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- (10) KESSLER, H.-G., GERNEDEL, C., NAKANISHI, K.: *Milchwissenschaft* 37 (10) 584-587 (1982)
- (11) KUO, K.P., CHERYAN, M.: *J. Food Sci.* 48 1113-1118 (1983)
- (12) LIM, T.H., DUNKLEY, W.L., MERSON, R.L.: *J. Dairy Sci.* 54 306-311 (1971)
- (13) LONSDALE, H.K.: In *Industrial Processing with Membranes* (Ed. R.E. Lacey, S. Loeb). Interscience, New York (1972)
- (14) LUSS, G.: In *Fouling and Cleaning in Food Processing* (Ed. D. Lund, E. Plett, C. Sander). Univ. of Wisconsin, Madison Extension Duplicating, Madison, WI, pp. 502-515 (1985)
- (15) MERIN, U., CHERYAN, M.: *J. Food Proc. Preserv.* 4 183-198 (1980)
- (16) NAKANISHI, K., KESSLER, H.-G.: *J. Food Sci.* 50 1726-1731 (1985)
- (17) PATEL, R.S., REUTER, H.: *Milchwissenschaft* 40 (12) 731-733 (1985)
- (18) PERI, C., DUNKLEY, W.L.: *J. Food Sci.* 36 25-30 (1971)
- (19) PORTER, M.C.: *Industrial and Engineering Chemistry - Prod. Res. Dev.* 11 234-248 (1972)
- (20) SKUDDER, P.J., GLOVER, F.A., GREEN, M.L.: *J. Dairy Res.* 44 293-307 (1977)
- (21) TONG, P.S., BARBANO, D.M., RUDAN, M.A.: *J. Dairy Sci.* 71 604-612 (1988)

6. Summary

ECKNER, K.F., ZOTTOLA, E.A.: *Effects of temperature and pH during membrane concentration of skim milk on fouling and cleaning efficiency.* *Milchwissenschaft* 48 (4) 187-191 (1993).

21 Ultrafiltration (skim milk)

Skim milk was concentrated using a DDS-20 UF/RO unit equipped with DDS HC-50 nanofiltration polyamide membranes (50 % passage NaCl). The effect of temperature and pH on concentration-polarization, fouling, and cleaning was studied. Milk temperature, milk pH, percent total solids, inlet and outlet pressures, and temperatures were monitored and controlled throughout the experiments. Flux was determined as a function of temperature, pH, acidulant, and applied pressure. Higher temperatures, pH, and pressures gave greater flux; lower temperatures, pH, and pressures gave lower flux. Resistance effects from membrane, con-

centration-polarization, and fouling could be elucidated by comparison to pure water flux, flux at 0.5 h, 1 h, terminal skim milk flux at 2 h, and water flux before cleaning and after cleaning regime. Mass transfer coefficients were correlated ($P = 0.05$) to process temperature, not pH. Fouling indices were calculated and corresponded ($P = 0.05$) to operational temperatures and pH. Approximately 70-85 % of flux decline with skim milk was related to concentration-polarization and an additional 5-30 % was from fouling. Process parameters affect cleaner efficacy. Cleaning cycles must be adjusted to account for processing conditions.

ECKNER, K.F., ZOTTOLA, E.A.: *Einflüsse von Temperatur und pH während der Membrankonzentration von Magermilch auf Belagbildung und Reinigungseffizienz.* *Milchwissenschaft* 48 (4) 187-191 (1993).

