Thermal Stability of Bovine Milk Immunoglobulin G (IgG) and the Effect of Added Thermal Protectants on the Stability

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ABSTRACT: D and z values and some thermodynamic parameters of immunoglobulin G (IgG) in phosphate buffer solution (PBS) (0.15 M NaCl/0.01 M phosphate buffer, pH 7.0) and colostral whey, with or without the presence of thermal protectants, were calculated in model systems. The D and z values for separated IgG in PBS were much lower than those for separated IgG in 20% glycerol, whey, and whey with 20% glycerol. IgG in colostral whey showed larger D and z values with the protectants. The heat denaturing rate constants at 70- 82 °C for separated IgG in PBS were larger than those of IgG in colostral whey; and the energies of activation for separated IgG in PBS, 0.2% glutamic acid, 10% whole milk, 20% maltose and 20% glycerol were also larger.

Key Words: bovine milk immunoglobulin G (IgG), colostral whey, thermal stability, D-value, z-value

Introduction

RECENTLY, SOME WOMEN HAVE GIVEN UP BREAST-FEEDING their offspring in favor of using infant formula whose composition is made as nutritious as possible compared to breast milk. However, there is the problem of severe denaturation of active antibodies and antibacterial components during the processing of formula which needs to be resolved (Kummer and others 1992). Active immunization as an appropriate immunoprophylaxis against infection has been a subject of intensive research (Kapikian and others 1980), and encouraging results have been obtained with a bovine vaccine (Vesikari and others 1984). However, because of problems arising from the practicability of actively immunizing infants, passive immunization remains of major importance. The fortification of immunity against diseases by oral administration (passive immunization) of immunoglobulin preparations containing antibodies against specific enteropathogenic (Mietens and others 1979) and enterotoxigenic (Tacket and others 1988) *Escherichia coli,* rotavirus (Hilpert and others 1987), and *Shigella flexneri* (Tacket and others 1992) has been found to be effective in experiments with animals as well as in human clinical trials. Thus, suggestions have been made to fortify the immunity of young children by adding immunoglobulins to infant formula and other foods (Goldman 1989; Facon and others 1993).

Immunization treatment elevates the specific antibody level in the serum and in milk. However, immunization of cows is costly. A number of studies have indicated that colostrum from non-immunized cows may also be effective. For instance, Bridger and Brown (1981) showed that colostrum from non-immunized cows was effective in protecting piglets against rotavirus infection. Yolken and others (1985), Musher and others (1990), and Brussow and others (1987) have determined the anti-rotavirus activity from non-immunized cows. Musher and others (1990) have pointed out that 50 ng of specific antibody is sufficient to protect a mouse against a lethal dose of *Streptococcus pneumoniae.* Based on that body weight, an estimated amount of 50 μg of a specific antibody would be required for a child weighing 20 kg to protect against the same bacterial infection.

Bovine immunoglobulin G (IgG) in colostrum is estimated to be about 50 mg/mL and is composed of 80 % IgG_1 (Jenness 1988). However, it is limited in quantity. Li-Chan and others (1994) reported surveys on the levels of IgG antibody in cow milk and indicated that the IgG content in normal milk was 0.03 to 0.71mg/mL from 254 samples in Canada in 1990 and 1991. Normal milk or whey is a more reliable source of immunoglobulins since it is available in large volumes despite the relatively low IgG content. Recently, immunoglobulins have been efficiently separated by metal chelating interaction chromatography (Al-Mashikhi and Nakai 1988); ultrafiltration and immobilized metal affinity chromatography (Fukumoto and others 1994); as well as protein G affinity chromatography (Akerstrom and others 1985), using cheese whey as the source of immunoglobulins. Thus, separating IgG from milk appears to be of great potential for its utilization in the immunological supplementation of foods (Facon and others 1993).

Thermal treatments, including sterilization, pasteurization, evaporation-concentration and spray-drying, are the usual methods of milk processing. However, immunoglobulins are thermolabile, and severe heat denaturation has been observed at temperatures above 75 °C (Glover 1985; Li-Chan and others 1995; Chen 1998). Goldsmith and others (1983) pointed out that no decline in IgG activity of breast milk was observed when heated at 62.5 °C for 30 min. Yolken and others (1985) measured the levels of antibodies against rotavirus in raw and pasteurized cow milk and stated that the level of antibodies was lowered during the heat treatment; however, it was still sufficient to provide protection against rotavirus. Canned evaporated milk and ultra heat treated (UHT) sterilized milk showed no detectable antibody activity (Li-Chan and others 1995; Kummer and others 1992). Thus, either modifications on heat treatments or the addition of thermal protectants should be considered in order to preserve the immunoprophylactic or therapeutic potential of IgG during all thermal processing.

