Journal of Food and Drug Analysis, Vol. 13, No. 1, 2005, Pages 57-63 藥物食品分析 第十三卷 第一期

Antioxidant Properties of Caseins and Whey Proteins from Colostrums

SHU-HUA CHIANG AND CHI-YUE CHANG*

Department of Bioindustry Technology, Da-Yeh University, 112 Shan-Jiao Rd., Dah-Tsuen Township, Chang-Hwa County 515, Taiwan, R.O.C.

(Received: May 21, 2004; Accepted: October 18, 2004)

ABSTRACT

The bovine colostrums collected within 7 days postpartum were used to prepare caseins and whey proteins. The caseins and whey proteins obtained by acid precipitation were freeze-dried and then dissolved respectively in 0.1 M and 0.05 M sodium phosphate buffer (pH 7.0) for electrophoresis and antioxidant activity analysis. The antioxidant activities, including reducing power, ferrous ion chelating ability, α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging activity and inhibitory effect on lipid peroxidation, were measured and compared with α-tocopherol, butylated hydroxyanisole (BHA) or ethylenediamine tetraacetic acid (EDTA, 2 Na-salt). Results showed that whey proteins at a concentration of 20 mg/mL had triple reducing power as that of the caseins. As for the ferrous ion chelating ability, both caseins and whey proteins exhibited increasing chelating ability as protein concentration increased. The whey proteins obtained from the colostrums collected on the first two days apparently exhibited relatively higher chelating ability. Similarly, DPPH radical scavenging activity of caseins and whey proteins increased as the protein concentration increased. Whey proteins from the colostrums collected between the second and the fourth days (Whey proteins 2, 3 and 4) and the caseins from the colostrums collected on the second day (Casein 2) had relatively higher radical scavenging activities, higher than 60% at a concentration of 20 mg/mL. The inhibitory effect on lipid peroxidation of caseins was higher than that of whey proteins, and was comparable to those of α-tocopherol and BHA within 8-hour reaction.

Key words: bovine colostrums, casein, whey protein, antioxidant activity

INTRODUCTION

Many human diseases are known to be related to free radicals. The free radical scavenging medicines are antioxidants in nature. Hence, the relationship between human health and the minor nutrients possessing antioxidant activity, such as vitamin C, vitamin E, β-carotene, glutathione, and lactoferrin, has always been a popular research subject[1-3]. Lactoferrin, a milk protein, exhibits high antioxidant activity and scavenges free radicals generated in the human body. It also has a direct and positive effect on inhibiting atherosclerosis and ageing, and thereby milk proteins have potential in the pharmaceutical industry in the future[1].

Milk proteins are divided into caseins and whey proteins, which are rich in essential amino acids and so are high-quality proteins. It is evident that there is a close relationship between the intake of milk and the human health. Maruyama *et al.*[4] reported that the active peptides in the milk caseins exhibited inhibitory activity on angiotensin I-converting enzyme. Mills *et al.*[5]reported that some peptides in the milk protein hydrolysate had immunological regulatory activity. Donnelly *et al.*[6] showed that whey protein isolate exhibited antioxidant activity in the Tween-20 oil emulsion system. Many studies also proved that whey proteins with molecular weight higher than 3,500 Da had inhibitory activity on lipid peroxidation[7,8].

Bovine colostrum is the milk secreted by cow during the first several days postpartum. Colostrum is not only a source of nutrients such as protein, carbohydrates, fat, vitamins and minerals, but also contains several biologically active molecules which are essential for specific functions. Migliore-Samour *et al.*[9] and Meisel[10] reported that the peptides derived from colostrum casein had immunological enhancing activity. Some peptides, such as Thr-Thr-Met-Pro-Leu-Trp, Pro-Gly-Pro-Ile-Pro-Asn, and Leu-Leu-Try, could help mice reduce the infection of *Klebsiella pneumoniae*[9]. Normally, colostrum is light yellow in color but becomes reddish yellow due to haemolysis. Colostrums are thicker and more viscous than normal milk, and has a distinct odor. From the standpoints of food processing and food hygiene, colostrum is not suitable to use as raw materials for dairy products. At the present, much colostrum has been secreted, and the farmers have little knowledge about colostrums and their usage. In order to evaluate the use of bovine colostrums, the colostrums collected on the different days postpartum are used in this study to prepare caseins and whey proteins, and their antioxidant activities are measured. All the results are expected to be references to produce high-valued products from bovine colostrums.

* Author for correspondence. Tel: +886-4-8511888 ext. 2281; Fax: 886-4-8511322; E-mail: feccy@mail.dyu.edu.tw

MATERIALS AND METHODS

I. *Materials*

Bovine colostrums collected within 7 days postpartum were obtained from the Chu-En Ranch at Hsiu-shui, Chang-Hwa County, Taiwan. The colostrums were collected at about 8 a.m. and then centrifuged at 10,000 × g for 30 min at 4˚C to remove fat. The colostrums after centrifugation were adjusted to pH 4.6 with 1.0 N HCl and allowed to rest in water bath at 30˚C for 30 min for the complete precipitation of caseins. The supernatant thus obtained was adjusted to pH 7.0 with 1.0 N NaOH and then centrifuged again at 10,000 × g for 30 min at 4˚C. The final supernatant after centrifugation twice is treated as whey proteins. Both of the caseins and whey proteins were freeze-dried and stored in a desiccator until use.