21 Ultrafiltration (Magermilch)

Magermilch wurde unter Verwendung einer DDS-20 UF/RO-Einheit, ausgerüstet mit DDS HC-50 Nanofiltrations-Polyamidmembranen (50 %ige NaCl-Passage) konzentriert. Untersucht wurden die Einflüsse von Temperatur und pH auf Konzentrationspolarisierung, Belagbildung und Reinigung. Milchttemperatur, Milch-pH-Wert, Anteil Trockenmasse, Einlaß- und Auslaßdruck und -Temperaturen wurden während der Versuche aufgezeichnet und kontrolliert. Der Flux wurde als Funktion von Temperatur, pH-Wert, Säuerungsmittel und angewendetem Druck bestimmt. Höhere Temperaturen, pH-Werte und Drücke bewirkten einen höheren Flux. Niedrigere Temperaturen, pH-Werte und Drücke ergaben einen niedrigeren Flux. Die Widerstandseffekte durch Membran, Konzentrations-Polarisierung und Belagbildung konnten durch Vergleich mit dem Flux von reinem Wasser, dem Flux nach 0,5 h, 1 h, dem terminalen Magermilchflux nach 2 h und dem Wasserflux vor und nach der Reinigung aufgeklärt werden. Die Massentransfer-Koeffizienten waren mit der Prozeßtemperatur korreliert ($P = 0,05$), nicht aber mit dem pH-Wert. Die Belagbildungsindizes wurden errechnet und entsprachen den Betriebstemperaturen ($P = 0,05$) und den pH-Werten. Etwa 70-85 % des Fluxabfalles bei Magermilch ließen sich auf die Konzentrations-Polarisierung zurückführen. Weitere 5-30 % ergeben sich aus der Belagbildung. Die Prozeßparameter beeinflussen die Reinigungseffizienz. Die Reinigungszyklen müssen den Prozeßbedingungen angepaßt werden.

pH-Dependent molecular weight changes of κ -casein glycomacropeptide and its preparation by ultrafiltration

By Y. KAWASAKI, H. KAWAKAMI, M. TANIMOTO, S. DOSAKO, A. TOMIZAWA, M. KOTAKE and I. NAKAJIMA

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1. Introduction

κ -Casein glycomacropeptide (GMP) is a hydrophilic glycopeptide released from bovine κ -casein by the action of chymosin at ^{105}Phe - ^{106}Met (1). Recently some biological functions of GMP have been reported, i.e. inhibition of gastric secretion (2), periodic contractions of the stomach and duodenum (3), aggregation of ADP-treated platelets (4), and adhesion of oral *Actinomyces* and streptococci to erythrocytes or polystyrene (5). Our previous study (6) indicated that GMP in-

hibited the binding of cholera toxin to its receptor. These functions encouraged us to utilize GMP as an ingredient in dietetic foods and pharmaceuticals.

We previously developed a procedure for isolating GMP on a pilot-plant scale from rennet casein whey, a by-product of manufacturing imitation cheese (7). However, cheese whey (whey) is more suitable for the production of GMP on an industrial scale, because a large amount of whey is produced in cheese making.

Several methods for isolating GMP from whey fractions have been reported. EUSTACHE (8) isolated GMP from the supernatant of whey coagulated upon heating and then concentrated the supernatant through a UF module, equipped with a membrane of molecular weight (MW) cut-off 3,000, to retain GMP. SAITO *et al.* (9) isolated GMP with alcohol precipitation and ion exchange chromatography from the supernatant of whey produced by heat coagulation of whey proteins. These methods seem reasonable because major impurities, such as β -lactoglobulin and immunoglobulin, are easily removed. But, the physical and nutritional properties of whey proteins coagulated upon heating are inferior to those of whey protein concentrates (WPC) produced by ultrafiltration.

In a preliminary test, we found the GMP solution to be turbid at neutral pH, while the turbidity disappeared at acidic pH. This suggests that the MW of GMP varies with pH; at neutral pH GMP possesses a considerably higher MW than the theoretical value (7 kDa) (1) based on the amino acid sequence of GMP. MORR and SEO (10) also reported that the MW of GMP determined with size exclusion HPLC was found to be 33 kDa at pH 7.5. Thus, we investigated the pH-dependent changes of apparent MW of GMP, to develop a simple procedure for isolating GMP from whey through an ultrafiltration system.

2. Materials and methods

Material

Ten liters of rennet casein whey were prepared from 1 kg of lactic casein (New Zealand Dairy Board) according to the method described by NISHIYA *et al.* (11). WPC (Sunlact N-2) was purchased from Taiyokagaku (Yokkaichi, Japan).

