

DAIRY FOODS

Use of Hydrolyzed Whey Peptide to Inhibit Culture Agglutination¹

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ABSTRACT

Papain was used to hydrolyze sweet whey to prepare peptides that were harvested with ultrafiltration membranes (molecular weight cutoffs of 10,000, 3000, and 1000). Insure[®] buffer salts were added to whey peptides (ratio 40:60% solids, respectively) to prepare media that were tested for their ability to inhibit culture agglutination. Commercial Insure[®] medium (75.7 g/l) was used as a control. Skim milk (240 ml) in 250-ml graduated cylinders was inoculated (4%) with *Lactococcus lactis* spp. *lactis* B62 or E72. Culture agglutination was determined by measuring upper center and bottom pH values of the skim milk column during 5 h of incubation. A pH differential was calculated by subtracting the bottom pH from the upper center pH. Cultures grown in media containing whey peptides agglutinated in skim milk to a lesser degree than when grown in the control medium. Culture agglutination was inhibited to a greater degree when cultures were grown in the 1000 molecular weight cutoff peptide medium than when grown in the 10,000 or 3000 molecular weight cutoff peptide medium. When culture E72 was grown in medium containing 1000 molecular weight cutoff peptides, culture agglutination was completely inhibited.

(Key words: whey peptides, *Lactococcus*, culture agglutination, papain)

Abbreviation key: FGM = fat globule membrane, MWCO = molecular weight cutoff.

INTRODUCTION

Culture agglutination is a problem that often occurs during cottage cheese production (14). Culture agglutination occurs during milk fermentation when lactic acid-producing bacteria bind with immunoglobulins (agglutinins) present in milk and form extended chains

of cells that clump together and cause localized acid production (2, 3). Acid produced by these aggregated cells causes protein (casein) to precipitate or coagulate around the culture (2, 6, 11). Thus, localized acid production enhances the mass of the culture aggregates to the degree that most aggregates fall to the bottom of the cheese vat and form a sludge layer and an uneven acid distribution throughout the skim milk (1, 2, 3). A large top-bottom differential of pH, cfu, and solids in the vat is exhibited when culture agglutination occurs (1, 4, 11).

Homogenization appears to affect culture agglutination by two methods. First, high pressure homogenization is necessary to reduce culture agglutination in skim milk (13). Evidently, high pressure (17.2 Mpa or greater) homogenization (13) of skim milk unfolds skim milk membrane or fat globule membrane (FGM) (12), exposing additional binding sites for the Fc region of Ig. Second, low-pressure homogenization will disrupt the interaction between the FGM and Ig (17). Walstra (17) noted that this interaction between the FGM and Ig could be easily disrupted with homogenization pressures as low as 1 Mpa.

Hicks and Hamzah (6) and Hicks and Ibramin (7) proposed that homogenization could also be used to disrupt the clumps of cells (agglutinated) in the bulk starter before they are added to the vat. However, like many other methods recommended to inhibit culture agglutination (such as high-temperature milk pasteurization and culture screening), homogenization of skim milk or bulk starter has not been accepted by the cottage cheese industry (16).

Ustunol and Hicks (16) theorized that culture agglutination may be inhibited by hydrolyzing the Ig in whey and using the binding components or fragments to block intact immune proteins from binding to the antigenic site on the culture cells surface. They prepared peptide fragments from whey with papain and harvested the peptides from a 10,000 molecular weight cutoff (MWCO) UF membrane. Results showed that these permeates contained a potent inhibitor of culture agglutination. They noted that whey peptides were able to reduce culture agglutination by 55 and 72 % for commercial cultures M30 and M37, respectively. The objec-

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tive of the present research was to determine whether smaller peptides would be as effective in reducing culture agglutination.