The caseins and the whey proteins obtained from colostrums collected within 7 days postpartum were designated as Casein 1~7 and Whey protein 1~7, respectively.

II. *Methods*

(I) *Electrophoresis*

Caseins and whey proteins were dissolved in 0.1 M[11] and 0.05 M[8] sodium phosphate buffer (pH 7.0), respectively. Freeze-dried caseins and whey proteins (0.1 g) were dissolved in 20 mL of 0.1 M and 0.05 M phosphate buffer (pH 7.0), respectively. Ten microliter of the protein sample liquid was added with 10 μL of lysis buffer containing 0.5 M Tris (pH 6.8), 2% bromophenol blue, 10% SDS, 75% glycerol, β-mercaptoethanol, and distilled water. The sample was then loaded on 15% acrylamide gel (gel concentration: stacking gel, 4.5%; resolving gel, 15.0%) for electrophoresis. Electrophoresis apparatus Model AE-6450 (ATTO, Japan) and power supplier PS500XT with a 2.5 AMP input (Hoefer scientific instruments, USA) were used. Electrophoresis started at 70 V and was tuned up to 140 V after a tracer dye entered the resolving gel. This voltage was held until the tracer dye reached the bottom of the gel. Gels were then immersed in Coomassie Blue for 2 hr and the color was stripped by a solution containing 7% methanol and 7% acetic acid.

(II) *Protein analysis*

The protein contents of the samples were determined in triplicate by a semimicro-Kjeldahl procedure[12]. Protein content was calculated as N × 6.38.

(III) *Preparation of sample solution*

Caseins and whey proteins were dissolved in 0.1 M[11] and 0.05 M[8] sodium phosphate buffers (pH 7.0), respectively. Freeze-dried casein or whey protein samples (100, 200, 400, 600, 800, and 1000 mg) were weighed and dissolved in 50 mL of sodium phosphate buffer by stirring with a magnetic stirrer. The concentrations of protein samples, α-tocopherol, BHA, and EDTA for antioxidant activity test were 2, 4, 8, 12, 16, and 20 mg/mL.

(IV) Test for reducing power

A method developed by Oyaizu[13] for reducing power test was used. The above sample solution (10 mL) together with α-tocopherol and BHA methanolic solutions were spiked with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was then kept in a 50˚C water bath for 20 min. The resulting solution was then cooled by placing it in a 20˚C water bath for 5 min, spiked with 2.5 mL of 10% trichloroacetic acid, and centrifuged at 800 × g for 10 min. The supernatant (5 mL) was then mixed with 5 mL of distilled water and 1 mL of 0.1% ferric chloride. The absorbance at 700 nm was then detected by a spectrophotometer after reaction for 10 min. Higher absorbance represents stronger reducing power.

(V) *Test for ferrous ion chelating ability*

A method by Decker and Welch[14] was adopted. Five mL of the test solutions, including protein sample and EDTA solutions, were spiked with 0.1 mL of 2 mM $FeCl_2$ and 0.2 mL of 5 mM ferrozine solutions. After reaction for 10 min, the absorbance at 562 nm of resulting solutions was recorded. Higher ferrous ion chelating ability of the test sample gives lower absorbance. The percentage of ferrous ion chelating ability is expressed as [1-(test sample absorbance / blank sample absorbance)] × 100.

(VI) *Test for DPPH radical scavenging activity*

A method given by Shimada *et al.*[15] was used to test for DPPH radical scavenging activity. Five milliliter of the sample solution accompanied with 5 mL of α-tocopherol, and BHA methanolic solutions were mixed with 1 mL of freshly prepared 1 mM DPPH methanolic solution. The resulting solutions were then allowed to rest for 30 min prior to spectrophotometer detection at 517 nm. The lower the absorbance at 517 nm, the higher DPPH scavenging activity. The percentage of DPPH scavenging activity is expressed as [1-(test sample absorbance / blank sample absorbance)] × 100.

(VII) *Test for anti-peroxidation activity*

The anti-peroxidation activity was assayed using a linoleic acid emulsion system. Sample (0.5 mL) and 2 mL of 0.2 M sodium phosphate buffer (pH 7.0) were mixed well with 0.02 M linoleic acid emulsion (2.5 mL), and then incubated at 37˚C. The degree of oxidation was measured according to the ferric thiocyanate (FTC) method[16] for measuring peroxides by reading the absorbance at 500 nm

Journal of Food and Drug Analysis, Vol. 13, No. 1, 2005

after coloring with $FeCl_2$ and ammonium thiocyanate. A control was performed with linoleic acid without sample solution.

