KOREAN J. FOOD SCI. TECHNOL.
Vol. 22, No. 7, pp. 774~779 (1990)

Effect of Thermalization and Ultrafiltration Membrane on the Increase of Cottage Cheese Yield Using Radiolabelled Protein

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방사성 표지단백질을 이용한 우유의 열처리 및 한외거르기가 코티지 치즈의 생산성 증대에 미치는 영향

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Abstract

[^{14}C]-radiolabelled β-lactoglobulin was used for the studies on the effect of thermalization and ultrafiltration for the increase of cheese yield. 4.33% of β-lactoglobulin was incorporated through thermalization. 3.20~3.65% of β-lactoglobulin was more incorporated with cheese curd in the thermalization and ultrafiltration than without ultrafiltration process. Comparing with protein increase, other whey proteins might be incorporated with casein micelles. Loss of [^{14}C]-β-lactoglobulin through processing and adsorption to membrane during ultrafiltration was only 1.03%.

Key words : β-lactoglobulin, radiolabelling, cheese yield

Introduction

The use of membrane systems to improve process efficiency in the dairy industry promises economic advantages to the consumer, the processor, and the farmer. Of late knowledge about how to use membrane on dairy farms is also becoming available. Maubois[1,2] in France, Amundson and Slack[3] in Wisconsin, USA and Zall and Chen[4-6] in New York are pioneers in this area. The first work with the on-farm concept of using membrane was carried on by Maubois[1] and his associates. They opted to concentrate and fractionate milk by first passing milk through the ultrafiltration (UF) membrane system and then thermalize the concentrate. Amundson and Slack[3] suggested there was no apparent advantage in thermalizing milk process in ultrafiltration membrane systems. However, Zall and Chen[5] preferred to thermalize milk prior to concentration and fractionation as they utilized concentrates in making cheese. Recently Zall and Chen[6] proposed that an increase cheese yield with Cheddar cheese of about 3% and more than 5% with Cottage cheese. Some researchers have questioned the validity of both reports.

One of the major factors in cheese yield is whey protein, especially, β-lactoglobulin, which is heat labile. β-Lactoglobulin can be denatured and associated with casein micelles under thermalization and pasteurization. Such heat treatments affect the milk proteins to varying extents, and this subject has been studied for some time and there are many reviews of the topic[7-10]. Particular interest has been directed to the interactions between the whey proteins in the milk serum and the casein micelles or colloidal phase of the milk.

For the present study, it was decided to examine the effect of thermalization and ultrafiltration membrane, namely, the formation of casein micelle-whey protein complex, especially, β-lactoglobulin into the cheese curd. It is very difficult to quantify heat-induced complex material in a sophiscatd system such as milk. However, tracer techniques allow the determination of very low concentration of a protein, even if the protein has been extensively modified by either hydrolysis or complex formation. Smits and Brouwershaven[11] successfully used ^{3}H-labelled β-lactoglobulin and centrifugation to examine some aspects of heat treatment

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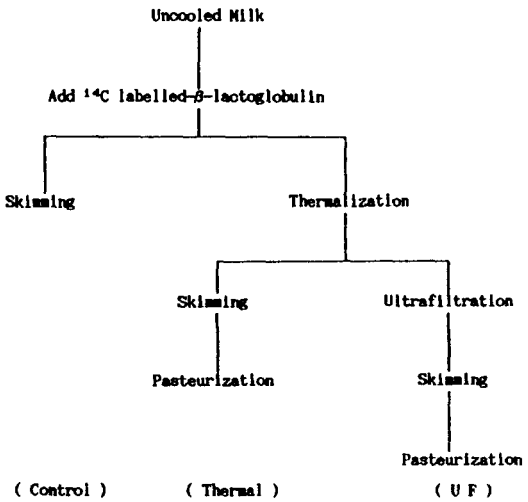