Gel filtration

Gel filtration was performed with fast protein liquid chromatography (FPLC) (Pharmacia) equipped with Superose 6 (12 x 30 mm, Pharmacia). Fifty μ l of the sample solution (10 mg/ml) were applied to a column equilibrated with 0.1 M Tris-HCl buffer (pH 7.0) or 0.1 M acetate buffer (pH 3.5) and eluted with the same buffer for 60 min at room temperature. MW calibration was performed using a gel filtration calibration kit (Pharmacia).

Quantitative analysis and purity of GMP

Quantitative analysis was performed and the purity of GMP determined according to the method described by KAWAKAMI *et al.* (12).

Gross composition, minerals, and amino acid composition

Minerals were determined by inductive coupled plasma atomic emission spectrometry (PS-3000 UV, Hitachi). After hydrolysis of GMP with 6 N HCl for 20 h at 110 °C, amino acid analysis was performed on an automatic amino acid analyzer (Hitachi model 835). The gross composition was determined by conventional methods.

Protein rejection of rennet casein whey during ultrafiltration

To determine the protein rejection (R_{pro}) of rennet casein whey during ultrafiltration (UF), a UF-module Lab-20 (DDS) with a UF-membrane GR51pp (MW 50 kDa cut off, 0.072 m², DDS), GR61pp (MW 20 kDa cut off, 0.072 m², DDS) or GR81pp (MW 8 kDa cut off, 0.072 m², DDS) was used. The UF operating conditions were as follows: flow rate, 15 l/min; temperature, 40 °C; and inlet-outlet pressure, 0.6–0.2 MPa. Ten l of rennet casein whey were adjusted to the desired pH with HCl and circulated continuously. R_{pro} was estimated with the following equation:

$$R_{pro} = 1 - (\text{Protein content of permeate} / \text{Protein content of retentate}).$$

3. Results

pH-Dependent MW change of GMP

The elution profiles of GMP through gel filtration are shown in Fig. 1. Although the theoretical MW of GMP was about 7 kDa, the retention time of the major peak indicated the MW of GMP distributed from 20 to 50 kDa at pH 7.0. This was in agreement with MORR and SEO (10). At pH 3.5, on the contrary, five major peaks were detected at MW ranging from 10 to 30 kDa. Fig. 2

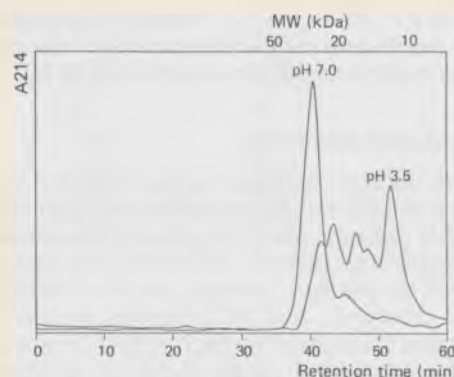


Fig. 1: Gel filtration chromatography of GMP. Sample: 50 μ l of sample solution (10 mg/ml); Column: Superose 6 (12 x 30 mm, Pharmacia); Elution: 0.1 M Tris-HCl buffer (pH 7.0) or 0.1 M acetate buffer (pH 3.5); Flow rate: 0.5 ml/min; Temperature: Room temperature; Detection: UV (214 nm).