(VIII) *Statistical analysis*

In this study, each experiment was conducted in triplicate. The significance of the difference between the protein contents of samples in this study were analyzed by one-way ANOVA in SAS(Statistic Analysis System)[17].

RESULTS AND DISCUSSION

I. *Protein Contents of the Casein and the Whey Protein Samples from the Colostrums Collected within 7 Days Postpartum*

The protein contents of the casein and the whey protein samples from the colostrums collected within 7 days postpartum are presented in Table 1. Results showed that protein contents of the casein samples ranged between 120.79 ± 0.20~114.98 ± 0.22%. The increasing sequence of the crude protein content of the casein samples from colostrums was Casein 7 ≒ Casein 2 ≧ Casein 6 ≧ Casein 5 ≧ Casein 4 ≧ Casein 1 ≧ Casein 3. The crude protein contents of the casein samples in Table 1 were all higher than 100%, apparently due to the presence of some non-proteinous-nitrogen compounds, such as amides, urea, nucleic acids and nitrogenous pigments. Urea contains 46.5% nitrogen and contributes greatly to the level of non-proteinous-nitrogen compounds. The protein contents of the whey protein samples ranged between 97.36 ± 0.93~19.26 ± 0.71%. The increasing sequence of the protein content of whey protein samples was Whey protein 1 ≒ Whey protein 2 > Whey protein 3> Whey protein 4 > Whey protein 5 > Whey protein 6≒Whey protein 7. The protein content of the whey sample decreased as the collection day increased. It could be due to the increasing percentage of lactose in colostrums as the collection day increased.

II. *SDS-PAGE of the Caseins and the Whey Proteins of the Colostrums Collected within 7 Days Postpartum*

SDS-PAGE (15% acrylamide gel) patterns of the caseins obtained from the colostrums collected within 7 days postpartum are shown in Figure 1. Comparing to the SDS-PAGE analysis by Burn and Dalgleish[18] and Hekmat and McMahon,[19] we assumed that the bands of 75-100, 30-35, 25-30, 23-25, 20-23, 15-20, and 10-15 kDa were lactoferrin, α_{s2}-casein, α_{s1}-casein, β-casein, κ-casein, β-lactoglobulin, and α-lactalbumin, respectively. Figure 1 exhibits that the band of 75-100 kDa is rich in the casein samples obtained from the colostrums collected during the beginning of the first 7 days, especially on the first 2 days. This might be due to that caseins obtained from the colostrums collected during the beginning of the first 7 days were not completely separated from whey proteins during the acid precipitation and centrifugation. It was also found in Figure 1 that the casein samples were not only rich in α_{s1}-casein and β-casein, but also in β-lactoglobulin and α-lactalbumin, suggesting that casein samples were contaminated with whey proteins during the acid precipitation and centrifugation.

SDS-PAGE (15% acrylamide gel) patterns of whey proteins obtained from the colostrums collected within 7 days postpartum are shown in Figure 2. Comparing to the SDS-PAGE analysis by Burn and Dalgleish[18] and Hekmat

Table 1. Protein contents of bovine colostrum caseins and whey proteins collected within 7 days postpartum

Collection day	Casein (%)	Whey protein (%)
1st day	114.98 ± 0.22^{cd}	97.36 ± 0.93^{a}
2nd day	120.74 ± 0.48^{a}	96.05 ± 0.11^{a}
3rd day	114.06 ± 1.02^{d}	37.71 ± 0.11^{b}
4th day	115.75 ± 2.11^{bcd}	28.33 ± 0.10^{c}
5th day	118.21 ± 1.12^{abc}	22.43 ± 0.13^{d}
6th day	118.89 ± 1.42^{ab}	20.55 ± 0.38^{e}
7th day	120.79 ± 0.20^{a}	19.26 ± 0.71^{e}

*Note: identical letter in the same column means values are not significantly different at $p > 0.05$.

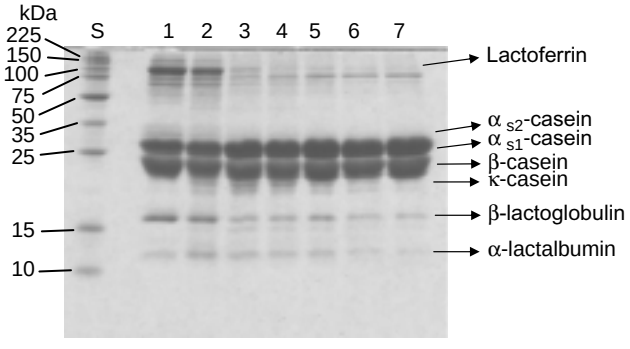

Figure 1. SDS-PAGE (15% acrylamide gel) patterns of the caseins from bovine colostrums collected within 7 days postpartum. Lane S: standard (225.0, 150.0, 100.0, 75.0, 50.0, 35.0, 25.0, 15.0 and 10.0 kDa), Number on the top of gel refers to the day of colostrums collected within 7 days postpartum.

