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Inhibitory Effects of Bovine Colostrum Protein Hydrolysates on Human Leukemic U937 Cell Growth

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ABSTRACT

Skimmed milk, caseins and whey proteins isolated from colostrums collected on the first to fifth days postpartum were hydrolyzed by different enzymes (alcalase, flavourzyme, and porcine small-intestinal enzymes). To investigate how different protein hydrolyastes affect cell death, leukemic U937 cells were treated with different protein hydrolysates at 100 - 1000 μg/mL for 24 to 120 h at 37°C in 5% CO_2 incubator. The results showed that protein hydrolysates leading to U937 cell death exhibited dose-and-time-dependent characteristics. This study also found that the proteins hydrolyzed by porcine small-intestinal enzymes have more significant effects on cell death than proteins hydrolyzed by other enzymes. In addition, the colostrum protein hydrolysates from skimmed milk were better than those from caseins and whey in inhibiting U937 cell growth.

Key words: colostrums, porcine small-intestinal enzymes, skimmed milk, protein hydrolysates, U937 cell

INTRODUCTION

Colostrums provide all the necessary nutrients, growth factors and immunological components a healthy term infant needs[(1)]. Bovine colostrums are the first milk produced postpartum, and are typically defined as the first six postpartum milkings collected during the period of transition from colostrums to milk[(2)]. Several researchers have compared the compositions of colostrums with those of mature milk and concluded that colostrums have higher protein, lower fat, and a lactose solution rich in immunoglobulins and other important immune factors and mediators[(1,3-5)]. The major differences between bovine colostrums and mature milk are that colostrums have higher levels of immunoglobulins, vitamins A and D, iron, calcium, as well as other vitamins and minerals[(6)]. Migliore-Samour *et al.*[(7)] and Meisel[(8)] 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, can help reduce the infection rate of *Klebsiella pneumoniae* in mice. Lindmark-Mansson and Akesson[(9)], Satue-Gracia *et al.*[(10)] and Al-Mashikhi and Nakai[(11)] reported that the lactoferrin derived from colostrums had a high antioxidant activity, could scavenge free radicals generated in the human body, and also had a direct and positive effect on inhibiting atherosclerosis and ageing.

Food protein hydrolysates have been widely accepted and applied in the field of cosmetics and healthcare products. Protein hydrolysate is a mixture of proteoses, peptones, peptides, and free amino acids. It is possible, depending largely on enzyme specificity and the degree of hydrolysis achieved, to generate hydrolysate products with either enhanced or reduced functionality. Significant interest exists in the hydrolytic release of bioactive peptide sequences encrypted within the primary structure of food proteins[(12)]. Several studies have confirmed that protein hydrolysates should be rich in low-molecular-weight peptides, such as di- or tri-peptides, with as little free amino acids as possible, to qualify as possessing high nutritional and therapeutic values[(13,14)].

Cell death is an essential process, being important not only for normal development but also for the adult life of many living organisms[(15,16)]. A vast amount of literature has reported on bovine colostrums and protein hydrolysates for nutrition and functional food. The literature, however, is limited in regard to bovine colostrums and protein hydrolysates for treating leukemia. Gill and Cross[(17)] reported on the relationship between the assimilated quantity of colostrums and anti-tumor results. Ma *et al.*[(18)] found, through an indirect model, that bovine colostrums were capable of enhancing the immunity for inducing human leukemic U937 cell death.

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The purpose of this study is to investigate the inhibitory effects of different bovine colostrum protein hydrolysates on human leukemic U937 cell growth through a direct model.

MATERIALS AND METHODS

I. *Reagent*

Roswell Park Memorial Institute (RPMI) 1640 medium and fetal bovine serum (FBS) were purchased from Hyclone (Logan, Utah, USA). Sodium bicarbonate, HEPES, L-glutamine, sodium pyruvate, alcalase (exo-peptidase; 2.4 Anson unit (AU)/g), flavourzyme (exo/endo-peptidase; 0.5 unit/mg) and N-benzoyl-L-arginine ethyl ester (BAEE) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Trypan blue was purchased from e-Bioscience (CA, USA).

II. *Preparation of Skimmmed Colostrums, Caseins and Whey Proteins*

The bovine colostrums used for this experiment were acquired from a cow within five days after parturition at the Chu-En Ranch, Hsiushui, Changhua, Taiwan. The colostrums were collected at about 8 a.m. and promptly centrifuged at 10,000 ×g for 30 min at 4°C to remove the fat. After centrifugation the colostrums were adjusted to pH 4.6 with 1.0 N HCl and kept in a 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 centrifuged again at 10,000 ×g for 30 min at 4°C. The final supernatant after two centrifugations was treated as whey proteins. Both the caseins and whey proteins were freeze-dried and stored in a desiccator at -80°C until used.