Fig. 1. Schematic milk preparation for the manufacturing of Cottage cheese

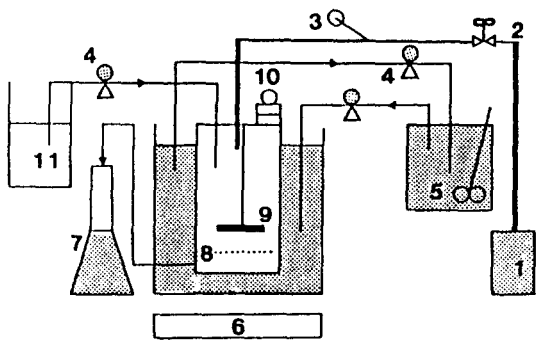

Fig. 2. Schematic experimental system used for the effect of ultrafiltration membrane of the thermalized milk

1. N_2 gas cylinder
2. valve
3. pressure gauge
4. peristaltic pump
5. water bath
6. stirrer
7. ultrafiltration
8. Amicon membrane(PM 10)
9. stirrer bar
10. pressure release valve
11. feed reservoir

in artificial milk systems. Olson *et al.*[12] showed that reductive methylation with ^{14}C-formaldehyde and sodium borohydride did not significantly alter either β-lactoglobulin or κ-casein. This result was confirmed by Rowley *et al.*[13,14] who showed that ^{14}C labelled β-lactoglobulin could be traced in heated milk systems.

In the present study, the effect of heat treatment and ultrafiltration on the transfer of β-lactoglobulin from the serum to the colloidal phase of milk during cheese processing has been measured using the mild reaction conditions by the methods described somewhere[15,16]. Also the effect of fouling on membrane system was studied using radiolabelled β-lactoglobulin.

Materials and Methods

One preparation of β-lactoglobulin(genetic variant B ; Sigma Co.) was radiolabelled with ^{14}C-formaldehyde(Amersham) using reductive methylation[17,18]. The modified test tubes(6×12 mm, thickness 1.0 mm) were used for thermalization. To measure the temperature at the geometric center in the modified test tube, the copper-constantan thermocouple(0.003 in. type K, Omega Engineering Inc.) was used and connected to Data Logger(Angus Co.). Data Logger printed out temperature at given interval time. For the lower temperature heat treatments an 1 ml aliquot of the milk mixture was heated in a thin-walled tube(made by sealing off Pasteur pippette) in a water bath controlled at 75.2 ℃, temperature that heats tube content to 74℃. After heat treatment, tube was cooled in an ice bath the each sample was withdrawn. The uncooled raw milk was treated differently according to the scheme outlined in Fig. 1. Thermalization was carried out at 74℃ for 10 sec. The thermalized milk was concentrated at about 40℃ by a laboratory-scale ultrafiltration system (Amicon Co. membrane type : PM 10) to approximately two-thirds of the original volume(Fig. 2). The concentration factor was calculated by testing the protein content in skimmed, thermalized, and ultrafiltered milk. The protein of the heated milk was estimated using the Bradford method(Bio-Rad Laboratories) with a sample of acid casein as a standard[19]. Samples were coagulated with rennet extract adjusted to clot the milk in 30 min at 30℃. The disrupted coagulum was centrifuged at 11,000g for 3 min in an Eppendorf centrifuge (Brinkman Instrument Co.) The clear supernatant was removed and the sediment was washed four times by resuspension followed by centrifugation. After freeze drying, dry curd(15~25 mg) was dissolved in 6 M guanidine hydrochloride(Sigma Co.), 0.05 M EDTA (Fisher Scientific Co.) and 0.03 M dithiothreitol(Sigma