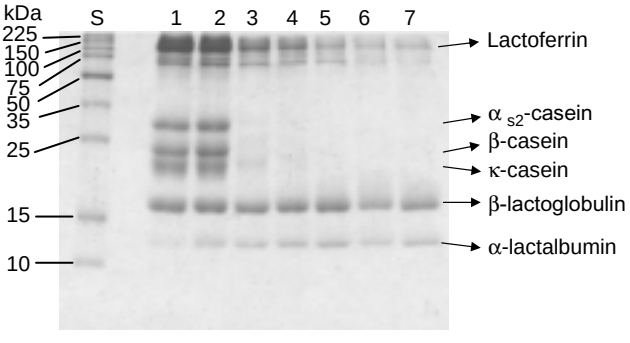

Figure 2. SDS-PAGE (15% acrylamide gel) patterns of the whey proteins from bovine colostrums collected within 7 days postpartum. Lane S: standard (225.0, 150.0, 100.0, 75.0, 50.0, 35.0, 25.0, 15.0 and 10.0 kDa), Number on the top of gel refers to the day of colostrums collected within 7 days postpartum.

and McMahon[19], we assumed that the bands of 75-150, 15-20, and 10-15 kDa were lactoferrin, β-lactoglobulin, and α-lactalbumin, respectively. Figure 2 also shows that the protein content of the band of 75-100 kDa decreased as the collection day increased, similar to the results in the protein content in Figure 1. In addition, it was apparent that whey proteins 1 and 2 contained proteins with molecular weights of 20-30 kDa, while those collected on the other days did not. This observation suggests that some caseins remain in the supernatant during the acid precipitation and centrifugation.

III. *Antioxidant Activity of the Caseins and the Whey Proteins of the Colostrums Collected within 7 Days Postpartum*

(I) *Reducing power*

The reducing powers of the colostrum caseins, whey proteins, α-tocopherol, and BHA are shown in Figure 3. At a concentration of 2 mg/mL, relatively high reducing powers of both α-tocopherol and BHA were observed, similar to the results of Hwang *et al.*[20] The reducing power of both caseins and whey proteins increased with the increasing sample concentration. As for the reducing power of caseins, absorbance at 700 nm ranged between 0.18 and 0.58 at a sample concentration of 20 mg/mL. The increasing sequence of reducing power of the caseins was Casein 2 > Casein 3 > Casein 4 ≧ Casein 1 ≒ Casein 5 ≧ Casein 6 > Casein 7.

For the whey proteins, absorbance at 700 nm ranged between 1.05 and 1.46 at a sample concentration of 20 mg/mL. The increasing sequence of reducing power of the whey proteins was Whey protein 6 > Whey protein 4 > Whey protein 2 ≧ Whey protein 3 ≒ Whey protein 5 ≧ Whey protein 7 ≒ Whey protein 1. Figure 3 presents that whey proteins had higher reducing power than caseins, and the reducing power of the former was 3 times higher than