In an attempt to investigate the thermal stability of IgG, bovine milk IgG in phosphate buffer and colostral whey, with added protectants, such as glutamic acid, maltose and glycerol, and in whole milk, were heated at temperatures ranging from 70 to 82 °C. The D-value, z-value, and some thermodynamic parameters, such as heat denaturing rate constant, enthalpy, free energy, and entropy of IgG in samples and in colostral whey were calculated, and the effects of the additives on IgG stability are discussed.

Results and Discussion

Thermal stability

Sugars that provide the apparent protection against freezing and thermal treatment in surimi processing were also reported (Shimizu and others 1994) to be effective in the protection of thermal denaturation of IgY (immunoglobulin in yolk) and IgG (Chen 1998). Separated IgG in PBS and colostral whey samples, with various thermal protectants added, were heated in model systems at 70, 72, 74, 78, and 82 °C to determine the D-values and z-values. Fig. 1 presents the semilogarithmic plots of IgG concentration measured by ELISA as a function of heating time. The residual IgG concentration decreases with increasing time and temperature and IgG mixed with 20% glycerol was observed to be the most stable followed by the sample with 20% maltose, 10% whole milk, and 0.2% glutamic acid. The loss of IgG activity could be due to heat denaturation or the unfolding of IgG molecules (Li-Chan and others 1995). Table 1 shows the decimal reduction time (D-value) calculated for each heating temperature. The higher D-values for IgG denaturation in 20 % maltose and 20% glycerol, compared with that in PBS, suggest a higher stability of IgG in sugar and sugar alcohol solutions than in buffer over this temperature range. Bovine serum IgG in PBS, boiled milk and UHT milk was stable when thermally treated at 62.7 °C for 30 min. However, it was less stable in PBS than in bovine milk over the temperature range of 72 to 80 °C (Li-Chan and others 1995). Similarly, IgG in breast milk has also been reported to be stable when heated at 62.5 °C for 30 min (Goldsmith and others 1983).

Semilogarithmic plots of the D-values vs temperatures yielded the following z-values for IgG: 7.1 °C in PBS, 7.8 °C in 0.2% glutamic acid, 8.1 °C in 10% whole milk, 8.3 °C in 20% maltose, and 8.8 °C in 20% glycerol. The higher z-values for IgG in sugar and sugar alcohol solutions suggest its higher stability in these solutions. Similar trends were observed (Fig. 2) when colostral whey was mixed with various protectants and thermally treated over the same temperature range. However, the decline of IgG activity in each whey sample was much slower than that in IgG PBS samples heated at the same temperature (Fig. 1), especially when colostral whey was heated to temperatures above 74 °C. Kummer and others (1992) reported that IgG is unstable when heated above 75 °C. Li-Chan and others (1995) reported a lower z-value (6.7 °C) for serum IgG in PBS compared with that in our study when samples were heated over a temperature range from 72 to 80 °C. Thus, it is obvious that the thermal protection of sugar and glycerol against milk IgG denaturation is remarkable when samples containing IgG are heated around the denaturing temperature. In addition, the components other than IgG in the whey and milk are beneficial in stabilizing the IgG during the thermal treatment. Morgan and others (1986) reported a z-value of 5.5 °C for the denaturation of IgA in breast milk. The slight difference in z-values may be in part due to the inherent differences in the thermal stability of bovine milk IgG in our study and bovine serum IgG (Li-Chan and others 1995) as well as breast milk IgA (Morgan and others 1986), and in part due to the possible variation in the determination of residual IgG concentration using ELISA as the assay technique. Table 1 presents the D-values vs temperatures, with the z-values for co-