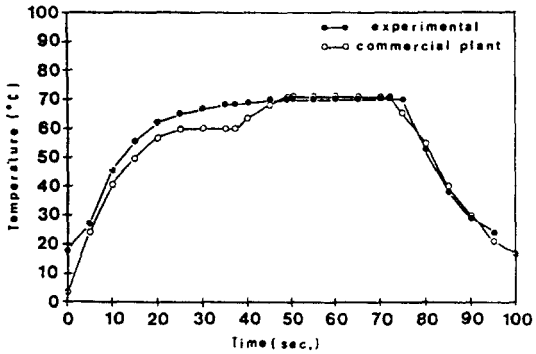

Fig. 3. Time-temperature profiles of the published commercial plant[20] and the geometric center of the modified tube under the heating conditions used in the present study

Co.). A 0.1 m*l* of this solution was added to 10 m*l* of Aquasol(New England Nuclear Co.) in counting vials, and their radioactivites were determined using a liquid scintillation counter(LS 7500, Beckman). These measurements of radioactivity allowed estimates of incorporation of radiolabelled β-lactoglobulin into the cheese curd. On occasions, all of washings were assayed for ^{14}C-methyl β-lactoglobulin so that a mass balance could be done. Quench correction curves and counting efficiencies were determined using ^{14}C toluene(Amersham) of known activity as a standard.

Where indicated the actual amount of β-lactoglobulin in the skim milk used in these studies was quantified in the whey after acid precipitation of the casein. SDS-polyacrylamide gel eletrophoresis(PAGE) of the whey samples and standard sample followed by staining and comparative densitometry(Ultrascan-XL LKB Laser Densitometer) allowed quantification of the β-lactoglobulin.

Trace amount of labelled ^{14}C-β-lactoglobulin and 0.04 g of ultrafiltration membrane(PM 10) were incubated in 5 m*l* of milk at 50℃ for 2, 5 and 12 hours. Membrane was washed with phosphate buffer(pH 6.7, 0.02 M) three times. Membrane was extracted with urea/sodium dodecyl sulfate(urea/SDS) buffer and radioactivity in the wash was determined. Membrane was also counted directly.

Results and Discussion

Considerable care was taken to try and mimic the time-temperature profiles used in commercial heat processing of milk. Fig. 3 showed a time-temperature profile between the reported data for commercial plant[20] and the present heating profile determined at the center of the heating tube with a thermocouple. Consequently, the samllest diameter and thinnest wall tubes that were readily available were used and bath temperatures were higher than the aspired milk temperatures. The data obtained in our system could be used for the prediction of the data from continuous flow plant scale.

Table 1. Incorporation of radiolabel into the cheese curd of unheated control and thermalized milk

Operation	Degree of incorporation
Control	100.00%
Thermal	104.33%

Effect of thermalization, ultrafiltration and pasteurization on incorporation of ^{14}C-β-lactoglobulin into curd

The specific activity of radiolabelled protein was 3.53 $\times 10^7$ disintegrations per minute(dpm) per mg of β-lactoglobulin. When a small quantity of ^{14}C-labelled β-lactoglobulin(3.53×10^7 dpm/mg) was added to milk, the mixture was heated. The heated milk was used for the preparation of Cottage cheese. After making the curd with rennet extract, the sample was separated into casein micelle fractions and the whey fractions which were then subjected to ^{14}C analysis. The results shown in Table 1 were obtained. In comparing unheated samples(control) with thermalization treatments, 4.33% increase of the incorporation of ^{14}C-β-lactoglobulin into the curd was observed(Table 1). This value is higher than the data of previous report[21] where heating temperature was lower (70℃, 45 sec : 1.16~1.99%).

