The Effects of Dairy Processes and Storage on Insulin-Like Growth Factor-I (IGF-I) Content in Milk and in Model IGF-I-Fortified Dairy Products

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ABSTRACT

The effects of several dairy processes on insulin-like growth factor-I (IGF-I) concentrations in milk and the storage stability of IGF-I-fortified dairy products were examined. The IGF-I content in raw milk determined by radioimmunoassay was significantly changed by the strength of heat treatments. In commercial manufacture of whole milk dry powder, IGF-I concentration was not significantly changed. A significant reduction in IGF-I content was found as the result of fermentation with a commercial starter culture. The IGF-I content in fortified milk and dried milk powder exhibited no significant changes over the tested storage periods (12 d for milk, 4 wk for dried milk powder), but the IGF-I content in the yogurt decreased significantly during storage. The use of IGF-I was varied by lactic strains and was apparent in the viable cells. When IGF-I was encapsulated using the surface-reforming process, the remaining IGF-I content after fermentation was significantly higher compared with that of the untreated control. Therefore, enteric coating of IGF-I before fermentation might be an effective method for the prevention of IGF-I degradation during fermentation.

Key words: insulin-like growth factor I, dairy process, lactic acid bacteria, encapsulation

INTRODUCTION

Insulin-like growth factors (IGF-I and IGF-II) comprise the principal growth factors in milk, and can be found in all mammalian species. Insulin-like growth factor I is a mitogenic polypeptide, the molecular structure of which is quite similar to that of insulin. This compound stimulates growth, differentiation, and metabolism in a variety of cell types, acting via IGF-I receptors (Zapf et al., 1984; Rechler and Brown, 1988).

Insulin-like growth factor I is a 7.5-kDa single chain peptide, which belongs to a family of growth factors that are identical in human, porcine, ovine, and bovine species (Tavakkol et al., 1988). Houle et al. (1997) reported on the effects of orally administered IGF-I with regard to the development of intestinal disaccharidase enzymes and villus height in pigs. Insulin-like growth factor I has also been reported to stimulate cellular growth and DNA synthesis in cultured bovine (Shamay et al., 1988) and ovine mammary tissues (Winder et al., 1989). In addition, IGF-I is a mammary apoptosis inhibitor (Neuenschwander et al., 1996; Rosfjord and Dickson, 1999). Burrin et al. (1994) reported that skeletal muscle and jejunal protein synthesis rates were higher in colostrum-fed piglets, and IGF-I in colostrum may be partially responsible for these effects. Insulin-like growth factor I content in bovine and porcine milk has been reported to be in the range of 22 to 26 ng/mL (Collier et al., 1991), and 1.27 to 8.10 ng/mL (Donovan et al., 1994), respectively. The concentration of IGF-I in bovine colostrum showed wide variation. Vega et al. (1991) reported that it was highest at 2 wk prepartum ($2,949 \pm 1,158$ ng/mL) and lowest in bovine milk at 49 d postpartum (5.0 ± 2.0 ng/mL). The IGF-I concentration was shown to increase in the final period of pregnancy (Donovan et al., 1994), and served an important function in the development of the postnatal gastrointestinal tract (Philipps et al., 1997). In this regard, supplementation with milk-borne IGF-I may prove to be therapeutic with regard to growth retardation in preterm infants.

Limited studies have been conducted regarding changes in IGF-I content during dairy processes. Juskevich and Guyer (1990) reported that the IGF-I contents in raw and pasteurized milk were 5.6 ± 0.56 and 8.2 ± 0.35 ng/mL, respectively, and that concentrations were reduced by 0.5 ng/mL or more when the same milk samples were subjected to the infant formula process. However, no other follow-up studies have been reported.

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The objectives of this study were to determine the effects of a variety of dairy processes, including homogenization, sterilization, spray drying, and fermentation on IGF-I contents in milk, and to monitor changes in IGF-I contents during the storage of model IGF-I-fortified dairy products.