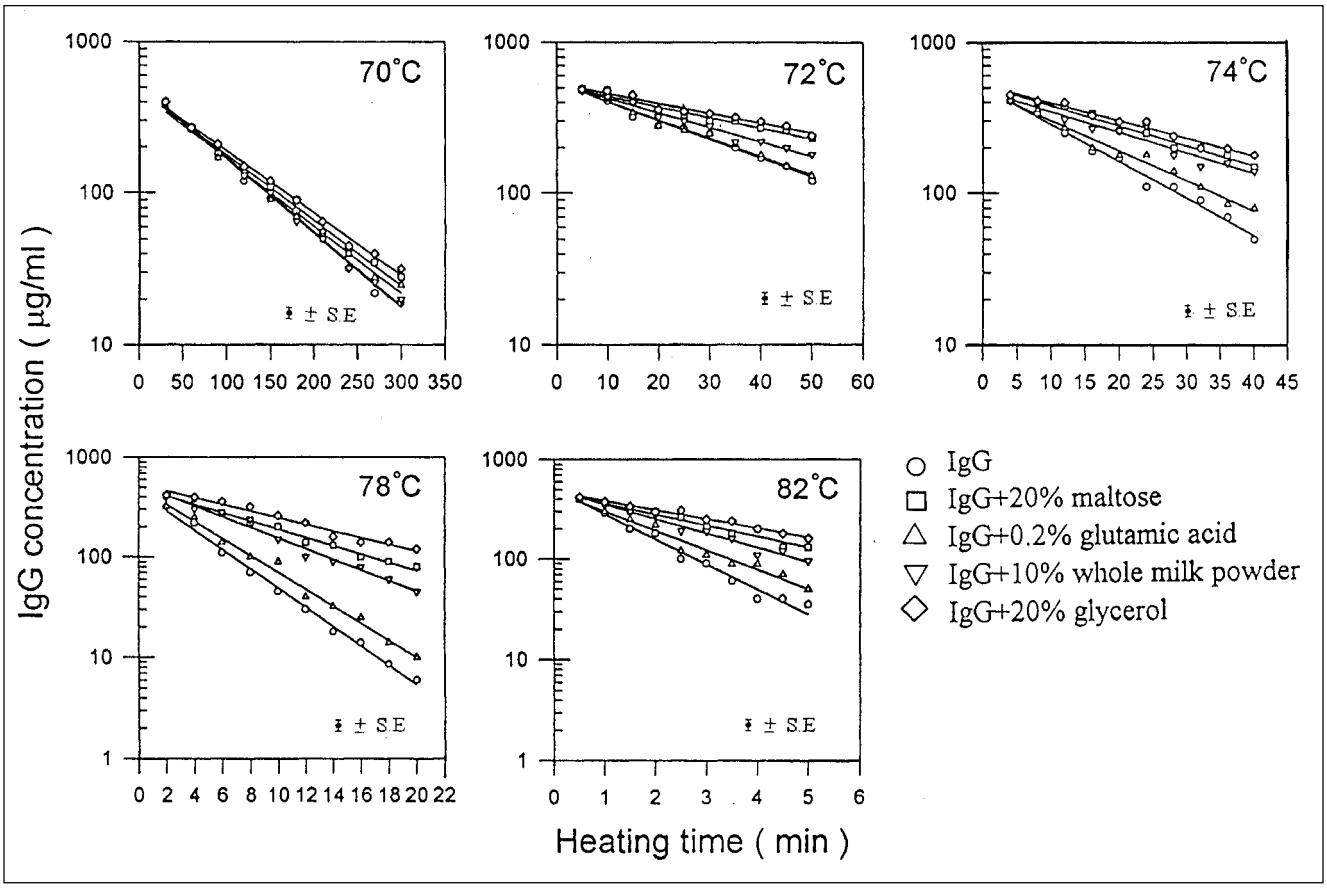

Fig.1—Semi-logarithmic plots of IgG concentrations in separated IgG with various protectants added as a function of heating time at temperatures ranging from 70 °C to 82 °C. Each value is the average of three determinations.

Table 1—D-values for thermal destruction of IgG in samples (4.2 mg/mL) and in colostral whey (4.8 mg/mL) with various protectants in model systems