The four degress increased in heating temperature seemed to have a substantial effect on the incorporation of β-lactoglobulin into the curd. Table 2 shows that the incorporation of label into the curd after ultrafiltration and pasteurization was increased 3.2~3.6%. This means that 3.2~3.6% of β-lactoglobulin in the thermalized milk was incorporated with ultrafiltration rather than without ultrafiltration. These values were only for β-lactoglobulin. The data using radiolabelled β-lactoglobulin suggested that thermalization at 74℃

Table 2. Incorporation of radiolabel into curd after ultrafiltration

Operation	C.F. [a]	Incorporation (%)	Actual incorporation [b]	Actual yield [c] (g/100 ml)	%increase of β-Lg in curd [d]
Thermal	1.000	—	—	—	—
UF	1.588	5.76%	3.62%	0.01086	0.38%
	1.632	5.96%	3.65%	0.01095	0.39%
	1.553	4.98%	3.20%	0.00960	0.34%

a) C.F. means concentration factor.
b) Actual incorporation compensates for concentration factor as following :
actual incorporation = incorporation/C.F.

c) $\text{Actual yield} = \frac{\text{Actual incorporation}(\%)}{100} \times \frac{0.3\text{g of } \beta\text{-Lg}}{100\ ml \text{ of S.M.}}$

whers S.M. means skim milk and β-Lg means β-lactoglobulin.
Also 0.3g of β-Lg in 100 ml of skim milk was determined by SDS-gel electrophoresis and densitometry.

d) Increase of β-Lg in curd

$$= \frac{\beta\text{-Lg(actual yield)}}{\text{Total curd(Thermal)}} \times 100(\%)$$

for 10 sec coupled with ultrafiltration and pasteurization at 74℃ for 16 sec may increase cheese yields slightly but less than the range claimed by Zall and Chen[6]. Slight variations in temperature and processing conditions appeared to have significant effects on incorporation of whey proteins into curd. Table 3 represents the protein amount of curd per unit of skim milk. The protein of the curd after UF increased 4.83% on the basis of thermalization. These values are higher than the 0.34~0.39% increase of β-lactoglobulin in curd. Since the number of replications was small and concentration factors were different(more concentrated), it was difficult to compare our data with values from Zall and Chen[6]. Only one time, 7.73% of increase of curd was close to his value. This value was not included in Table 3, which was average value. However, the radioactivity data would suggest substantially lower yields than Zall and Chen issue. As stated under Table 3, we will place more credence in the radioactivity measurements. This means that the increase of protein of curd also results from other proteins such as α-lactalbumin, immunoglobulins and serum albumin. These proteins contain also disulfide bonds or sulfhydryl groups, which react via disulfide interchange reaction

Table 3. Incorporation of protein into the curd during thermalization

Operation	Protein(%)	C.F, [a]	Actual protein increase [b]
Control	2.73%	1.000	—
Thermal	2.80%	1.000	2.56%
UF	4.77%	1.625	4.83%

a) C.F. means concentration factor.
b) Actual protein increase was calculated as following

$$\frac{(\text{Treatment/C.F.}) - \text{Control}}{\text{Control}} \times 100(\%)$$

Three times of replication was done.
Protein was determined using Bradford method.

Table 4. Distributions of radioactivity in the permeated milk, the retentate and the membrane filter after treatment of ultrafiltration membrane

Location	Radioactivity (dpm) [a]	Distribution (%)
Total added radioactivity	5.230×10^6	100.00
Retentate	5.146×10^6	98.39
Permeate	7.008×10^4	1.34
UF membrane filter	1.412×10^4	0.27
Total loss(permeate + filter)	8.420×10^4	1.61 (1.03) [b]

a) dpm : disintegrations per minute
b) Concentration factor was taken into consideration.

with other proteins. The studies of Elfagm and Wheelock[22,23] and Shalabi and Wheelock[24] suggested that the sequence of reations leading to heat-induced complex in milk involved α-lactalbumin as well as β-lactoglobulin and κ-casein and that these reactions were complex and temperature dependent. Noh *et al.*[25] showed that α-lactalbumin and β-lactoglobulin probably denatured(unfolds) independently and either simultaneously, or consequently, reacted via disulfide interchange reactions with κ-casein and became attached to the casein micelle. Therefore, when radiolabelled α-lactalbumin, bovine serum albumin or immunoglobulin is used for other replication for β-lactoglobulin, the influence of ultrafiltration on cheese yield should become more clear.