MATERIALS AND METHODS

Milk and Colostrum

Raw bulk milk and colostrum were obtained at dairy farms in the northern Kyung-ki province of South Korea. Colostrum samples were collected from Holstein cows within 24 h postpartum, and were immediately frozen and stored at -40°C . After thawing, samples were skimmed by centrifugation at $9,000 \times g$ for 20 min at 4°C . The skimmed samples were diluted twice with distilled water and casein was removed from the samples by adjusting to pH 4.6 using 2 N HCl. Whey was obtained by centrifugation at $1,500 \times g$ for 15 min. The colostrum whey was then freeze-dried, and used as a source of crude IGF-I.

Effects of Homogenization and Heating on IGF-I Concentrations in Milk

Raw bulk milk was homogenized at 70°C with a homogenizer (APV-1000, APV, Silkeborg, Denmark) at a pressure of 150,000 kPa. The homogenized milk sample was divided into 3 portions. Two portions were heated at 75 and 85°C for 15 min, respectively, using a tubular-type heat exchanger (Kirchfeld, Germany), and the remaining portion was autoclaved at 121°C for 20 min.

Changes in IGF-I Concentration During Whole Dried Milk Powder Process

Whole dried milk was prepared using commercial spray dryer (APV) at the Yang-ju plant of Seoul Dairy Co. (Seoul, Korea). Raw milk was clarified, preheated at 55°C , homogenized at a pressure of 100,000 to 120,000 kPa using an homogenizer (Type 1030 MC 18-5TPS, APV), and then UHT-pasteurized (130°C for 2 s). The pasteurized milk was concentrated in a 4-effect falling film vacuum evaporator (evaporator type-2 TVR F IV, APV) at a maximum feed rate of 15,000 L/h. The temperatures of the first, second, third, and fourth effects were 73, 71, 60, and 55°C , respectively. The concentrated milk containing 40 to 45% of total milk solids was subjected to spray drying. During spray drying, the inlet chamber temperature was maintained at 140 to 150°C , and the outlet air temperature was maintained at about 85°C . Samples were

collected from raw milk, after the concentration step, and from the final whole milk powder. The IGF-I concentrations in all of the samples were then analyzed.

Changes in IGF-I Concentrations During Fermentation

Skim milk powder (Seoul Dairy Co.) was reconstituted in distilled water to give 10% total solids before pasteurization. The pasteurized reconstituted milk was inoculated with 1.5% commercial yogurt starter culture (*Lactococcus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus*, Culture Systems, Inc., Mishawaka, IN). During the fermentation process, aliquots of the samples were collected every 3 h until the pH of samples was close to 4.0. All of the collected samples were immediately frozen at -40°C , until the analysis of IGF-I concentration was conducted.

Use of Recombinant IGF-I by Single Lactic Strains

Because IGF-I concentration was decreased significantly during the fermentation process, IGF-I availability by single lactic strains was assessed. The lactic strains used in this study were obtained from the Food Microbiology Laboratory at Korea University (Seoul, Korea). *Lactococcus delbrueckii* ssp. *bulgaricus*, and *Lactococcus acidophilus* 4356 were grown at 37°C in de Man, Rogosa, and Sharpe (MRS) broth (Difco, Detroit, MI) for 18 h, and *S. salivarius* ssp. *thermophilus* ABT-4 was incubated at 42°C in M17 broth with 0.5% lactose for 24 h. Before their use in experiments, the lactic strains were subcultured at least 3 times. Recombinant human IGF-I (500 ng/mL, Gropep Pty. Ltd., Adelaide, Australia) was added to the MRS broth, M17 broth, and cell-free spent broth at lag phase, log phase (12 h after fermentation), and death phase (18 h after fermentation), respectively. All of the collected samples were immediately frozen at -40°C , until the analysis of IGF-I concentration was conducted.