	D-value (min)[a]								
	Bovine milk IgG in					Whey in			
Temperature (°C)	PBS[b]	0.2% glutamic acid	10% whole milk	20% maltose	20% glycerol	control	0.2% glutamic acid	20% maltose	20% glycerol
70	200.0 ± 2.5	208.3 ± 2.6	217.3 ± 2.4	232.5 ± 2.7	245.1 ± 3.4	234.1 ± 3.1	252.2 ± 3.7	257.2 ± 3.4	276.0 ± 3.8
72	80.6 ± 3.1	83.3 ± 2.4	107.6 ± 2.1	142.9 ± 2.4	152.0 ± 2.4	97.2 ± 2.4	110.0 ± 2.6	160.1 ± 2.7	180.1 ± 2.7
74	40.9 ± 1.3	55.5 ± 1.7	66.0 ± 1.3	76.9 ± 1.5	88.2 ± 1.8	56.7 ± 1.1	58.7 ± 1.4	90.2 ± 1.8	102.0 ± 1.9
78	10.4 ± 0.8	11.8 ± 0.8	18.7 ± 0.6	23.9 ± 0.8	30.1 ± 1.0	11.6 ± 0.9	12.6 ± 0.5	28.3 ± 0.6	40.1 ± 1.3
82	4.0 ± 0.5	5.7 ± 0.4	6.9 ± 0.5	8.9 ± 0.4	10.9 ± 0.6	6.5 ± 0.7	6.8 ± 0.5	9.1 ± 0.4	11.1 ± 0.7

[a] These values are averages of three determinations
[b] 0.15 M NaCl/0.01 M phosphate buffer (pH 7.0)

lostral whey being 7.4, 7.5, 8.5, and 9.0 °C for the thermal destruction of IgG in whey, whey with 0.2% glutamic acid, 20% maltose, and 20% glycerol, respectively. Addition of 20% glycerol or maltose helps stabilize colostral IgG remarkably during thermal processing, similar to IgY being stabilized (Ou-Yang 1997).

Sugars show a remarkable stabilizing effect on IgY molecules under various physical and chemical conditions by increasing the hydrophobic interactions in the protein molecules (Shimizu and others 1994). The extent of stabilization of proteins and enzymes by different sugars and polyols is explained by their different influences on the changes of preferential solvation of protein molecules (Back and others 1979; Ismond and others 1988; Timasheff 1993). However, the extent of stabilization of proteins is variable. For example, glycerol shows less protective effect than maltose in the heat denaturation of fish myofibrils (Ooizumi and others 1981), while it was observed to be a strong protectant against the thermal denaturation of milk IgG in our study. The magnitude of thermal stabilization of proteins by glycerol, sugars, and sugar alcohols could be related to the extent of molecular interactions between them. Besides, the number of hydroxy groups in a single sugar or sugar alcohol molecule has also been reported to be closely related to the thermal protective effect in protein denaturation (Ooizumi and others 1981).

Thermodynamic parameters

Table 2 shows the calculated heat denaturing rate constants of IgG, with various amounts of protectants added and heated at temperatures ranging from 70 to 82 °C, by using the Arrhenius equation (Sanchez and others 1992). The rate constant increased with increasing temperature and that of the IgG in PBS was observed to be the highest, followed by those of IgG with 0.2% glutamic acid, 10% whole milk, 20% maltose, and 20% glycerol. The order in magnitude was opposite to that in Table 1, which indicated a reverse relationship between the D-value