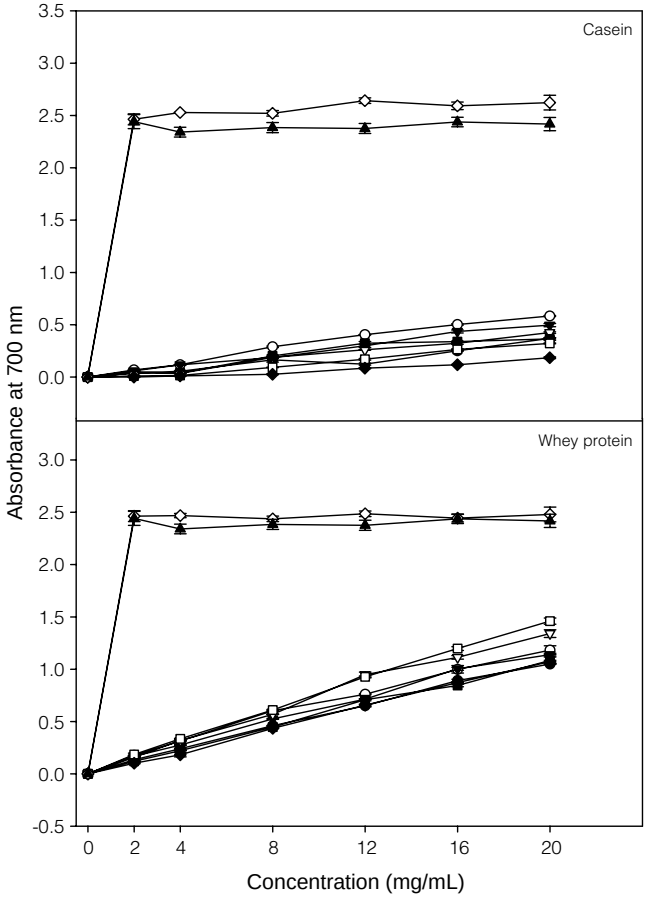

Figure 3. Reducing power of caseins and whey proteins from bovine colostrums collected within 7 days postpartum, in comparison with BHA and α-tocopherol. -●-, casein 1/whey protein 1, obtained on the first day; -○-, casein 2/whey protein 2, obtained on the second day; -▼-, casein 3/whey protein 3, obtained on the third day; -▽-, casein 4/whey protein 4, obtained on the fourth day; -■-, casein 5/whey protein 5, obtained on the fifth day; -□-, casein 6/whey protein 6, obtained on the sixth day; -◆-, casein 7/whey protein 7, obtained on the seventh day; -◇-, BHA; -▲-, α-tocopherol.

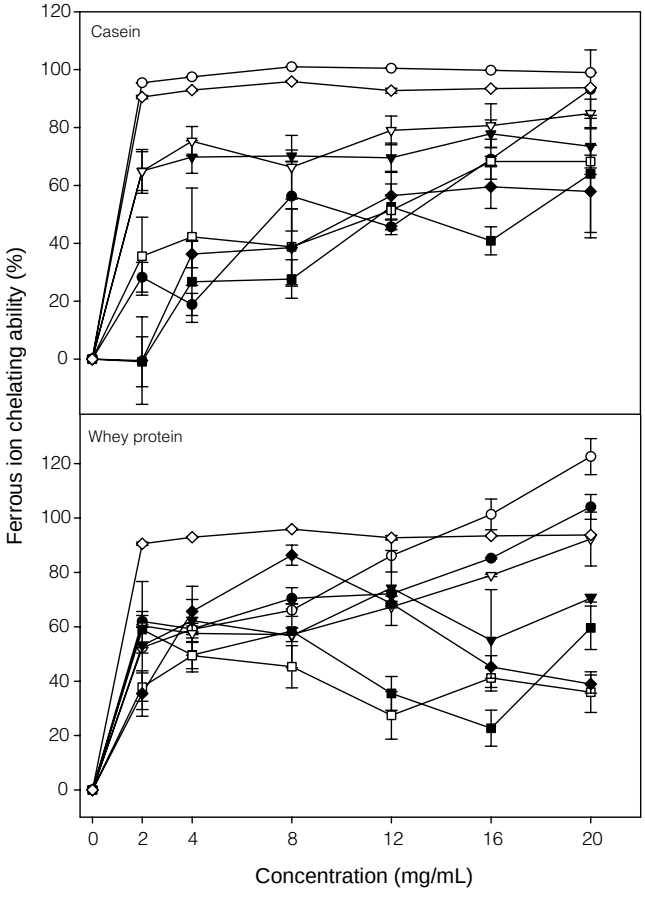

Figure 4. Ferrous ion chelating ability of caseins and whey proteins from bovine colostrums collected within 7 days postpartum, in comparison with EDTA. -●-, casein 1/whey protein 1, obtained on the first day; -○-, casein 2/whey protein 2, obtained on the second day; -▼-, casein 3/whey protein 3, obtained on the third day; -▽-, casein 4/whey protein 4, obtained on the fourth day; -■-, casein 5/whey protein 5, obtained on the fifth day; -□-, casein 6/whey protein 6, obtained on the sixth day; -◆-, casein 7/whey protein 7, obtained on the seventh day; -◇-, EDTA.

Journal of Food and Drug Analysis, Vol. 13, No. 1, 2005

those of the latter at a concentration of 20 mg/mL. Lindmark-Mansson and Akesson,[1] Satue-Gracia *et al*.,[21] and Al-Mashikhi and Nakai[22] reported that the antioxidants in cow milk included lactoferrin, vitamin C, E, β-carotene, and so on. Based on the results of electrophoretic analysis and reducing power test, we concluded that the higher reducing power of whey proteins was due to the abundance of lactoferrin in whey proteins.

(II) *Ferrous ion chelating ability*

Figure 4 shows the ferrous ion chelating ability of the colostrum caseins, whey proteins, and EDTA. Due to the mutual interference between the phosphate buffer in the sample solution and the ferrous ion in the testing solution, the ferrous ion chelating ability of the caseins and the whey proteins appeared irregularly, but still showing an increasing trend as the sample concentration increased. For caseins at a concentration of 20 mg/mL, the increasing sequence of ferrous ion chelating ability was Casein 2 > Casein 1 > Casein 4 > Casein 3 > Casein 6 > Casein 5 > Casein 7. For whey proteins, the increasing sequence of ferrous ion chelating ability at a concentration of 20 mg/mL was Whey protein 2 > Whey protein 1 > Whey protein 4 > Whey protein 3 > Whey protein 5 > Whey protein 7 ≧ Whey protein 6. Results in Figure 4 shows that whey proteins 1 and 2 had relatively high ferrous ion chelating ability. It could also be due to that whey proteins 1 and 2 contained higher amount of lactoferrin, which is capable of absorbing ferrous ion and catalyzing the synthesis and the transportation of ferrous proteins. Besides ferrous ion, lactoferrin also can combine with other cations, such as Zn^{2+}, Cr^{3+}, Cu^{2+}, Co^{3+} and Mn^{3+}[23,24]. Colbert and Decker[25] reported that whey protein could inhibit 90% of the lipid peroxidation catalyzed by ferrous ion, and was a natural and effective antioxidant.