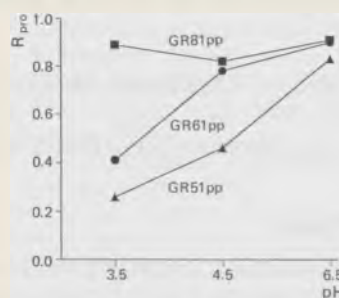


Fig. 2: Relationship between protein rejection (R_{pro}) and pH in UF of rennet casein whey. Sample: 0.2 % GMP solution (10 l); UF-module: Lab-20 (DDS); UF-membrane: GR51pp (MW 50 kDa cut off, 0.072 m², DDS); GR61pp (MW 20 kDa cut off, 0.072 m², DDS); GR81pp (MW 8 kDa cut off, 0.072 m², DDS); Flow rate: 15 l/min; Temperature: 40 °C; Inlet-outlet pressure: 0.6–0.2 MPa.

shows the relationship between R_{pro} and pH of rennet casein whey during UF with either one of the three different membranes, GR51pp (MW 50 kDa cut off), GR61pp (MW 20 kDa cut off) or GR81pp (MW 8 kDa cut off). Since the majority of proteins in the rennet casein whey is GMP (7), R_{pro} represents the rejection of GMP. More than 88 % of the protein were rejected by GR81pp and GR61pp at pH ranging from 4.5 to 6.5, independent of the MW cut off of membranes. GR51pp also rejected 80 % of protein at pH 6.5. At pH 3.5, R_{pro} with GR51pp was 0.23 and GR61pp was 0.41, whereas a much higher R_{pro} was observed when GR81pp was used. These findings suggested that the apparent MW of GMP ranged from 20 to 50 kDa at pH > 4.5, while it became lower at pH < 4.5. The pH-dependent MW changes in GMP were completely reversible.

Isolation of GMP from WPC

The pH-dependent MW change of GMP led to the development of a simple procedure for isolating GMP from WPC by UF (Fig. 3). As a preliminary test, we examined the effects of WPC concentration on the recovery of GMP from acidic solutions of WPC. The recovery decreased with increasing WPC concentration, probably due to the fouling of the UF membrane. We, therefore, fixed the WPC concentration at 2 %.

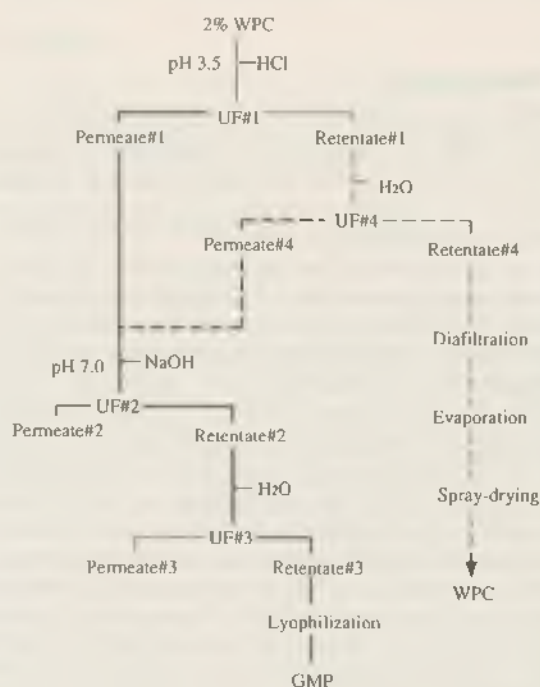


Fig. 3 Procedure to isolate GMP by UF

Fifty l of 2 % WPC solution (pH 3.5) were concentrated to 10 l by UF (UF#1) with a UF-membrane under the following operating conditions: flow rate, 15 l/min; temperature, 40 °C; and inlet-outlet pressure, 0.6–0.2 MPa. After adjustment of pH to 7 with NaOH, the permeate (40 l, Permeate #1) was concentrated to

(Retentate #2) through the same UF membrane (UF#2). In order to desalinate, diafiltration of Retentate #2 (UF#3) was continuously performed until the electric conductivity (ECV) of the retentate became constant (95 μ S/cm). The total volume of water added for diafiltration was 25 l. After concentration of the retentate to 1 l (Retentate #3), Retentate #3 was lyophilized.