MATERIALS AND METHODS

Preparation of Whey Peptides

Raw milk was obtained from the University of Kentucky Dairy Farm (Lexington). Milk was pasteurized at 63°C for 30 min and cooled to 31°C. Pasteurized milk was coagulated by adding 0.5% double-strength chymosin, EC 3.4.23.4, isozyme B (CHY-MAX®; Pfizer, Milwaukee, WI). Coagulum was cut (6 mm³) after 20 min, heated to 37°C, and stirred for 1 h to aid whey expulsion. Whey was recovered by filtering the curd-whey mixture through five layers of cheese cloth. Recovered whey (37°C) was hydrolyzed with crude papain (0.15%, papaya latex, crude Type II; Sigma Chemical Co., St. Louis, MO) while being ultra filtered (10,000 MWCO) with a hollow fiber cartridge (Supelco model HF2-20-PM10; Romicon, Inc., Woburn, MA). A centrifugal pump (model 135-114-10; FASCO Industries, Inc., Cass, MO) was used to force the whey through the UF cartridge. Permeate (ca. 65% of initial vol, contained 1.86% TS) was collected over 5 h and was cooled to less than 2°C in an ice bath. One-half of the 10,000 MWCO permeate (ca. 2 L) was subsequently fractionated by passing the permeate through a YM3 UF Diaflo membrane (3000 MWCO). A portion (ca. 1 L) of the permeate from the YM3 membrane was passed through a YM1 (1000 MWCO) membrane (Amicon, Inc., Beverly, MA) under nitrogen gas (0.38 MPa). Permeates from the 10,000, 3000, and 1000 MWCO membranes were freeze-dried and used to prepare culture media.

Culture Propagation

Frozen, commercial lactic, single-strain *Lactococcus lactis* ssp. *lactis* and *cremoris* cultures (4C8, 422, 3854, 194, 874, MS2, B62, E72, 478, WWA, W, MS4, WWQ, and 620A) were obtained from Chr. Hansen's Laboratory, Inc. (Milwaukee, WI). APT broth (45 g/L with 1.5% bacto agar) slants (7 ml) were prepared (10 slants per culture), inoculated (streaked with a sterile loop), and incubated (48 h at 26°C). Colonies were washed off (1 ml sterilized 10% solids reconstituted skim milk) the slants and frozen (-86°C) in 1.1-ml sterile cryogenic vials. Cryogenic cultures were used to prepare other starter cultures.

Elliker broth was prepared (48.5 g/L of distilled water), steamed (20 min), and pH-adjusted (6.8 ± 0.2 at 25°C), and 7-ml aliquots (10-ml tubes) were sterilized at 121°C for 15 min. Medium in each tube was inocu-

lated with a different 1-ml cryogenic culture and incubated at 30°C overnight to prepare bulk starters.

Culture Agglutination Test

Screening cultures for sensitivity to agglutination. Skim milk used in agglutination tests was prepared from raw milk (5°C) by centrifugation (Sorvall, Model R-C 5-B Automatic Refrigerated Superspeed Centrifuge; DuPont Co., Newtown, CT) at 12,400 × *g*. Skim milk was drawn off by puncturing the solidified fat cap with a 10-ml pipette and aspirating the serum into a 500-ml Erlenmeyer flask. Skim milk was passed through several layers of cheese cloth to remove remaining fat granules and was pasteurized (63°C for 30 min). Pasteurized skim milk (240 ml) was inoculated with 4% bulk starter (10 g) and incubated at 31°C in 250-ml graduated cylinders. All 15 cultures were tested for their sensitivity to agglutination by monitoring the pH (pH II; American Scientific Products, McGaw Park, IL, with an 33 cm Orion Research pH 912600 combination electrode; Boston, MA) in the upper center (7.5 cm under surface) and bottom (9) of the skim milk column at hourly intervals for 6 h. The pH differential was determined by subtracting the bottom pH from the upper center pH. Cultures producing a pH differential ≥ 0.1 was categorized as agglutinating and those that produced a pH differential < 0.1 was denoted as resistant to agglutination (non-agglutinating) (7).