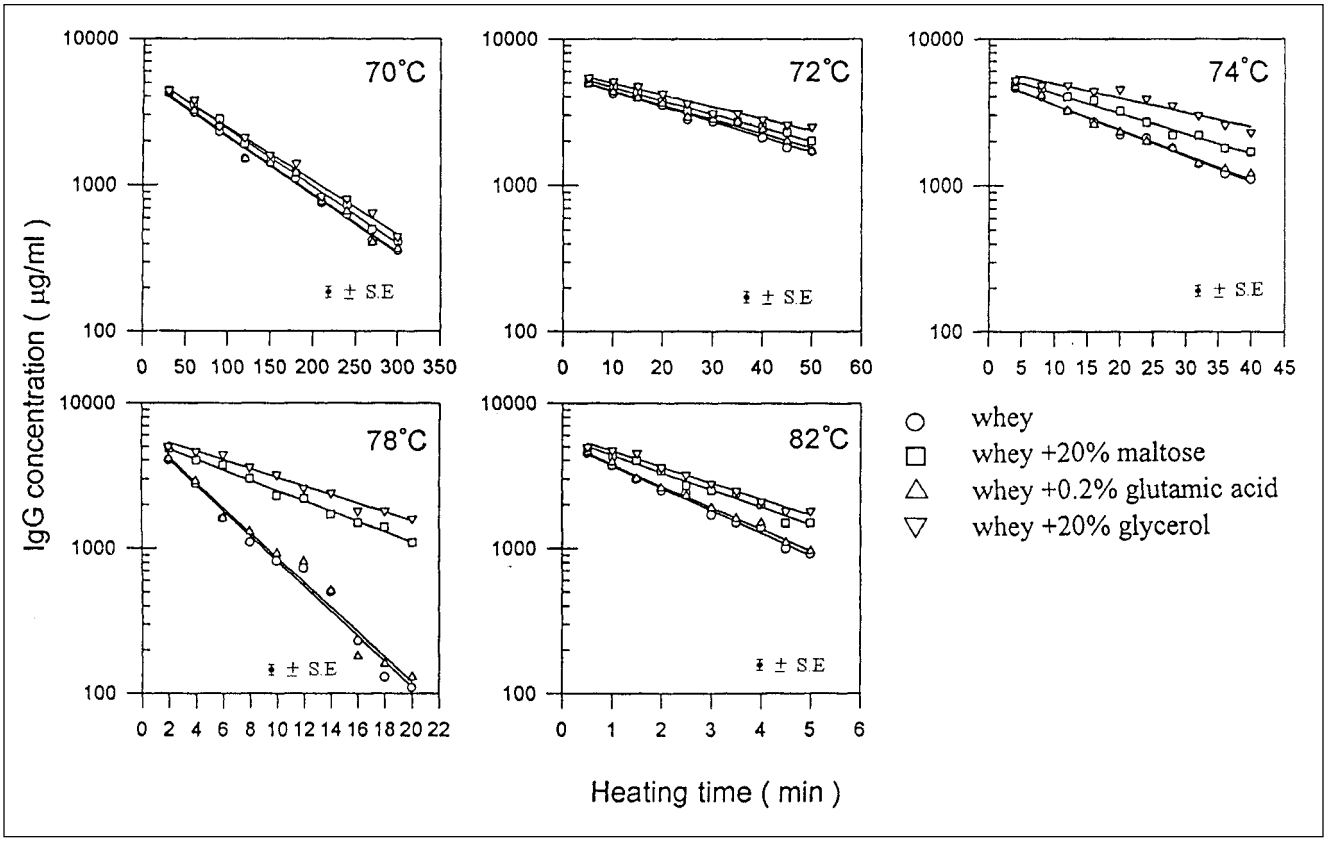

Fig. 2—Semilogarithmic plots of IgG concentrations in colostral whey with various protectants added as a function of heating time at temperatures ranging from 70 °C to 82 °C. Each value is the average of three determinations.

Table 2—Heat denaturation rate constants (min^{-1}) of IgG in samples (4.2 mg/mL) and in colostral whey (4.8 mg/mL) with various protectants added

	Rate constant (k, × 10^2)[a]								
	IgG in						Whey in		
Temperature (°C)	PBS[b]	0.2% glutamic acid	10% whole milk	20% maltose	20% glycerol	control	0.2% glutamic acid	20% maltose	20% glycerol
70	1.15 ± 0.05	1.10 ± 0.04	1.05 ± 0.04	0.99 ± 0.04	0.94 ± 0.03	1.02 ± 0.15	1.00 ± 0.04	0.89 ± 0.03	0.83 ± 0.04
72	2.87 ± 0.11	2.76 ± 0.12	2.14 ± 0.08	1.71 ± 0.06	1.50 ± 0.06	2.37 ± 0.11	2.21 ± 0.11	1.52 ± 0.07	1.38 ± 0.54
74	5.63 ± 0.24	4.68 ± 0.21	3.11 ± 0.12	2.89 ± 0.13	2.62 ± 0.14	4.06 ± 0.18	3.92 ± 0.17	2.80 ± 0.12	2.25 ± 0.11
78	22.14 ± 1.01	19.52 ± 0.84	12.31 ± 0.51	9.63 ± 0.35	7.67 ± 0.35	18.72 ± 0.86	17.85 ± 0.74	8.14 ± 0.33	5.75 ± 0.24
82	57.57 ± 2.34	38.37 ± 1.63	31.04 ± 1.24	25.87 ± 1.18	21.13 ± 1.02	35.32 ± 1.14	33.96 ± 0.015	23.93 ± 1.11	20.93 ± 1.00

[a]These values are averages of three determinations
[b]0.15 M NaCl/0.01 M phosphate buffer (pH 7.0)

Table 3—Energies of activation of IgG in samples (4.2 mg/mL) and in colostral whey (4.8 mg/mL) with various protectants added