When WPC solution was concentrated through UF-membrane GR61pp at pH 3.5, the majority of whey proteins such as β -lactoglobulin, α -lactalbumin, immunoglobulin and bovine serum albumin were rejected by the membrane, whereas GMP, lactose and minerals permeated (Permeate #1). After neutralization, Permeate #1 was concentrated and desalinated through the same UF membrane (UF#2, UF#3). GMP was concentrated into the resultant retentate (Retentate #3) because lactose and minerals passed through the membrane. Table 1 summarizes the material balance of each fraction. At the process UF #1, 18.4 % of GMP were recovered from WPC in Permeate #1, but Retentate #1 still contained 77.9 % of GMP. process UF#2 for the recovery of GMP in Retentate #2 was successful with lactose and minerals being permeated into Permeate #2. After diafiltration and concentration (UF#3), 18 g of GMP were retained; the final recovery yield was 18 %.

Table 1: Recovery of GMP in each fraction during processing from WPC*

Fraction	Content of GMP (g)
2 % WPC	100.1
Retentate #1	77.9
Permeate #1	18.4
Permeate #2	17.8
Permeate #2	0.1
Retentate #3	17.8
GMP powder	18.0

* GR61pp was employed.

Effects of MW cut off of UF membranes and diafiltration of Retentate #1

Since the recovery yield with UF membrane GR61pp was low, the GR61pp was replaced with GR51pp (MW cut off 50 kDa) to improve the recovery yield. Although the recovery of GMP increased to 23 g with GR51pp (Table 2), we were not satisfied with the recovery yield. In this procedure, UF #1 seemed to be the most important step for recovering GMP, because

Table 2: Effects of diafiltration and MW cut off of UF membrane on recovery and purity of GMP

MW cut off (kDa)		Diafiltration (UF #4)	Recovery (%)	Purity (%)
UF #1	UF #2			
20	20	no	18	86.4
50	50	no	23	84.8
50	50	yes	63	81.0

Retentate #1 contained 77.9 % of GMP when GR61pp was used (Table 1). Therefore, we attempted to recover the residual GMP from Retentate #1 by diafiltration with GR51pp. After diafiltration with additional 40 l of water, the recovered permeate (Permeate #4) was mixed with Permeate #1 (Fig. 3). This additional diafiltration (UF #4) improved the recovery of GMP to 63 % (Table 2). The purity of the recovered GMP was higher than 80 %, independent of the UF-membranes employed (Table 2). Taking the recovery and purity of GMP into consideration, GR51pp appeared to be a more suitable membrane than GR61pp.

Analysis of recovered GMP

The elution profile of the recovered GMP on gel filtration chromatography is shown in Fig. 4. The GMP

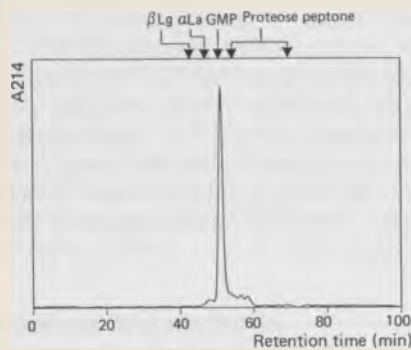


Fig. 4: Gel filtration profile of recovered GMP
Sample: 20 µl of sample solution (0.5 mg/ml); Column: Two coupled TSK gel G3000 PWxL (7.8 x 300 mm, Tosoh); Elution: 55 % (v/v) Acetonitrile containing 0.1 % (v/v) trifluoroacetic acid; Flow rate: 0.3 ml/min; Temperature: Room temperature; Detection: UV (214 nm).

fraction contained a small amount of impurities such as α-lactalbumin and proteose peptones. Table 3 compares the amino acid compositions of the recovered GMP with theoretical values. The amino acid composition was similar to that calculated from the amino acid sequence of GMP, except for the lower

Table 3: Amino acid composition of GMP		
Amino acid*	Cal. (%)**	Found (%)
Asx	8.5	8.5
Thr	18.2	14.6
Ser	7.8	8.5
Glx	19.2	21.0
Pro	11.6	11.0
Gly	0.9	1.0
Ala	5.3	5.4
Val	8.9	7.7
Met	2.0	1.4
Ile	10.1	9.4
Leu	1.7	3.1
Tyr	0.0	0.3
Phe	0.0	0.6
Lys	5.7	5.7
His	0.0	0.5
Arg	0.0	1.0

* Cys and Trp were not analyzed
** The theoretical value were based on the primary structure of GMP.