Changes in IGF-I Concentration in Model IGF-I-Fortified Dairy Products During Storage

Model IGF-I-fortified milk and whole milk powder were prepared by the addition of crude IGF-I (freeze-dried colostrum whey) to local city milk (10%, wt/vol) or whole milk powder (10%, wt/wt), respectively. For the preparation of yogurt, crude IGF-I (10%, wt/vol) was added to yogurt premix (14% total solids) before fermentation. Commercial starter culture (1.5%, Culture Systems, Inc.) was inoculated, and fermentation continued in a 42°C incubator until the pH reached 4.0.

Table 1. Changes in IGF-I content of milk by homogenization and by heat treatments¹

	Process				
	Raw milk	Homogenized	Heat treatment		
			75°C, 15 min	85°C, 15 min	Autoclaved
IGF-I (ng/mL)	36.5 ± 8.4 ^a	33.4 ± 7.7 ^a	20.1 ± 5.0 ^b	20.0 ± 4.5 ^b	ND

^{a,b}Different superscripts indicate significant differences at $P < 0.05$.

¹All values are expressed as mean ± (n = 52); ND = not detected.

After fortification, the model products were vigorously stirred to ensure complete mixing, and then sealed tightly in cap tubes. The IGF-I-fortified milk and the whole milk powder were stored at 4°C for 12 d, and at 25°C for 4 wk, respectively. The yogurt samples were then stored at 4°C for 18 d. Throughout the storage period, changes in IGF-I concentrations were monitored. All treatment and analytical measurements were repeated 3 times, using different samples.

IGF-I Analysis

The IGF-I concentrations in the samples were analyzed using the method of Donovan et al. (1991). Insulin-like growth factor-binding proteins were removed by acid-ethanol treatment (HCl:ethanol = 12.5:87.5), and then neutralized. After removal of IGF-binding proteins, the sample was mixed with 0.1 mL of radioimmunoassay (RIA) buffer (30 mM sodium phosphate, 0.02% protamine sulfate, 10 mM EDTA, 0.05% Tween-20, 0.02% sodium azide, pH 7.5), containing rabbit antihuman IGF-I polyclonal antiserum (GroPep Pty., Ltd.) and [¹²⁵I] IGF-I, and incubated for 16 h at 4°C. After the incubation, 0.1 mL of goat antirabbit IgG antibody (GroPep Pty., Ltd.) was added, and the mixture was incubated for 1 h, followed by an additional 1 h of incubation with 0.1 mL of normal rabbit serum at 4°C. After the addition of 1 mL of RIA buffer, the tubes were centrifuged for 10 min at 3,000 × *g* at 4°C. The supernatant was aspirated, and the pellets were counted with a gamma counter (COBRA, Packard Instrument Co., Meriden, CT) for 1 min. All determinations were performed in triplicate.

Encapsulation of Crude IGF-I

The effects of encapsulation on IGF-I degradation during the fermentation process were also determined. The surface of freeze-dried colostrum whey (crude IGF-I) was reformed with the enteric coating ingredient, Eudragit L100-55 (Röhm GmbH, Darmstadt, Germany), in a hybridization system (model NSH-0, Nara Machinery Co., Ltd., Tokyo, Japan). In a preliminary experiment to optimize the encapsulation process, we

had determined an optimal formulation ratio of 9:1 (wt/wt, crude IGF-I: Eudragit L100-55), a running time of 3 min, and a rotor speed of 17,500 × *g*. The temperature of the hybridization chamber was maintained below 30°C by the circulation of cooled water within a jacket. During the surface-reforming process, fine wall materials adhered to the surfaces of bacteriocin particles in the dry state by friction and collision as described by Ishizaka et al. (1989).

Microstructure of Encapsulated Crude IGF-I

The microstructure of the encapsulated crude IGF-I was visualized with a scanning electron microscope (Hitachi S-2380, Ltd., Tokyo, Japan). The samples were coated for 60 s with gold-palladium in an E-1010 ion sputter coater (Hitachi Ltd.), and the topography of the particles was observed at 15 kV.

Statistical Analyses

All data were analyzed using the GLM procedure of SAS (SAS Institute, 1985). Significant differences ($P < 0.05$) between treatment means were assessed using the LSD (least significant difference) method.