Sample	Energy of activation (kJ/mole)[a]
IgG in PBS[b]	328.4 ± 1.5
IgG + 0.2% glutamic acid	300.5 ± 2.1
IgG + 10% whole milk	289.4 ± 1.4
IgG + 20% maltose	280.3 ± 1.6
IgG + 20% glycerol	265.4 ± 2.0
whey	316.1 ± 1.7
whey + 0.2% glutamic acid	292.8 ± 1.3
whey + 20% maltose	273.6 ± 1.8
whey + 20% glycerol	257.3 ± 1.6

[a] These values are averages of 3 determinations
[b]0.15 M NaCl/0.01 M phosphate buffer (pH 7.0)

and the heat denaturing rate constant. Similar trends in IgG of colostral whey with added sugar, sugar alcohol, and amino acid were observed over the same temperature range (Table 2).

Calculation of the energies of activation (Ea) using the Arrhenius equation resulted in the values of 328.4, 300.5, 289.4, 280.3, and 265.4 kJ/mole for IgG in PBS, 0.2% glutamic acid, 10% whole milk, 20% maltose, and 20% glycerol, respectively. The energies of activation of colostral whey with glutamic acid, maltose, and glycerol added were lower compared with those of separated IgG with the corresponding added protectants (Table 3). The magnitude of energy of activation of milk IgG in PBS is close to that of serum IgG as reported by Li-Chan and others (1995) and of the other whey components, including lactoferrin (Sanchez and others 1992), β-lactoglobulin A and B (Dannenberg and Kessler 1988), over a similar temperature range. Therefore, it is evident that the addition of certain kinds of sugar and amino acid can lower the energy of activation and, thus, lead to the stabilization of IgG.

Higher thermal stability of bovine milk IgG in 20% maltose or glycerol than in PBS was indicated by the larger D-values (Table 1) for thermal denaturation at temperatures ranging from 70 to 82 °C. In our study, the higher z-value and lower Ea suggest a higher thermal stability of IgG in sugar solutions than in PBS, which is consistent with the results reported by Sanchez and others (1992) for the destruction of iron-saturated lactoferrin heated at temperatures ranging from 72 to 85 °C. Besides, similar results (Li-Chan and others 1995) were presented when bovine serum IgG in PBS, boiled milk, and UHT milk was heated over a similar temperature range (72 to 80 °C). The larger D-values, higher z-values, and lower energies of activation for IgG in milks than in PBS suggest a smaller temperature dependence of the rate of IgG destruction in milks than in PBS.

The changes of enthalpy, free energy, and entropy of IgG (Table 4) and colostral whey (Table 5), with or without the presence of various protectants, were calculated. Generally, a higher value of enthalpy calculated during the thermal treatment indicates a more severe conformation change. Separated IgG in PBS displayed the highest value of enthalpy change at any temperature during the thermal treatment followed by the IgG with 0.2% glutamic acid, 10% whole milk, 20% maltose, and 20% glyc-

Table **4—Changes[a] of enthalpy (H*), free energy (G*) and entropy (S*) of IgG in samples with various protectants added**

	IgG in														
	PBS			0.2% glutamic acid			10% whole milk			20% maltose			20% glycerol		
Temperature (°C)	ΔH*	ΔG*	ΔS*	ΔH*	ΔG*	ΔS*	ΔH*	ΔG*	ΔS*	ΔH*	ΔG*	ΔS*	ΔH*	ΔG*	ΔS*
70	325.51	97.14	0.66	297.70	97.51	0.58	286.58	97.26	0.55	277.42	97.57	0.52	262.54	97.71	0.48
72	325.49	95.09	0.67	297.68	95.21	0.59	286.56	95.94	0.55	277.40	96.76	0.52	262.52	96.96	0.48
74	325.47	93.72	0.67	297.66	94.95	0.58	286.54	95.43	0.55	277.38	95.55	0.52	262.50	95.93	0.48
78	325.44	90.84	0.67	297.63	91.21	0.59	286.51	92.55	0.55	277.35	93.27	0.52	262.47	93.93	0.48
82	325.41	84.41	0.68	297.60	90.61	0.58	286.48	90.73	0.55	277.32	91.45	0.52	262.44	92.05	0.48

Standard deviation of each value is less than 1.0%
[a] These values are averages of three determinations

Table 5—Changes[a] of enthalpy (H*), free energy (G*) and entropy (G*) of IgG in colostral whey with various protectants added