Thr content, which may be attributable to excessive hydrolysis (20 h) with 6 N HCl (13). Although GMP contains neither Tyr, Phe, His, Arg, nor Trp, small amounts of these were detected. The Leu content of the GMP was twice the theoretical value. These amino acids probably originated from contaminated peptides and/or proteins. The gross and mineral compositions are summarized in Table 4. The high calcium and phosphorus content may be responsible for the colloidal calcium phosphates which were not excluded into permeate during UF #2 and UF #3.

Table 4: Gross and mineral compositions of GMP	
Component	Content (in sample 100 g)
Protein*	76.71 g
Carbohydrates	14.87 g
Fat	0.47 g
Ash	7.95 g
Water	5.33 g
Na	130 mg
K	183 mg
Ca	2,600 mg
Mg	240 mg
P	850 mg

* Nitrogen coefficient = 6.65, based on the primary structure of GMP

4. Discussion

The mechanism for the pH-dependent MW changes in GMP is unclear. One possibility is that GMP forms associations that form oligomers at neutral pH through non-covalent interactions, and the oligomers partially dissociate at acidic pH. A similar phenomenon is known with the wheat protein gliadin (14). Gliadin associates at pH 5.0 through hydrogen bonding and hydrophobic interaction. However polymeric gliadin dissociates into the monomeric form at pH 3.0, because the neutralization of negatively charged carboxyl groups diminishes the interaction between positively and negatively charged groups on gliadin (14). The mechanism of association and dissociation with gliadin may be applicable to GMP. Another possibility is the hydration of GMP at neutral pH, which is supported by gel formation at low temperatures (15). The proposed process for isolation of GMP is easily scaled up, because the UF of whey has been well established on an industrial scale at WPC processing plant. Additional advantage is the production of WPC from Retentate #4 (Fig. 3) without deteriorating physical functions and nutritional values. Thus, the process is highly beneficial for large scale production of GMP in the food industry.

5. References

(1) MACKINLAY, A.G., WAKE, R.G.: In Milk Protein II (Ed. H.A. Mackenzie), Academic Press, New York, pp. 367-418 (1971)
(2) CHERNIKOV, M.P., STAN, E.Y.: In XXI International Dairy Congress, Moscow, Vol. 1, Book 2, 161 (1982)

- (3) STAN, E.Y., GROISMAN, S.D., KRASIL'SHCHIKOV, K.K., CHERNIKOV, M.P.: Bull. Exp. Biol. Med. **96** 889-891 (1983)
- (4) JOLLES, P., LEVY-TOLEDANO, S., FIAT, A.-M., SORIA, C., GILLESSEN, P., THOMADIS, A., DUNN, F.W., CASCEN, J.P.: Eur. J. Biochem. **158** 379-382 (1989)
- (5) NEESER, J.-R., CHAMBAZ, A., VEDOVO, S.D., PRIGENT, M.-J., GUGGENHEIM, B.: Infect. Immun. **56** 3201-3208 (1988)
- (6) KAWASAKI, Y., ISODA, H., TANIMOTO, M., DOSAKO, S., AHIKO, K.: Biosci. Biotech. Biochem. **56** 195-198 (1992)
- (7) TANIMOTO, M., KAWASAKI, Y., DOSAKO, S., AHIKO, K., NAKAJIMA, I.: Biosci. Biotech. Biochem. **56** 140-141 (1992)
- (8) EUSTACHE, J.-M.: U.S. Pat. 4,042,576 (1977)
- (9) SAITO, T., YAMAJI, A., ITOH, T.: J. Dairy Sci. **74** 2831-2837 (1991)
- (10) MORR, C.V., SEO, A.: J. Food Sci. **53** 80-87 (1988)
- (11) NISHIYA, T., TATSUMI, K., IDO, K., TAMAKI, K., HANA-
WA, N.: Nippon Nogeikagaku Kaishi **63** 1359-1364 (1989)
- (12) KAWAKAMI, H., KAWASAKI, Y., DOSAKO, S., TANIMO-
TO, M., NAKAJIMA, I.: Milchwissenschaft **47** 688-693 (1992)
- (13) CRESTFIELD, A.M., MOORE, S., STEIN, W.H.: J. Biol. Chem. **238** 622-627 (1963)
- (14) KASARDA, D.D., BERNARDIN, E., NIMMO, C.C.: In Advances in Cereal Science and Technology (Ed. Y. Pomeranz), Amer. Ass. Cereal Chem. Inc., St. Paul, 158-236 (1976)
- (15) MARSHALL, C.S.: Food Research Quality **51** 86-91 (1991)