Agglutination tests with fractionated peptides. Agglutination sensitive cultures were used to inoculate media containing different sized peptides. Fractionated whey peptides (10,000, 3000, and 1000 MWCO) as freeze-dried permeates were used to replace the whey solids in an Insure® medium system (Gist-brocades, Millville, UT). The ratio of freeze-dried whey peptide to salt buffer mixture was adjusted as 60:40 on a solids basis. Commercial Insure® medium that contained whey solids at the same ratio was used as a control medium. All media were prepared (75.7 g/L) and heat treated at 85°C for 45 min. Media (10 ml) in graduated cylinders (25-ml) were cooled to 26°C, inoculated with 4% agglutinating culture, and incubated (with continuous agitation) at room temperature (23 to 25°C) for 16 to 18 h or until pH of 5.30 was reached. Bulk starters (10 ml) were used to inoculate 240 ml of pasteurized skim milk. Severity of agglutination in skim milk was monitored by measuring the upper center and bottom pH at 1-h intervals for 6 h and computing the pH differentials.

Experimental Design

A randomized block design was used for all culture agglutination tests. Effect of whey peptide size (whey

control, 10,000, 3000, and 1000 MWCO) on culture agglutination was tested for each agglutinating culture. The pH differentials were monitored for 6 h at 1-h intervals. Changes in upper and lower pH and pH differentials, as affected by peptide size, were statistically analyzed to determine the influence of peptide size on agglutination sensitivity. All experiments were replicated three times. Least significant differences and least square means were computed.

RESULTS AND DISCUSSION

Hydrolyzed whey peptides as originally developed by Ustunol and Hicks (16) were fractionated to determine whether smaller peptide fractions could be economically important in inhibiting culture agglutination. Three peptide fractions were prepared by filtering hydrolyzed whey through 10,000, 3000, and 1000 MWCO membranes, consecutively. Each peptide fraction was used to prepare an internal pH control medium for the growth of lactic cultures. Resultant bulk cultures were inoculated into a model skim milk system developed by Ustunol and Hicks (16) to determine the effect of peptide size on culture agglutination.

Culture Agglutination

Culture agglutination occurs when culture cells adhere to other chains or clumps of cells (3, 4, 7, 11, 13). The mass of cells start to fall in a skim milk system when a chain length or a clump size exceeds approximately eight cells (11, 14). Clumps of cells can produce acid so fast that casein precipitates around the clumps (2, 5). Rate of sedimentation increases as size of the cell and casein complex increases. Severely agglutinated cultures have over 90% of their bacteria cells on the bottom of the fermentation vessel after a few hours (2, 7). Ustunol and Hicks (16) theorized that whey peptides could inhibit culture agglutination by binding to the antigenic sites that normally bind with intact Ig, thus interfering with the bridging process. Severity of agglutination can be determined from slow or uneven acid production during cultural growth (3, 8, 10, 11, 16).

Culture screening. Commercial single strains of *Lactococcus lactis* ssp. *lactis* and *cremoris* were screened to select strains that were sensitive and resistant to agglutination. Sensitivity of cultures to agglutinate was determined by calculating a pH differential (from the upper center and bottom pH). Cultures that agglutinated (pH differential ≥ 0.1) were E72, B62, MS2, 478, and 874. All other cultures had a pH differential < 0.1 and were categorized as nonagglutinating cultures.

Some cultures (620, 422, 3854, 874, 4c8, 478, 194, W, 867, and MS2) did not decrease the pH below 6.0 at the

upper center or bottom of the skim milk column during 6 h of incubation. MS4 and WWQ cultures were categorized as moderate acid producers. Hicks and Hamzah (6) suggested that the pH should drop to 4.6 or 4.7 within 5 h of incubation before cottage cheese curd can be cut. The pH of E72, B62, and WWA cultures was < 5 , but only E72 and B62 showed high pH differentials (0.76 and 0.31, respectively). E72 and B62 cultures were selected for this study and were categorized as severely agglutinating cultures (6, 7). Microscopic observations of the sediments showed E72 to form tighter clumps of cells, whereas B62 formed slightly longer chains and looser clumps of cells. Both cultures were typical of chaining and clumping cultures (9) and would represent a worst case scenario if used in the manufacture of cottage cheese.