III. *Preparation of Porcine Small-Intestinal Enzymes*

The porcine small-intestines used for this experiment were obtained from the Chin-Ku packinghouse in Beitoe, Changhua, Taiwan. The extraction method was modified from Simpson and Haard[19]. Three hundred grams of the porcine small-intestines were homogenized with 100 mL of 0.5 M Tris-HCl buffer (pH 7.5, containing 0.5 M of NaCl and 0.2 M of $CaCl_2 \cdot 2H_2O$) for 1 min. The homogenate was incubated for 4 h at 4°C, centrifuged and defatted twice (12,000 ×g, 30 min, 4°C). The supernatant was collected and treated as a crude enzyme preparation. A portion of the enzyme sample was taken for an activity test; the remainder was freeze-dried and powdered for protein hydrolysis and stability tests, as well as electrophoresis analysis.

IV. *Assay for Porcine Small-Intestinal Enzyme Activity with BAEE*

The activity test for the crude enzyme preparations was implemented with the method developed by Hummel[20]. The rate of hydrolysis of *N*-benzoyl-arginine ethyl ester (BAEE) was determined by the change in absorbance at 253 nm, under a pH of 8.0, a temperature of 25°C and a 1.0 mM concentration of substrate. The BAEE unit is defined as the amount of enzyme that will cause a change in absorbance of 0.001/min under testing conditions. The BAEE unit converting method is calculated by the following formula:

$$(A_1 - A_0) / \text{min} \times (1 \text{ BAEE unit} / 0.001 \times \text{min}^{-1})$$

A_1: absorbance of sample

A_0: absorbance of control

V. *Enzymatic Hydrolysis*

The hydrolysis methods developed by Morato *et al*.[21], Pintado *et al*.[22] and Spellman *et al*.[23] were summarized and adopted. Two grams of protein samples were dissolved in 100 mL of 0.01 N sodium phosphate buffer (pH 7.0) at room temperature. The mixture, with a pH of around 4.6 to 7.0, was placed in a water bath maintained at 90°C for 30 min to enable protein denaturation. The reaction pH levels were adjusted to 8.0 for hydrolysis of porcine small-intestinal enzyme perparations (enzyme/substrate (E/S) = 5%), pH 8.5 for alcalase hydrolysis (E/S = 3%) and pH 7.5 for flavourzyme hydrolysis (E/S = 1.5%) with 2 N NaOH or HCl. These three enzymes were added to the mixture at temperatures of 37, 50 and 40°C, respectively. The reactions were terminated by immersing the beaker into a water bath at 85°C for 5 min[24]. The degree of hydrolysis (DH) of colostrum protein hydrolysate was calculated as (amino nitrogen/total nitrogen) × 100%, where the total nitrogen and amino nitrogen content were determined by the semimicro-Kjeldahl method[25] and the formol titration method[26], respectively.

VI. *Preparation of Sample Solution*

Bovine colostrums, caseins, whey proteins and their hydrolysate samples were dissolved in a phosphate buffer solution (8 g NaCl, 1.15 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.2 g KCl dissolved 1 L dd. H_2O, pH 7.0). The sample concentrations were prepared at 100, 200, 400, 500, 800 and 1000 μg/mL. Concurrently, penicillin and streptomycin (20 μL/mL) were added to each sample, all of which were filtered by a 0.22-μm filter for the cell experiment. The abbreviations, PIS, ALS, FLS, PIC, ALC, FLC, PIW, ALW and FLW, denote the hydrolysates of skimmed colostrums (S), caseins (C), and whey (W) obtained by porcine small-intestinal enzymes (PI), alcalase (AL), and flavourzyme (FL), respectively.

VII. *Protein Analysis by SDS-PAGE*

The sample (0.03 g) was dissolved in 2 mL of 0.5 M Tris-HCl buffer (pH 7.5). Ten μL of the protein-sample liquid was added with 10 μL lysis buffer containing 0.5 M Tris (pH 6.8), 2% bromophenol blue, 10% SDS, 75% glycerol, 5% β-mercaptoethanol and distilled water. The sample was then loaded on a 15.0% acrylamide gel (gel concentration: stacking gel, 4.5%; resolving gel, 15.0%) for electrophoresis.

An electrophoresis apparatus Model AE-6450 (ATTO, Japan) and a power supplier PS500XT with a 2.5 AMP input (Hoefer scientific instruments, USA) were used. The electrophoresis was started at 70 V and subsequently turned up to 140 V after a tracer dye was added to the resolving gel. This voltage was maintained until the dye reached the bottom of the gel. The gels were then immersed in coomassie blue for 2 h and the color was stripped by a solution containing 7% methanol and 7% acetic acid.