	Whey in											
	Control			0.2% glutamic acid			20% maltose			20% glycerol		
Temperature (°C)	ΔH*	ΔG*	ΔS*	ΔH*	ΔG*	ΔS*	ΔH*	ΔG*	ΔS*	ΔH*	ΔG*	ΔS*
70	313.21	97.84	0.63	287.98	97.80	0.62	270.79	97.87	0.50	254.49	98.07	0.46
72	313.19	95.65	0.63	287.96	95.85	0.62	270.77	95.98	0.51	254.47	96.37	0.46
74	313.17	94.66	0.63	287.94	94.77	0.62	270.75	95.39	0.51	254.45	96.37	0.46
78	313.14	90.89	0.63	287.91	91.16	0.62	270.72	93.76	0.51	254.42	94.77	0.46
82	313.11	90.53	0.63	287.88	90.65	0.62	270.69	91.33	0.51	254.39	92.07	0.46

Standard deviation of each value is less than 1.0%
[a]These values are averages of three determinations

erol added. Results in Table 6 also show a similar trend in enthalpy change when colostral whey samples were heated over the same temperature range.

Ovalbumin, lysozyme, conalbumin, and α-chymotrypsin were heated at a constant rate and the temperatures of the maximum rate of denaturation were raised in the presence of sugars and polyols (Back and others 1979). The magnitude of the stabilizing effect depends on both the nature of the protein and the nature of the sugar or polyol. The stabilization of proteins and enzymes is due to the effects of sugars and polyols on the enhancement of hydrophobic interactions in protein molecules (Back and others 1979; Shimizu and others 1994). Thus, the heat denaturing rate constants, changes of enthalpy, free energy, and entropy of milk IgG were all lowered in the presence of sugar or glycerol.

Conclusions

CERTAIN KINDS OF ADDITIVES SUCH AS SUGAR, AMINO ACID, and sugar alcohol were found to be effective in stabilizing IgG activity; however, the mechanisms could not be fully explained. Components other than IgG, such as fats, lactose, and proteins, in whey and whole milk appear to be helpful in the stabilization of antibody during the thermal treatment in model systems. Although the application of high levels of sugar and sugar alcohol, up to 20%, is impractical in food processing, the use of such additives suggests the beneficial effectiveness in preserving the IgG activity during thermal processing which would enhance the therapeutic potential of immunoglobulins in cow milk.

Materials and Methods

Materials

Bovine colostrum milk was collected within 6 d postpartum from the Taiwan University dairy herd and kept frozen at -20 °C until used. Frozen colostrum was thawed in running tap water and then centrifuged (10,000 ∞ *g*, 30 min, 4 °C) to remove the upper cream layer; and the pH of the colostrum was adjusted with 1 N HCl to pH 4.6. The colostrum thus obtained was kept for 30 min in a 40 °C water bath to facilitate curd formation. Centrifugation was performed again in order to collect the clear whey. The pH of the whey was raised to 7.0 by 1 N NaOH to protect the immunoglobulins from denaturation. Subsequent centrifugation was repeated to remove the suspended precipitants formed during the pH neutralization, and the supernatant (4.8 mg active IgG/mL) finally collected was used as the experimental material. Active IgG concentration was determined with the enzyme-linked immunosorbent assay (ELISA) method.

The whole milk used was reconstituted from a commercial product (Klim Co., Taiwan). Rabbit anti-bovine IgG whole serum, bovine serum IgG, p-nitrophenyl phosphate, agarose type IV, and alkaline phosphatase conjugated rabbit anti-chicken IgG were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Radial immunodiffusion plates were purchased from Fisher Scientific (Ottawa, Ont., Canada). All other reagents were of analytical grade.

Separation of IgG from colostrum

IgG was prepared according to the method described by Akerstrom and others (1985) as well as Fredriksson and Nilsson (1987). Protein G Sepharose 4 Fast Flow (Pharmacia, Sweden), packed in the column (1.0 cm ∞ 10 cm) and equilibrated with binding buffer (0.02 M phosphate buffer, pH 7.0), was charged with 4 mL (approximate 19 mg IgG) of colostral whey and was subsequently eluted with binding buffer until the absorbance at 280 nm was approximately zero. Glycine buffer (0.1 M glycine, pH 2.8) was, then, applied to the column, and the collected effluent was neutralized with 1 M Tris-HCl (pH 9.0) to approximately pH 7.0 to protect IgG from denaturation. The IgG thus obtained was dialyzed against 100 volumes of PBS (0.15 M NaCl/0.01 M phosphate buffer, pH 7.0) at 4 °C for 24 h and was then freeze-dried. The IgG powder with a recovery and purity of 97.1% and 86.6% (Chen 1998), respectively, was dissolved to give a final concentration of 4.2 mg active IgG/mL in PBS before being used in the stability study. Active IgG concentration was determined with ELISA, and the total protein concentration was measured by the method described by Bradford (1976), using the Bio-Rad protein assay dye reagent.