RESULTS

Changes in IGF-I Concentrations During Dairy Processes

Changes in IGF-I concentration during the homogenization and heating are shown in Table 1. The IGF-I concentration in raw milk whey was found to be 36.5 ± 8.4 ng/mL, which was only slightly altered (33.4 ± 7.7 ng/mL) by homogenization. When the milk was heated at either 75°C or 85°C for 15 min, the IGF-I concentration was significantly decreased by 45.0 and 45.2%, respectively, compared with that of unheated raw milk ($P < 0.05$). When milk was autoclaved (121°C for 15 min), no IGF-I was detected in the sample. This indicates that the native IGF-I concentration in the samples was affected by heating strength. The spray-drying step, in combination with pasteurization, re-

Table 2 Changes in IGF-I concentration during the production of whole milk powder¹

	Process ²		
	Raw milk	Concentrated	Spray-dried
IGF-I (ng/mL)	47.2 ± 6.9 ^a	69.5 ± 8.2 ^b	42.5 ± 7.3 ^a

^{a,b}Different superscripts indicate significant differences at $P < 0.05$.

¹All values are expressed as mean ± SE (n = 19).

²Raw milk was concentrated yielding 40 to 45% of total milk solids. Whole milk powder was reconstituted to the same solid content as in raw milk.

sulted in no substantial changes in IGF-I concentration (Table 2). Only a minor reduction of IGF-I content could be observed when whole milk powder was reconstituted to the same solid content as in city milk.

Interestingly, IGF-I concentrations decreased dramatically, from 30.3 ± 7.5 to 5.0 ± 2.2 ng/mL, after the completion of fermentation (Table 3).

Changes in IGF-I Concentrations During the Storage of IGF-I-Fortified Dairy Products

The freeze-dried colostrum whey and raw milk whey contained about 2,473 and 32.8 ± 14.5 ng/mL of IGF-I, respectively. The IGF-I concentration in raw milk whey after fortification (10%, wt/vol) was approximately 274.4 ± 23.9 ng/mL. The IGF-I concentration in the samples exhibited no significant changes for up to 12 d of storage at 4°C (Figure 1). The IGF-I-fortified whole milk powder contained 125.5 ± 6.6 ng/mL of IGF-I after fortification, and there were no significant differences in IGF-I concentration occurring after 4 wk of storage at 25°C (Figure 2).

For IGF-I-fortified yogurt, however, IGF-I concentrations decreased significantly; only about 20% of the initial IGF-I remained after the completion of fermentation. No further decreases in IGF-I concentrations were detected after 18 d of storage at 4°C (Figure 3).

Use of IGF-I by a Single Lactic Acid Bacteria Strain

To determine the reason for the decrease in IGF-I concentrations during fermentation, recombinant human IGF-I was added to both MRS and M17 broths.

Figure 1. Changes of IGF-I concentration in IGF-I-fortified milk during storage at 4°C. Each bar represents the mean ± SE (n = 13). The raw milk was fortified with crude IGF-I at the level of 10% (wt/vol).

Each lactic acid strain was then separately inoculated, at either log phase or death phase. As shown in Figure 4, a marked decrease in the IGF-I concentration was observed in both log phase and death phase. Among the tested strains, *L. delbrueckii* ssp. *bulgaricus* and *L. acidophilus* 4356 used IGF-I more readily than did *S. salivarius* ssp. *thermophilus* ABT-4. The recovery rates of IGF-I associated with *L. delbrueckii* ssp. *bulgaricus* and *L. acidophilus* 4356 were in the range of 22 to 33%, whereas that of *S. salivarius* ssp. *thermophilus* ABT-4 ranged from 65 to 67%.

Significant reductions in IGF-I concentrations were not observed in the cell-free supernatant, regardless of the inoculated strain. This demonstrates that IGF-I use occurred principally as an activity of lactic acid bacteria, and that the extent to which it occurred varied depending on the characteristics of the lactic strain used.