6. Summary

KAWASAKI, Y., KAWAKAMI, H., TANIMOTO, M., DOSAKO, S., TOMIZAWA, A., KOTAKE, M., NAKAJIMA, I.: pH-Dependent molecular weight changes of κ -casein glycomacropeptide and its preparation by ultrafiltration. Milchwissenschaft **48** (4) 191-196 (1993).

24 Casein glycomacropeptide (ultrafiltration)

κ -Casein glycomacropeptide (GMP) is a biologically active peptide released from κ -casein by chymosin. Although the theoretical molecular weight (MW) of GMP was about 7 kDa, the apparent MW of GMP on gel filtration was changed depending on pH. The distribution of the apparent MW ranged from 20 to 50 kDa at pH 7, but from 10 to 30 kDa at pH 3.5. GMP was rejected at pH 6.5 with ultrafiltration (UF) membranes with MW cut off of 50 or 20 kDa, but was mostly permeated through the same UF membranes at pH 3.5. We developed a simple procedure using UF for isolating GMP from whey protein concentrate (WPC), taking the pH-dependent change in the apparent MW into account. The WPC solution was subjected to UF and diafiltration under acidic conditions using UF membrane with MW cut off of 50 kDa. After the permeate was neutralized, the second UF with the same UF membrane was carried out to retain GMP in the retentate. A lyophilized GMP powder was recovered with 63 % yield and 81 % purity.

KAWASAKI, Y., KAWAKAMI, H., TANIMOTO, M., DOSAKO, S., TOMIZAWA, A., KOTAKE, M., NAKAJIMA, I.: pH-Abhängige Molekulargewichtsveränderungen des κ -Casein-Glykomakropeptids und die Präparation durch Ultrafiltration. Milchwissenschaft **48** (4) 191-196 (1993).

24 Casein-Glykomakropeptid (Ultrafiltration)

κ -Casein-Glykomakropeptid (GMP) ist ein biologisch aktives Peptid, das aus κ -Casein durch Chymosin freigesetzt wird. Obgleich das theoretische Molekulargewicht (MW) von GMP etwa 7 kDa beträgt, verändert sich das MW

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von GMP bei der Gelfiltration in Abhängigkeit vom pH-Wert. Die Verteilung der Molekulargewichte variiert zwischen 20 und 50 kDa bei pH 7, und von 10 bis 30 kDa bei pH 3,5. GMP wurde bei pH 6,5 bei Ultrafiltrationsmembranen mit einem MW-cut-off von 50 oder 20 kDa zurückgehalten. Bei der gleichen Ultrafiltrationsmembran kam es jedoch bei pH 3,5 zu einem Durchtritt. Die Autoren entwickelten ein einfaches Verfahren unter Verwendung der Ultrafiltration zur Isolierung von GMP aus Molkenproteinkonzentrat