Effect of Different Peptide Sizes on Culture Agglutination

Media (Insure[®]-type media) were prepared with (10,000, 3000, and 1000 MWCO) and without fractionated hydrolyzed peptides. Prepared media were inoculated with B62 (first study) or E72 (second study) culture and were incubated for 16 to 18 h at 23 to 25°C to prepare bulk starter cultures. Bulk starters were used to inoculate (4%) skim milk to determine the rate of culture agglutination in the skim milk. All cultures grown in media containing peptides were less agglutinated ($P < 0.01$) when cultured in skim milk at 5 h than those grown in the whey (control) medium (Table 1).

Culture B62. Culture B62, grown in whey (control) medium, became severely agglutinated ($P < 0.0001$) by the fourth hour of incubation and developed a pH differential > 1.0 by the fifth hour (Table 1). Culture on the bottom of the cylinder appeared to be acid inhibited by the fifth hour of incubation (4). Acid development slowed after the pH dropped to 4.96 (fifth hour; Table 1) at the bottom of the cylinder. Hicks and Hamzah (6) data revealed that when the pH differential exceeds 0.3, more than 90% of the lactic bacteria are agglutinated and in the sludge layer at the bottom of the vessel.

When culture B62 was grown in media containing the 3000 and 10,000 MWCO peptide fractions growth appeared to be slightly inhibited (Table 1) because upper center and bottom pH values did not fall as rapidly as would have been expected. Growth of B62 culture grown in 10,000, 3000, and 1000 MWCO peptide media did not proceed (as monitored by acid production in skim milk) until after the fourth, third, and 0 h, respectively (Figure 1). Culture B62 from medium containing the 1000 MWCO whey peptides started producing acid immediately after inoculation and produced acid in the top of the skim milk column faster ($P < 0.01$) than the

Table 1. pH differentials during a 6-h incubation of cultures (B62 and E72) grown in media containing various peptide sizes.

Cultures	Peptide size	pH location	Time (h)					
			1	2	3	4	5	6
B62	Control	Top	6.58	6.56	6.46	6.32	6.13	5.95
		Bottom	6.65	6.46	6.36	5.65	4.96	4.76
		δ pH ¹	-0.07	0.10	0.10	0.67	1.17	1.19
	10,000	Top	6.64	6.67	6.65	6.67	6.54	6.47
		Bottom	6.71	6.74	6.71	6.64	5.91	5.54
		δ pH ¹	-0.07	-0.07	-0.06	0.03	0.63	0.96
	3000	Top	6.66	6.66	6.65	6.61	6.37	6.10
		Bottom	6.71	6.71	6.59	6.42	5.95	5.53
		δ pH ¹	-0.05	-0.05	0.06	0.19	0.42	0.57
	1000	Top	6.65	6.48	6.34	6.21	6.00	5.78
		Bottom	6.60	6.54	6.00	5.68	5.51	5.40
		δ pH ¹	-0.05	-0.06	0.34	0.53	0.49	0.38
E72	Control	Top	6.87	6.85	6.83	6.82	6.75	6.53
		Bottom	6.84	6.83	6.81	6.77	6.67	5.98
		δ pH ¹	0.03	0.02	0.02	0.05	0.08	0.55
	10,000	Top	6.30	5.69	5.10	4.74	4.62	4.56
		Bottom	6.27	5.60	4.66	4.43	4.43	4.45
		δ pH ¹	0.03	0.09	0.44	0.31	0.19	0.11
	3000	Top	6.63	6.23	5.46	5.12	4.83	4.68
		Bottom	6.59	6.04	4.83	4.54	4.55	4.47
		δ pH ¹	0.04	0.19	0.63	0.58	0.28	0.21
	1000	Top	5.92	5.29	4.76	4.56	4.46	4.40
		Bottom	5.89	5.26	4.74	4.54	4.44	4.37
		δ pH ¹	0.03	0.03	0.02	0.02	0.02	0.03

¹pH differential, least significant difference = 0.12; replications = 3.