(III) *DPPH radical scavenging activity*

The DPPH radical scavenging activities of the colostrum caseins, whey protein, α-tocopherol, and BHA are presented in Figure 5. DPPH radical scavenging activities of the caseins and the whey proteins increased as the sample concentration increased. For caseins at a concentration of 20 mg/mL, the DPPH radical scavenging activities ranged between 12.5 and 74.7%. The increasing sequence of DPPH radical scavenging activities of the caseins was Casein 2 > Casein 3 > Casein 4 ≒ Casein 6 > Casein 7 > Casein 5 > Casein 1. For whey proteins at a concentration of 20 mg/mL, the DPPH radical scavenging activities ranged between 42.3 and 86.8%. The increasing sequence of DPPH radical scavenging activities of whey proteins was Whey protein 3 > Whey protein 2 > Whey protein 4 > Whey protein 1 > Whey protein 6 ≒ Whey protein 7 > Whey protein 5.

Figure 5 also shows that the DPPH radical scavenging activities of whey proteins were higher than those of caseins. This finding was consistent with the results of Shinmoto *et al*.,[26] who also indicated that whey proteins had relatively high radical scavenging activities and lactoferrin was the key component for high scavenging activity. Therefore, we can conclude that lactoferrin in the whey proteins is also the key component for the high scavenging activity in colostrums.

(IV) *Anti-peroxidation effect*

The anti-peroxidation activity of the colostrum caseins, whey proteins, α-tocopherol, and BHA are presented in Figure 6. Results showed that caseins from the colostrums collected within 7 days postpartum exhibited high inhibitory activity on lipid peroxidation during the early period (the first 8 hr) of incubation at 37˚C, which was comparable to those of α-tocopherol, and BHA. On the other hand, whey proteins exhibited much lower inhibitory activity on lipid peroxidation than the caseins, α-tocopherol, and BHA.

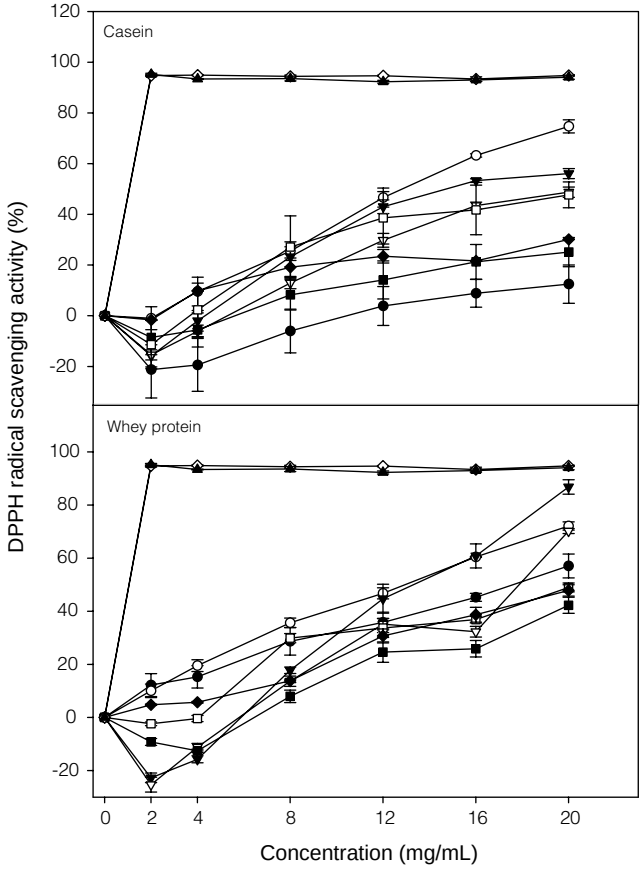

Figure 5. DPPH radical scavenging activity of caseins and whey proteins from bovine colostrums collected within 7 days postpartum, in comparison with BHA and α-tocopherol. -●-, casein 1/whey protein 1, obtained on the first day; -○-, casein 2/whey protein 2, obtained on the second day; -▼-, casein 3/whey protein 3, obtained on the third day; -▽-, casein 4/whey protein 4, obtained on the fourth day; -■-, casein 5/whey protein 5, obtained on the fifth day; -□-, casein 6/whey protein 6, obtained on the sixth day; -◆-, casein 7/whey protein 7, obtained on the seventh day; -◇-, BHA; -▲-, α-tocopherol.