(WPC). Dabei wurde die pH-abhängige Veränderung des Molekulargewichtes herangezogen. Die WPC-Lösung wurde ultra- und diafiltriert unter sauren Bedingungen bei Verwendung einer Ultrafiltrationsmembran mit einem MW-cut-off von 50 kDa. Nach Neutralisierung des Permeats wurde eine zweite Ultrafiltration mit der gleichen Membran durchgeführt, um die GMP im Retentat zu halten. Ein lyophilisiertes GMP-Pulver konnte mit einer 63%igen Ausbeute und einer 81%igen Reinheit gewonnen werden

Behaviour of *Listeria* spp. during the production of Camembert cheese under various conditions of inoculation and ripening*

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1. Introduction

During the 1980's *Listeria monocytogenes* emerged as a potential foodborne pathogen. In several large scale outbreaks of Listeriosis, coleslaw, pasteurized milk, and several types of soft cheese were the vehicles for the transfer of *L. monocytogenes* to consumers causing Listeriosis (1, 2, 3, 4, 9). Associated with these outbreaks were extensive losses in fetuses, newborns, infants and adults that have compromised immune systems (6). Subsequent monitoring of food, especially of milk and milk products, found soft cheeses to contain *Listeria* spp. more frequently than all other types of cheese (10). Prior to the mid 1980's only limited information existed concerning the fate of *L. monocytogenes* in cheese. Reports by TERPLAN *et al.* (6) and by RYSER and MARTH (8) provided basic information on the behaviour of the germ during production and ripening of the two predominant types of soft cheese – white mould cheese (type Camembert) and red smear cheese. Both working groups investigated the development of *Listeria* in the two types of cheese when the vat milk was inoculated with *Listeria*.

The present study examined the behaviour of *Listeria* spp. on Camembert cheese under various conditions of inoculation and ripening. The main concern of this study was to examine the behaviour of *Listeria* spp. throughout the whole period from the production up to the consumption of Camembert cheese.

2. Materials and methods

Cheese production and ripening

White mould cheese (type Camembert) was manufactured using a laboratory scale cheese production facility with 4 vats with a maximum capacity of 8 l each. Cheese production and ripening were carried out as outlined in Table 1. Starter culture OP1 and the culture medium ECO6+ were donated by the Marschall section of Bayer Diagnostics, Frankfurt/M., FRG.

Table 1: Manufacturing and ripening schedule for Camembert cheese

Process step	Time/ duration	Process parameters Temp. (°C)	pH	RH (%)
Warming pasteurized vat milk	08:00	4–33	6.70	—
Add starter and mould culture	08:15	33	6.70	—
Preacidification	ca. 45 min	33		
Add rennet	09:00	33	6.45	—
Coagulation period	ca. 45 min	33		
Cutting	09:45	33	6.40	
Curd handling in the vat	ca. 60 min	33		
Draining of whey	10:45	33	6.30	
Moulding	10:50	33	6.30	
1st Turn	11:00	33	6.30	
2nd Turn	12:30	26	6.00	
3rd Turn	17:00	26	5.30	
Brine 18 % (w/w) NaCl	07:00	20	4.90	
0.22 % (w/w) CaCl	next day			
Drying	24 h	19	4.90	85
Ripening	7 d	17	4.9–7.5	95
Packing (aluminum foil)	d 8 after production			
Storage	up to 6 weeks	6		

RH = relative humidity

The mould culture was supported by Laboratorium Dr. Drewes, Seesen, FRG.

All pieces of equipment (vats, knives, moulds, hurdles, etc.) and the brine, which had direct contact to vat milk, cheese curd and cheese were steam sterilized before use to avoid any kind of microbial recontamination. The cheese was ripened in glass desiccators (No. 5070250, Witeg, Wertheim/M., FRG) each containing 8 to 10 single pieces of cheese (diameter 70 mm, ca. 80 g). The relative humidity (RH) was controlled with solutions of sodium chloride in different concentrations in the bottom of the desiccators (19 % w/w) NaCl solution with a resulting RH of 85 % and 7.95 % (w/w) NaCl solution with an RH of 95 %, respectively). The desiccators were stored in cooling incubators (Type BKE50, Memmert GmbH, Schwabach, FRG). Besides cheese from laboratory production, cheese from three differences caused

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