Stability of Encapsulated IGF-I During Fermentation

To prevent IGF-I loss during fermentation, crude IGF-I was encapsulated by a surface-reforming pro-

Table 3. Changes in IGF-I concentration during fermentation of milk¹

	pH				
	6.32	5.02	4.45	4.20	4.06
IGF-I (ng/mL)	30.3 ± 7.5 ^a	31.8 ± 5.9 ^a	31.3 ± 5.3 ^a	26.0 ± 4.0 ^a	5.0 ± 2.2 ^b

^{a,b}Different superscripts indicate significant differences at $P < 0.05$.

¹All values are expressed as mean ± SE (n = 28).

Figure 2. Changes of IGF-I concentration in IGF-I-fortified whole milk powder during storage at 20°C. Each bar represents the mean $\pm$ SE (n = 15). The raw milk was fortified with crude IGF-I at the level of 10% (wt/wt).

cess (hybridization) using enteric coating materials (Eudragit L100-55). Figure 5 shows the size and shape of the encapsulated IGF-I. The encapsulation process resulted in smooth-surfaced spherical beads, each about 20 μ m in diameter. The microencapsulated IGF-I was then used to fortify the yogurt premix and changes in IGF-I concentrations during fermentation were monitored.

As shown in Figure 6, fermentation resulted in a 90% decrease in IGF-I concentration in the control, but only about a 20% decrease in the IGF-I concentration of the yogurt fortified with the encapsulated IGF-I. Although a gradual decrease in IGF-I concentration was observed during storage, about 58% of the initial

Figure 3. Changes of IGF-I concentration in IGF-I-fortified yogurt during storage at 4°C. Each bar represents the least squares mean $\pm$ SE (n = 18).

Figure 4. The changes of IGF-I concentration during lactic fermentation using single strain lactic acid bacteria. Recombinant human IGF-I (500 ng/mL) was added at each stage of fermentation. Each bar represents the least squares mean $\pm$ SE (n = 60). LB = *L. delbrueckii* ssp. *bulgaricus*; LA = *L. acidophilus* 4356; ST = *S. salivarius* ssp. *thermophilus*.

IGF-I concentration remained in the yogurt fortified with the encapsulated IGF-I after 18 d of storage.

DISCUSSION

Milk and colostrum contain valuable biologically active substances, in addition to their essential nutrients. Milk proteins are one of the richest sources of functional substances present in milk and colostrum. Milk peptide and growth factors constitute 2 major groups of biologically active dairy proteins. Several studies have pointed to the prospective biological activities of milk peptides (Clare and Swaisgood, 2000; Gobetti et al., 2002), whereas the application and efficacy of milk-derived growth factors, including IGF-I and transforming growth factor β , remain controversial. However, Howarth et al. (1996) reported that the oral administration of growth factor extracted from cheese whey might serve to ameliorate intestinal damage in methotrexate-treated rats. Although the biological activities exhibited by milk-derived growth factors may not be wholly analogous to their human counterparts, some efficacy should be expected, as a great deal of structural homology is shared between cow and human growth factors.

To date, reports regarding the effects of dairy processing on the concentrations of IGF-I have been quite limited. Previously, Donovan et al. (1991) and Collier

A

B

Figure 5. Morphology of crude IGF-I before and after encapsulation. A) Before encapsulation (crude IGF-I); B) after encapsulation with Eudragit L100-55.

et al. (1991) investigated the effect of heating on IGF-I concentration. According to these studies, IGF-I concentration in both human and cow's milk were not changed under normal pasteurization conditions, such as exposure to a temperature of 56°C for 30 min or to 79°C for 45 s. However, the above reports did not address the effects of other heating conditions on IGF-I concentration. More recently, Elfstrand et al. (2002) attempted to determine the effects of various processes, including filtration, pasteurization, and freeze-drying, on immunoglobulins, growth factors, and growth hormone content in bovine colostrums. They reported that heating (60°C for 45 min) and freeze-drying of colostrum whey resulted in a 75% reduction in immunoglobulin content, but the content of the growth factors remained unaffected. Our results demon-

Figure 6. The changes of IGF-I concentration in yogurt containing encapsulated IGF-I. Each bar represents the least squares mean $\pm$ SE (n = 40).

strated significantly decreasing patterns of IGF-I concentrations when raw milk was heated at 75 and 85°C for 15 min (Table 1). In the present study, IGF-I concentrations were unaffected by homogenization.