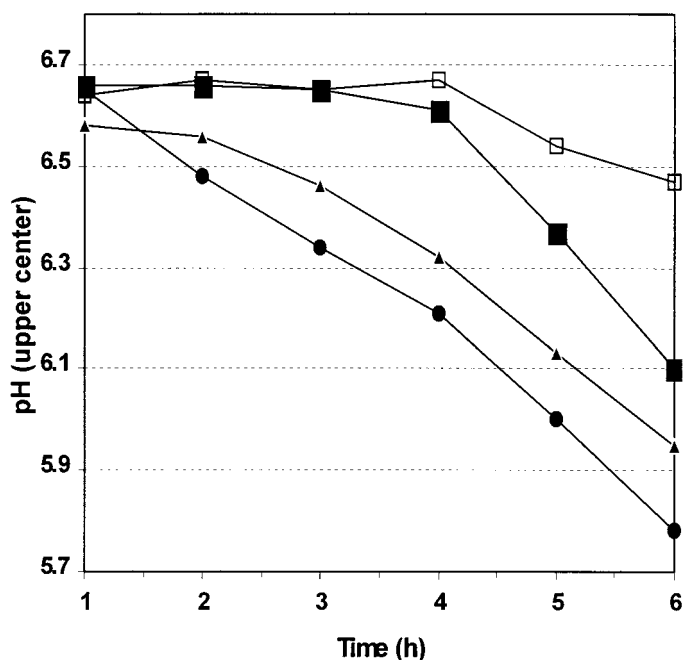


Figure 1. Growth of B62 culture in skim milk from media containing whey (▲, control) and 10,000 (□), 3000 (■), and 1000 (●) molecular weight cutoff whey peptides as monitored by the upper center pH of the skim milk column. Replications = 3.

B62 culture grown in media containing the 10,000 and 3000 MWCO peptides. They appeared to be about as active as B62 grown in whey medium. Ustunol and Hicks (16) also noted that some cultures were slightly inhibited when grown in medium containing the 10,000 MWCO whey peptides.

B62 culture grown in whey peptide media produced lower ($P < 0.001$) pH differentials than when grown in the whey (control) medium (Figure 2). Culture grown in peptide media (10,000, 3000, and 1000 MWCO) reduced culture agglutination as calculated from pH differentials by 19, 52, and 68%, respectively, after 6 h of incubation. Culture B62 grown in the 1000 MWCO media was more resistant ($P < 0.03$) to culture agglutination than cultures grown in the 10,000 and 3000 MWCO media. However, onset of culture agglutination was inversely related to peptide size. Culture B62 grown in the 1000 MWCO peptide medium showed signs of agglutination (LSD = 0.12 pH differential) by the third hour, whereas cultures grown in the 3000 and 10,000 MWCO media did not show signs of agglutination until the fourth and fifth hours, respectively, but produced a much greater pH differential (Figure 2). Culture B62 grown in whey medium (control) did not begin to agglutinate until the fourth hour. The lower molecular weight peptides actually caused or allowed some early culture agglutination to occur, but the severity of agglutination was less than