Thermal treatment of IgG samples in model systems

Thermal destruction of IgG was calculated using the thermal death time method described by Morgan and others (1986), as well as Ramaswamy and others (1989), assuming first-order destruction. The D-value, representing the time required for 90% denaturation of IgG, was calculated for each heating temperature from semilogarithmic plots of concentration of active IgG vs heating time. The z-value, representing the temperature change (°C) required to reduce the D-value in one logarithmic cycle in the temperature range of 70 to 82 °C under investigation in this study, was calculated by the negative reciprocal slope of the semilogarithmic plot of D-values vs heating temperature.

Aliquots (200 μL) of IgG samples (IgG in PBS, 0.2% glutamic acid, 10% whole milk, 20% maltose, and 20% glycerol) and colostral whey samples (colostral whey with 0.2% glutamic acid, 20% maltose, and 20% glycerol) in disposable glass tubes (40 mm ∞ 3.5 mm inner diameter) were heated in temperature-controlled water baths at 70, 72, 76, 78, and 82 °C to investigate the relation of residual IgG activity to changes in thermal temperature and time. The temperature of the samples was measured by an electronic thermometer (model Tc-100, Line Seiki, Singapore). The time taken for the solutions to equilibrate to the temperature of the water bath ranged from 7 to 9 s. At specified time intervals, individual test tubes were removed from the water bath and immediately cooled in an ice water bath. Each thermal treatment was done in triplicate.

Thermal kinetic analysis was conducted according to the method described by Sanchez and others (1992) to calculate the heat denaturing rate constant (k), activation energy (Ea), enthalpy change (ΔH*), free energy change (ΔG*), and enthalpy change (ΔS*) using the Arrhenius equation, $\ln k = \ln s - (Ea-/R)\ (1/T)$, where k is a reaction rate constant calculated from the D-value using the relationship $k = 2.303/D$; s is a frequency factor; Ea is the energy of activation; R is the universal gas constant (8.314 joul/deg^{-1} mole^{-1}); T is the temperature in K. Furthermore, $\Delta H^* = Ea-RT$, $\Delta G^* = RT(\ln K_B/hp + \ln T - \ln k)$, where K_B = Boltzmann constant ($1.38 \infty 10^{-16}$ erg/ K); and h_p = Planck constant ($6.62 \infty 10^{-27}$ erg/ K); and $S^* = (\Delta H^* - \Delta G^*)/T$.

Least squares linear regression analysis of the data on semilogarithmic plots were performed using SYS-TAT/SY-

GRAPH (Wilkinson 1990a, b).

Determination of protein

Protein content was determined according to the method described by Bradford (1976), using the Bio-Rad protein assay dye reagent. Various concentrations (100 to 500 μg/mL) of bovine serum IgG (Sigma Chemical Co.) was used to obtain the calibration curve.

Determination of IgG concentration

The single radial immunodiffusion method (SRID) was used according to the procedures described by Fukumoto and others (1994). Rabbit anti-bovine IgG (1.0 mg/mL) was mixed with agarose type IV and PBS to make a gel. Bovine serum IgG was diluted with PBS to various concentrations (0.25, 0.5, 0.75,1.0, 1.5, and 2.0 mg/mL) in order to establish the standard curve for the quantification of IgG in the samples. Triplicate samples were analyzed twice for IgG by SRID.

ELISA (Kummer and others 1992) was conducted to quantify the IgG content in IgG and colostral whey samples during thermal treatment. For the analysis of thermal destruction of IgG in model systems, standard curves for ELISA were constructed, using bovine serum IgG (Sigma Chemical Co.) of known concentration as determined by the Bio-Rad protein assay dye reagent (Bradford 1976) to prepare a standard solution of IgG in PBS. Triplicate samples of each temperature-time combination were analyzed twice for IgG by ELISA.

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