Incorporation of radiolabelled ^{14}C-β-lactoglobulin into UF membrane as an index of membrane fouling

β-Lactoglobulin was expected to be a significant mem-

Table 5. The comparison of extraction and direct counting methods in incorporation(adsorption) of ^{14}C-β-lactoglobulin onto the PM-10 membrane(Molecular weight cut-off 10,000 daltons) at various incubation times

Time of incubation at 50℃ (hours)	Extraction			Direct counting		
	milk	Urea/SDS extract + filter	%of Incorporation [a]	milk	filter	%of Incorporation [a]
		dpm [b]			dpm	
2	7.55×10^6	5.72×10^4	0.751%	7.10×10^6	3.68×10^4	0.516%
5	7.35×10^6	4.80×10^4	0.648%	6.85×10^6	3.92×10^4	0.569%
12	7.10×10^6	6.00×10^6	0.838%	6.50×10^6	5.60×10^4	0.854%

a) % of Incorporation $= \frac{\text{extract}+\text{filter}}{\text{milk}+(\text{extract}+\text{filter})}\times 100$

b) dpm : disintegrations per minute

brane foulant in the ultrafiltration process. For this study, it was a very low level because of short operation time. The amount of radiolabelled protein attached to the membrane after UF was also determined. About 0.4 mg of protein per 30 ml of milk was bound to the membrane. This was less than 0.05% of protein added to the UF unit. The remaining radioactivity in the membrane was about 14,000 dpm, equal to 0.27% of total radioactivity added to the UF unit. This suggested that β-lactoglobulin be selectively incorporated into the membrane. The radioactivity of permeate through the UF membrane was a 70,000 dpm and was a 1.34% of total added radioactivity. Therefore, the loss of radioactivity in the ultrafiltration process was 1.61%(Table 4). When the concentration factor was taken into consideration, in reality, the loss of radioactivity was 1.03%(= 1.61%/1.553). So membrane fouling of ultrafiltration could be negligible. The degree of incorporation to the filter was calculated by the direct counting method. As an alternative, the filter was extracted with urea /SDS for the given times and the extracted was counted. Two methods showed similar data. Incubation was carried out at higher temperature than that of ultrafiltration conditions(40℃). In addition, severe condition such as longer operation time was evaluated for fouling effect. As the incubation time increased, the radiolabelled ^{14}C-β-lactoglobulin was more bound to the membrane filter(Table 5). 0.838~0.854% of radioactivity was expected to transfer from milk fraction to the membrane filter after 12 hours of incubation. These values could be negligible. However, operation time for changing membrane filter could be longer, then degree of fouling should be reestimated. The low value at 5 hours of incubation(4.8×10^4 dpm) in the extraction method might be experimental error taking into consideration of the increasing values with increasing incubation time. As shown in Table 5 ^{14}C labelled β-lactoglobulin can be used for the prediction of the degree of fouling during the operations even if small degreee of incorporation.

요 약

치즈 제조시 열처리와 한외거르기를 통하여 생산량이 증가되는 정도를 방사성 동위원소 [^{14}C]가 표지된 락토글로불린을 이용하여 분석하였다. 그 결과 열처리를 통하여 4.33%의 β-락토글로불린이 치즈 커드에 결합되었으며, 한외거르기를 통하여 3.20~3.65%의 β-락토글로불린이 생산량 증대에 관여하였다. 단백질의 증가와 비교해 본 결과, 다른 유청단백질도 한외거르기, 열처리 효과에 의하여 치즈 커드와 결합하는 것으로 추측되었으며 한외거르기시 막에 흡착되거나 하여 손실된 양은 1.03% 이었다.

Acknowledgement

This paper was supported by RESEARCH FUND for JUNIOR SCHOLARS, Korea Research Foundation.

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(1990년 7월 16일 접수)