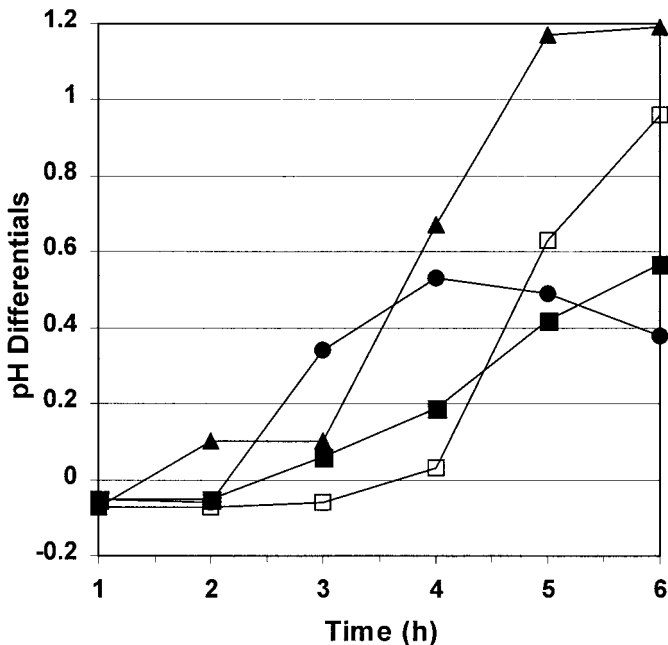


Figure 2. Sensitivity (pH differentials) of *Lactococcus lactis* ssp. *lactis* B62 to culture agglutination grown in medium containing whey (control) (▲) and 10,000 (□), 3000 (■), and 1000 (●) molecular weight cutoff peptides hydrolyzed from whey. Replications = 3. Least significant difference = 0.12.

that observed for the other cultures (Figure 2), and the pH in the upper center portion of the skim milk column was still decreasing at the sixth hour (Figure 1). The upper center pH of the culture grown in the 3000 MWCO medium dropped at an accelerated rate between the fourth and sixth hours (Figure 1).

Hicks and Hamzah (6) observed that as the pH differential increased so did yield losses, sedimentation depth, and the concentration (number of cells per g) of culture bacteria found in the sediment layer. Sedimentation rates are affected by the number of cells in the chain or clump. Large clumps of cells from the bulk starter generally sediment within a short period after they are added to the vat (7, 11). Lactic bacteria such as culture B62 that form looser complexes tend to produce longer chains. As the chain length increases, sedimentation rate increases. Because culture B62 grown in the 1000 MWCO medium agglutinated earlier, but to a lesser extent, in skim milk than the other B62 cultures, it might be speculated that the smaller peptides bound less water than the larger peptides. Thus, the chains that were initially large enough to sediment did so initially at a faster rate, with little additional agglutination occurring later because interaction between cells was inhibited. Culture B62 grown in media containing larger peptides sedimented at slower rate but to a greater extent than the culture grown in the medium

containing the 1000 MWCO whey peptides and to a lesser extent than the control.

Culture E72. Culture E72 forms dense clumps of bacteria when it agglutinates. These clumps are rather large (many clumps exceed 300 cells per clump), and the cells within the clump are packed together in a tight sphere as if the surface of the lactic cells in the clump had a high affinity for each other. Some clumps of bacteria appeared to be a composite of smaller clumps that stuck together as the culture sedimented. This physical configuration that was observed for agglutinated culture E72 may explain why this culture had an extremely limited growth in skim milk when grown in a whey medium (control bulk starter) as compared to E72 cultures grown in the other media containing whey peptides (Table 1). Note that culture E72 grown in the whey medium (control) did not appear to develop any acid or agglutinate until the sixth hour (Figure 3). However, a light brown color was observed on the bottom of the graduated cylinder after 1 h of incubation. Acid development of culture E72 was much slower than that of culture B62 when grown in the same whey media (Table 1).

When dense clumps of lactic bacteria from the bulk starter are added to skim milk, they often settle to the bottom of the vessel within a very short period of time (7, 11). Cultures that settle to the bottom of the vessel