Cervato *et al.*[27] have demonstrated the inhibitory activity of caseins, lactoferrin and lactoglobulin on lipid peroxidation using a linoleic acid emulsion system, and the results showed that caseins had the highest inhibitory activity. They also demonstrated the inhibitory activity of α-, β-, and κ-caseins on lipid peroxidation using the same system. Again, they showed that α-casein had the highest inhibitory activity, followed by β-casein and κ-casein. Cervato *et al.*[27] concluded that caseins had higher inhibitory activity on lipid peroxidation than whey proteins, and among the caseins tested, α-casein was the key component for the inhibitory activity. Similar results were also reported by Laakso[28]. The results of this study also confirmed that caseins were more effective on the inhibition of lipid peroxidation than whey proteins.

From the results of electrophoresis of protein and antioxidant activity analysis, it was revealed that the antioxidant activities of colostrum caseins and whey proteins not only depend on their protein quantity, but also on their protein composition.

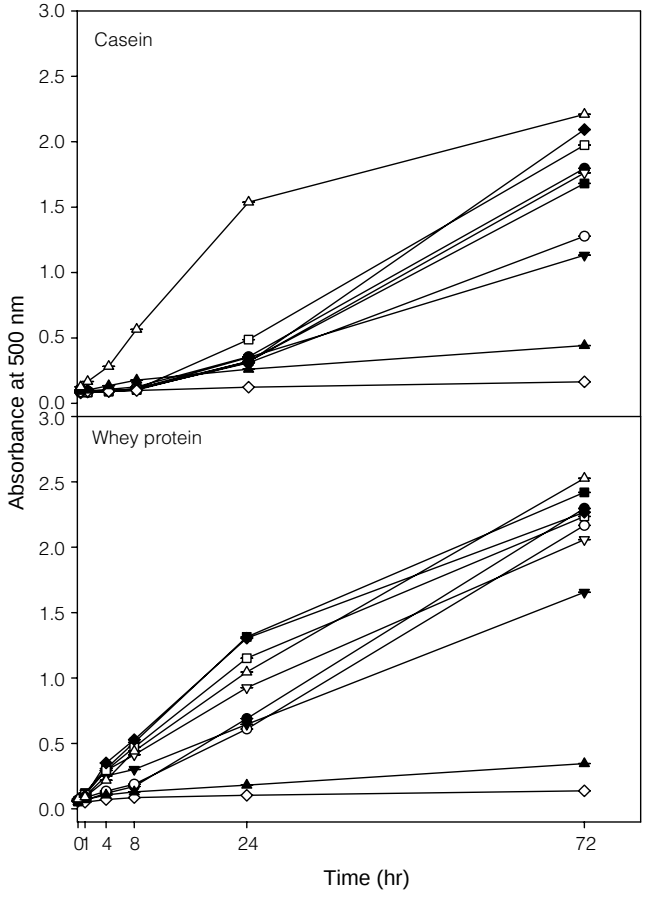

Figure 6. Antiperoxidative activity of caseins and whey proteins from bovine colostrums collected within 7 days postpartum, in comparison with BHA and α-tocopherol as measured by the ferric thiocyanate method. -●-, casein 1/whey protein 1, obtained on the first day; -○-, casein 2/whey protein 2, obtained on the second day; -▼-, casein 3/whey protein 3, obtained on the third day; -▽-, casein 4/whey protein 4, obtained on the fourth day; -■-, casein 5/whey protein 5, obtained on the fifth day; -□-, casein 6/whey protein 6, obtained on the sixth day; -◆-, casein 7/whey protein 7, obtained on the seventh day; -◇-, BHA; -▲-, α-tocopherol, -△-, control.

CONCLUSIONS

In this study, we found that colostrum whey proteins had higher reducing power, ferrous ion chelating power and DPPH radical scavenging activity than colostrum caseins. Among the whey proteins tested, those collected on the first 4 days postpartum had relatively higher antioxidant activity. Based on the results of electrophoresis in this study, we concluded that lactoferrin was the key component for the high antioxidant activity of whey proteins. Present study also showed that the inhibitory activity of caseins on lipid peroxidation was higher than that of whey proteins. All the results of this study reveal that bovine colostrums are potential materials for high-valued products, such as antioxidants, even though colostrums are unsuitable for food processing.

ACKNOWLEDGEMENTS

This work was supported by a grant (NSC 93-2214-E-212-003) from the National Science Council, Executive Yuan, Republic of China.

