CM-chitosan solution. It has been shown that CM-chitosan acts as a protein binding agent and coating agent in cheese (25,26-28). Chitosan and various chitosan derivatives are used as a coating to preserve apples (25,29). It is believed that CM-chitosan decreases contact with air and inhibits microorganisms in cheese, since CM-chitosan has known antibiotic properties and similar results are observed in fruit packaging.

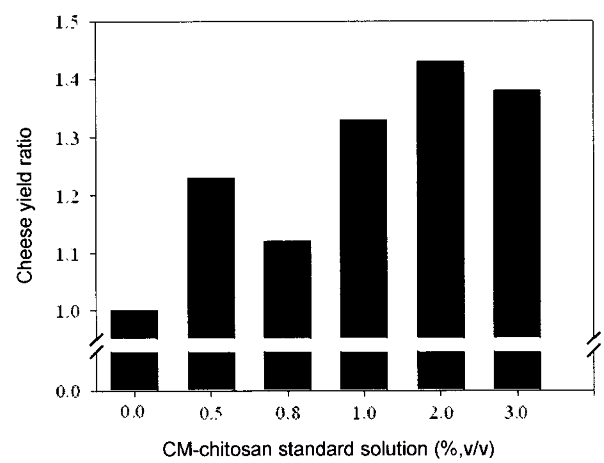

Fig. 8. Change in cheese yield due to addition of CM-chitosan standard solution to milk. Cheese yield ratio means increase for CM-chitosan-containing cheese relative to control cheese.

Improvement of cheese yield by CM-chitosan solution

The yield of CM-chitosan cheese was 11~42% greater than that of control cheese and the increase depended somewhat on CM-chitosan concentration (Fig. 8). This result well consistent with total protein content of CM-chitosan cheese. If the result of 0.8% (v/v) CM-chitosan cheese is excluded, cheese yield will increase 24~42% by CM-chitosan solution.

Table 4. Effect of CM-chitosan on the elasticity and cohesiveness of cottage cheese

Weeks of storage	CM-chitosan level[1)]					
	0%	0.8%	2%	0%	0.8%	2%
	Elasticity			Cohesiveness		
0	3.0	4.0	5.0	0.9	0.6	0.9
1	4.0	6.5	5.0	0.8	0.8	0.9
2	5.0	6.0	6.0	0.8	0.9	1.0
3	5.7	4.0	5.0	0.7	0.5	0.9
4	4.5	3.0	5.8	0.7	0.5	0.9

1)v/v addition of 1% CM-chitosan standard solution to milk during cheese production.

Rheological and sensory properties

Rheological properties of cottage cheese affect a consumer preference via mouth feeling and reflect adherence to process standards. In this study, we considered elasticity and cohesiveness because they are the most representative rheological properties of cheese. Elasticity is the tendency to return to original shape after biting while cohesiveness is resistance to spreading. The appropriate characteristics for Cheese are high elasticity and low cohesiveness. Rheological studies aim both to improve and to stabilize these properties.

Elasticity was initially proportional to the amount of CM-chitosan solution added (Table 4). At 0% or 0.8% (v/v) CM-chitosan solution, elasticity was unstable over time, but was relatively stable at 2% (v/v).

Cohesiveness decreased for 4 weeks in control cheese, while 0.8% CM-chitosan solution level produced an increase for 2 weeks followed by a decrease and 2% CM-chitosan solution kept it relatively constant.

Polysaccharides including chitosan are used to improve rheological properties (5,6,30). Stretching ability and bread quality are improved by chitosan (31).

Elasticity is perceived during chewing and cohesiveness is the spreading property of cheese, also perceived in the mouth. High elasticity and low cohesiveness make cheese suitable to apply to bread and other foods. Although only one CM-chitosan cheese had both higher elasticity and lower cohesiveness than the control cheese, the rheological properties of cheese are slightly improved (elasticity) and stabilized (cohesiveness) by CM-chitosan solution.

CONCLUSION

There are various ways to improve cheese yield, rheological properties and protein concentration. In this study, we attempted to find a simple and economical approach suitable for industrial-scale cheese production. This objective was achieved by the addition of CM-chitosan solution.

Dispersing CM-chitosan solution in milk produces a net-like structure. This structure increases the protein ratio in cheese by decreasing whey protein loss. This network forms between amide groups of CM-chitosan and negative charges groups of protein (5). Rheological properties were slightly improved by the network structure. This network provided protection against impact and reduced the destruction of the protein structure.

In view of the results of this study, it is considered that 2% by volume of a 1% w/v CM-chitosan standard solution added to milk is optimal for cheese manufacture. The solid CM-chitosan required is thus 0.2 g per kg of milk. CM-chitosan may thus be considered a very efficient food additive for the cheese industry.

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