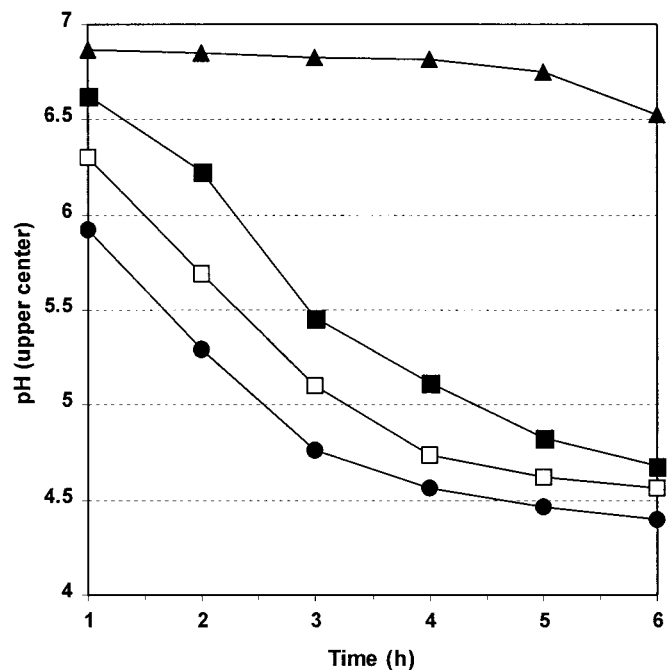


Figure 3. Growth of E72 culture in skim milk from media containing whey (▲, control) and 10,000 (□), 3000 (■), and 1000 (●) molecular weight cutoff whey peptides as monitored by the upper center pH of the skim milk column. Replications = 3.

this fast generally do not collect much casein around the clumps, because the organism is in the lag phase when it reaches the bottom of the skim milk column (7). Cultures in this state form a light brown sediment (same as color as in the bulk starter sediment) because no casein (causes a white sediment) precipitates around the clumps of bacteria (7). True pH measurements of the fine sediment layer on the bottom of the vessel are difficult to determine (6) because the bulb of the pH probe (combination electrode) is two to three times the height of the brown sediment layer. Thus, the bottom pH remains undetectable for an extended period (6) or until the sediment layer becomes deep enough to cover the pH probes bulb (about 2.5 mm). This hypothesis is supported by all E72 bulk starter cultures reaching their target pH (5.3) before being inoculated into the skim milk and showing no upper center pH activity until the fifth hour (Figure 3). Also, these events occurred during all three replications, suggesting that the inoculum was highly agglutinated coming from the bulk starter and fell to the bottom of the skim milk vessel while in the lag phase.

When E72 culture was grown in media containing 1000 MWCO whey peptides and inoculated in skim milk, culture agglutination was completely inhibited during 6 h of incubation. The peptides in the 1000 MWCO containing medium protected E72 culture better ($P < 0.001$) than when E72 was grown in media containing the 10,000 or 3000 MWCO peptides. However, when E72 culture was grown in medium containing the 10,000 MWCO peptides (the second best medium) and was inoculated into skim milk, the upper center pH decreased faster ($P < 0.01$) than when grown in media containing the 3000 MWCO whey peptides. The decrease was not as fast ($P < 0.04$) as when culture grown in medium containing the 1000 MWCO whey peptide (Figure 3).

Culture agglutination was not observed for E72 culture grown in the media containing 1000 MWCO whey peptides, even after incubating in skim milk for 6 h (Figure 4). The pH at the upper center and bottom of the skim milk column was nearly the same during the 6 h of incubation, and the pH differential did not exceed 0.03 pH units (Table 1). E72 culture agglutination was apparent (LSD = 0.12) after 2 and 3 h of incubation in skim milk when the E72 culture was grown in media containing the 10,000 and 3000 MWCO whey peptides, respectively (Figure 4).

In general, culture E72 was protected to a greater extent by the whey peptides than culture B62 (Table 1). Although the 1000 MWCO whey peptide medium protected both cultures better than the 10,000 and 3000 MWCO peptides, the E72 culture was completely protected by the 1000 MWCO peptide. It is interesting to