REFERENCES

1. Lindmark-Mansson, H. and Akesson, B. 2000. Antioxidative factors in milk. Br. J. Nutri. 84: S103-S110.
2. Gill, H. S. and Cross, M. L. 2000. Anticancer properties of bovine milk. Br. J. Nutri. 84: S161-S166.
3. Cross, M. L. and Gill, H. S. 2000. Immunomodulatory properties of milk. Br. J. Nutri. 84: S81-S89.
4. Maruyama, S., Nakagomi, K., Tomozuka, N. and Suzuki, H. 1985. Isolation and characterization of angiotension I-converting enzyme inhibitor derived from an enzymatic hydrolysate of casein. II. Isolation and bradykinin-potentiating activity on the uterus and the ileum of rats. Agric. Biol. Chem. 49: 1405-1409.
5. Mills, E. N. C., Alcocer, M. J. C. and Morgan, M. R. A. 1992. Biochemical interactions of food-derived peptides. Trends Food Sci. Technol. 3: 64-68.
6. Donnelly, J. L., Decker, E. A. and McClements, D. J. 1998. Iron-catalyzed oxidation of menhaden oil as affected by emulsifiers. J. Food Sci. 63: 997-1000.
7. Sasaki, A., McClements, D. J. and Decker, E. A. 2000. Antioxidant activity of whey in a salmon oil emulsion. J. Food Sci. 65: 1325-1329.
8. Tong, L. M., Sasaki, S., McClements, D. J. and Decker, E. A. 2000. Mechanisms of the antioxidant activity of a high molecular weight fraction of whey. J. Agric. Food Chem. 48: 1473-1478.

9. Migliore-Samour, D., Floch, F. and Jolles, P. 1989. Biologically active casein peptide implicated in immunomodulation. J. Dairy Res. 56: 357-362.
10. Meisel, H. 1998. Overview on milk protein-derived peptides. Int. Dairy J. 8: 363-373.
11. Rival, S. G., Boeriu, C. G. and Wicher, H. J. 2001. Casein and casein hydrolysates. II. antioxidative properties and relevance to lipoxygenase inhibition. J. Agric. Food Chem. 49: 295-302.
12. AOAC. 1995. Official Methods of Analysis of the AOAC, 16th ed. Association of Official Analytical Chemists. Washington, DC, U. S. A.
13. Oyaizu, M. 1986. Studies on products of browning reaction: antioxidative activities of products of browning reaction prepared from glucosamine. Jan. J. Nutri. 44: 307-315.
14. Decker, E. A. and Welch, B. 1990. Role of ferritin as a lipid oxidation catalyst in muscle food. J. Agric. Food Chem. 38: 674-677.
15. Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. 1992. Antioxidative properties of xanthane on the autoxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food Chem. 40: 945-948.
16. Mitsuda, H., Yasumoto, K. and Iwami, K. 1966. Antioxidative action of indole compounds during the autoxidation of linoleic acid. Eiyo to Shokuryo 19: 210-214.
17. SAS. 1985. SAS User's guide: statistics version. 5th ed. SAS Inst. Cary, NC, U. S. A.
18. Brun, J. M. and Dalgleish, D. G. 1999. Some effects of heat on the competitive adsorption of caseins and whey proteins in oil-in-water emulsions. Int. Dairy J. 9: 323-327.
19. Hekmat, S. and McMahon, D. J. 1998. Distribution of iron between caseins and whey proteins in acidified milk. Lebensm.-Wiss. u.-Technol. 31: 632-638.
20. Hwang, J. Y., Shue, Y. S. and Chang, H. M. 2001. Antioxidative activity of roasted and defatted peanut kernels. Food Res. Int. 34: 639-647.
21. Satue-Gracia, M. T., Frankel, E. N., Rangavajhyala, N. and German, J. B. 2000. Lactoferrin in infant formulas: effect on oxidation. J. Agric. Food Chem. 48: 4984-4990.
22. Al-Mashikhi, S. A. and Nakai, S. 1987. Isolation of bovine immunoglobulins and lactoferrin from whey protein by gel filtration techniques. J. Dairy Sci. 70: 2486-2492.
23. Arnold, R. R., Brewer, M. and Gautier, J. J. 1980. Bactericidal activity of human lactoferrin: sensitivity of a variety of microorganisms. Infect. Immun. 28: 893-898.
24. Ainscough, E. W., Brodie, A. M. and Plowman, J. E. 1979. The chromium, manganese, cobalt and copper-complexes of human lactoferrin. Inorg. Chim. Acta. 33: 149-153.
25. Colbert, L. B. and Decker, E. A. 1991. Antioxidant activity of ultrafiltration permeate from acid whey. J. Food Sci. 56: 1248-1250.
26. Shinmoto, H., Dosako, S. and Nakajima, I. 1992. Antioxidant activity of bovine lactoferrineon iron/ ascorbate induced lipid peroxidation. Biosci. Biotech. Biochem. 56: 2079-2080.
27. Cervato, G., Cazzola, R. and Cestaro, B. 1999. Studies on the antioxidant activity of milk caseins. Int. J. Food Sci. Nutr. 50: 291-296.
28. Laakso, S. 1984. Inhibition of lipid peroxidation by casein: evidence of molecular encapsulation of 1, 4-pentadiene fatty acids. Biochim. Biophys. Acta. 792: 11-15.