Collier et al. (1991) reported that the use of higher temperatures (121°C for 5 min) during the preparation of infant formula resulted in the denaturation of IGF-I to the extent that IGF-I was no longer recognized by the antibodies used during the RIA procedure. However, IGF-I concentrations showed little changes under commercial whole dried milk processing (Table 2).

The most significant reduction of IGF-I concentration was observed during fermentation. Our IGF-I recovery test confirmed that IGF-I was primarily used by lactic acid bacteria; substantial IGF-I loss did not occur in the cell-free spent broth. This result suggested that the observed reduction in IGF-I content during fermentation might be attributable to the activities of lactic acid bacteria, many of which are able to use IGF-I or IGF-binding protein complex as their sole nutrient source. The extent to which IGF-I was used varied depending on the bacterial strain used; IGF-I was used preferentially by *L. delbrueckii* ssp. *bulgaricus* and *L. acidophilus* 4356 compared with *S. salivarius* ssp. *thermophilus*. It is presumed that the observed reductions in IGF-I concentrations were not due to acid production by lactic acid bacteria, but instead to use of IGF-I as a nitrogen source by lactic acid bacteria.

The stability of IGF-I during storage was evaluated using model crude IGF-I-fortified dairy products. We detected no significant changes in the IGF-I concentrations of market milk or whole milk powder under typical storage conditions. However, the same pattern of IGF-I loss was found in the IGF-I-fortified yogurt, and

only 20% of the initial IGF-I concentration remained immediately after the completion of fermentation.

To prevent IGF-I loss during fermentation, a new food matrix was generated using microencapsulation. The enteric coating material Eudragit L100 was selected and used to protect the IGF-I from the acidic environment during fermentation. This enteric coating effectively reduced IGF-I degradation during fermentation; about half of the fortified IGF-I remained after storage, compared with what was observed in the uncoated treatment. This result implies that lactic acid bacteria are unable to use encapsulated IGF-I for their growth. The gradual decrease in IGF-I concentration observed during storage might be attributable to use of insufficiently coated IGF-I.

It is generally believed that lactic acid bacteria exhibit very limited proteolytic activity (Axelsson, 1998). Beshkova et al. (1998) demonstrated that *S. thermophilus* 13a possesses poor proteolytic properties, and that the proteolytic activity exerted during lactic acid fermentation is important in that it requires an exogenous nitrogen source, and affects the use of peptides and proteins from the growth medium. Our recovery test indicated that IGF-I use in the 3 selected strains was much greater during the logarithmic phase. The utilization of IGF-I by *S. salivarius* ssp. *thermophilus* ABT-4 was found to be much lower than that of the above 2 strains (66.9 and 64.8% recovery rates of *L. delbrueckii* ssp. *bulgaricus* and *L. acidophilus* 4356, respectively).

CONCLUSIONS

Insulin-like growth factor I appears to have some potential as a nutraceutical in the food industry, or as a pharmaceutical agent, akin to insulin for diabetes. Two dairy processes critically affected IGF-I concentration in milk and dairy products. Both homogenization and commercial whole dried milk process scarcely affected IGF-I concentration but it was significantly decreased either by heat treatment (75 and 85°C for 15 min) and fermentation. The decreased IGF-I content determined in the fermented products might be related to lactic acid bacteria, which are capable of utilizing either IGF-I or IGF-binding protein complex as their nutrition source. The microencapsulation of colostrum whey with enteric coating materials before fermentation yielded good results with regard to the maintenance of IGF-I content during shelf life.

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