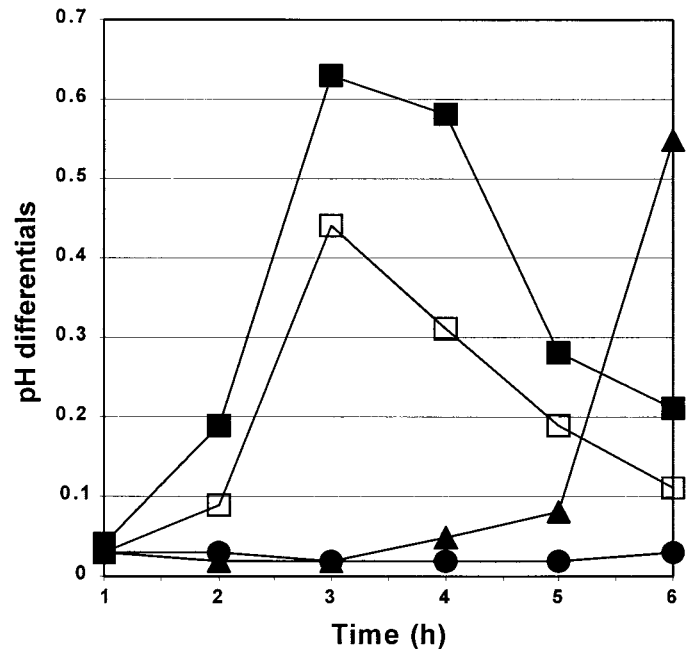


Figure 4. Sensitivity (pH differentials) of *Lactococcus lactis* ssp. *lactis* E72 to culture agglutination grown in medium containing whey (control) (▲) and 10,000 (□), 3000 (■), and 1000 (●) molecular weight cutoff peptides hydrolyzed from whey. Replications = 3. Least significant difference = 0.12.

note that this technology worked best with a “clumping organism” (7), in that E72 was so severely agglutinated in the control bulk starter that cottage cheese could not have been manufactured. When the 1000 MWCO peptide was present in the bulk starter, however, cottage cheese could have been produced without yield losses.

Peptide Activity

These data indicate that the two cultures had different sensitivities to agglutination and different growth performances with and without the presence of peptides in the media. These observations are similar to others reported in the literature. Scheuble et al. (14) observed that severity of agglutination of any particular strain depended on several factors, which included the frequency that a specific antigenic determinant was expressed on the cell surface, the agglutinin titer, or the specificity of the antibody to antigenic determinant on the cell surface.

Ustunol and Hicks (16), reported that peptide size, configuration, or association affected inhibition of culture agglutination. Whey peptides liberated by trypsin, chymotrypsin, and pepsin did not inhibit agglutination and, in some cases, increased culture agglutination. When Ustunol and Hicks (16) tested papain-hydrolyzed

wey that had been ultrafiltered through a 100,000 MWCO membrane, almost no inhibition of culture agglutination was observed. Only the fraction that was collected through a 10,000 MWCO membrane inhibited culture agglutination. Stryer (15) states that agglutination reactions stop once the F_{ab} fraction (50 KDa) has been liberated by papain. Ustunol and Hicks (16) observed that the peptide fraction had to be substantially smaller (<10 KDa) to inhibit lactic culture agglutination and proposed a different agglutination mechanism. Bridging reactions would have to be possible between a single F_{ab} molecule and a bridging component such as milk FGM.

Stryer (15) reported that peptides of less than 10 amino acids can be recognized by helper T cells; however, most protein receptor sites are larger. The smallest fraction collected in this research was from a 1000 MWCO membrane and would contain an active peptide of less than eight amino acid units, assuming the average molecular weight was 120. Evidently, this small peptide was able to bind to the antigenic site on the surface of the cell sufficiently tight to inhibit intact immune proteins from binding to these sites.

Because the pepsin hydrolysis that produces numerous F_c fractions did not inhibit culture agglutination (16), it is probable that the inhibition activity of the fractions (10,000, 3000, and 1000 MWCO) used in this research came from the F_{ab} units. F_c regions generally contain a complimentary binding site that is important in secondary bridging reactions (15). The F_{ab} fragments contain the variable regions that bind to the antigenic sites (15). The findings of this research are consistent with the literature in that the hydrolyzed peptides are probably from the variable region of an intact immune protein.

CONCLUSIONS

This study suggests that fractionated hydrolyzed wey peptide is a potential agglutination inhibitor during skim milk fermentation. Fractionated peptides that passed through 10,000, 3000, or 1000 MWCO membranes were effective culture agglutination inhibitors. However, the 1000 MWCO peptides inhibited agglutination better than larger MWCO sizes. The 1000

MWCO peptide completely inhibited culture E72 agglutination, suggesting that the smaller peptide may have greater commercial potential than a higher molecular weight fraction.

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