VIII. *Cell Cultures*

U937 cell lines were obtained from the Bioresource Collection and Research Center (BCRC). The cells were cultured in RPMI 1640, supplemented with 10% heat inactivated fetal bovine serum, penicillin (100 units/mL), streptomycin (100 μg/mL), HEPES (100 μg/mL), sodium pyruvate and sodium bicarbonate, and subsequently incubated at 37°C in a humidified atmosphere containing 5% CO_2.

IX. *Bovine Colostrums Affecting Cell Death as Determined by Trypan Blue*

U937 cells at 1×10^5 cells/mL treated with different protein hydrolysates at 100, 200, 500, 800 and 1000 μg/mL were placed into a 12-well microtiter plate for 24, 72 and 120 h, respectively, at 37°C in 5% CO_2 (cell/samples ratio = 4/1, v/v). The control was treated with both the medium and a PBS. The number of dead cells was counted in a trypan blue dye exclusion test[27].

Growth inhibition (%) = (1-(Number of live cells / total cells)) × 100%

X. *The Growth of Human Mononuclear Cells (MNC) as Determined by MTT Assay*

Human peripherical blood was obtained from healthy adult volunteers between the ages of 22 and 25. MNCs were recovered by adding the blood to Ficoll-Hypaque solution (1.077 g/mL) (from Sigma Chemical Co., USA) and centrifuging at 400 ×g for 30 min. A suspension of 1.0×10^7 cells/mL in an RPMI 1640 medium was prepared.

Human MNCs were incubated with colostrum proteins and their hydrolysates, mediums, PBS (control) and PHA (phytohemagglutinin, positive control, 5 μg/mL) at 37°C in 5% CO_2 for 1 and 3 days (cell/samples ratio = 4/1, v/v). Then, 25 μL of MTT reagent was added and the cells were incubated for another 4 h at 37°C in 5% CO_2. One hundred μL of MTT lysis buffer (25 mL N,N-dimethyl formamide (DMF) and sodium dodecyl sulfate 10 g/ mL were mixed) was then added and the cells incubated for 16 - 18 h at 37°C in 5% CO_2. The absorbance was measured at 570nm. The growth index was calculated by applying the following equation:

Growth index = (MTT value of sample stimulated cells / MTT value of medium control cells)

XI. *Statistical Analysis*

In this study, each experiment was conducted in triplicate and the results analyzed by one-way analysis of variance in SAS (Statistic Analytic System) (SAS, 2001).

RESULTS AND DISCUSSION

I. *Activity and Stability of Porcine Small-Intestinal Enzymes*

In this study the activity of a crude enzyme preparation from porcine small-intestine was first assayed. Trypsin, the major enzyme in the intestine, was chosen as the enzymatic activity indicator. Since the enzyme preparation was not purified beforehand, various proteins were present in the mixture. The BAEE unit converting method was employed to calculate the activity of the porcine small-intestinal enzymes, measured at 516.60 BAEE units/mg solid. After the freeze-dried crude enzyme preparations were stored at -20°C for 14 days the activity decreased by 9.88%. On the 21st day, the activity had decreased by 17.63% (data not shown). Hence, to ensure the enzymatic activity remained at high level during protein hydrolysis, the porcine small-intestinal enzyme preparation used in the experiment was stored for not longer than 14 days after preparation.

II. *Electrophoresis Analysis*

Figure 1 depicts the electrophoresis analysis of the protein samples and crude enzyme preparation extracted from porcine small-intestines. Figure 1 (A) indicates that the enzyme preparation contains several proteins because this preparation is crude. After comparing the results with the trypsin electrophoresis results obtained by Kurtovic *et al.*[28], Francis *et al.*[29], and Keryn *et al.*[30], it is evident that the band of 25 - 35 kDa in the electropherogram is trypsin. Moreover, after comparing the electrophoresis reports by Burn and Dalgleish[31] and Hekmat and McMahon[32] regarding bovine colostrums proteins, it is also evident that the bands of 75 - 100, 30 - 35, 25 - 30, 23 - 25, 20 - 23, 15 - 20, and 10 - 15 kDa in Figure 1 (B), (C), and (D) are lactoferrin, αs_2-casein, αs_1-casein, β-casein, κ-casein, β-lactoglobuin, and α-lactalbumin, respectively. An examination of Figure 1 (E) reveals that lanes 2, 3, and 4 have more low-molecular weight bands than lane 1 and exhibit different distributions of these bands. These results indicate that different enzymes exhibit different capabilities in terms of hydrolysis.

III. *Hydrolysis of Bovine Colostrum Proteins*

Most protein hydrolysates are obtained by using commercial enzymes from microorganisms. Commercial enzymes are often in pure form and have high activity. Although only small amounts are usually needed for effective results, these enzymes are, nevertheless, expensive.

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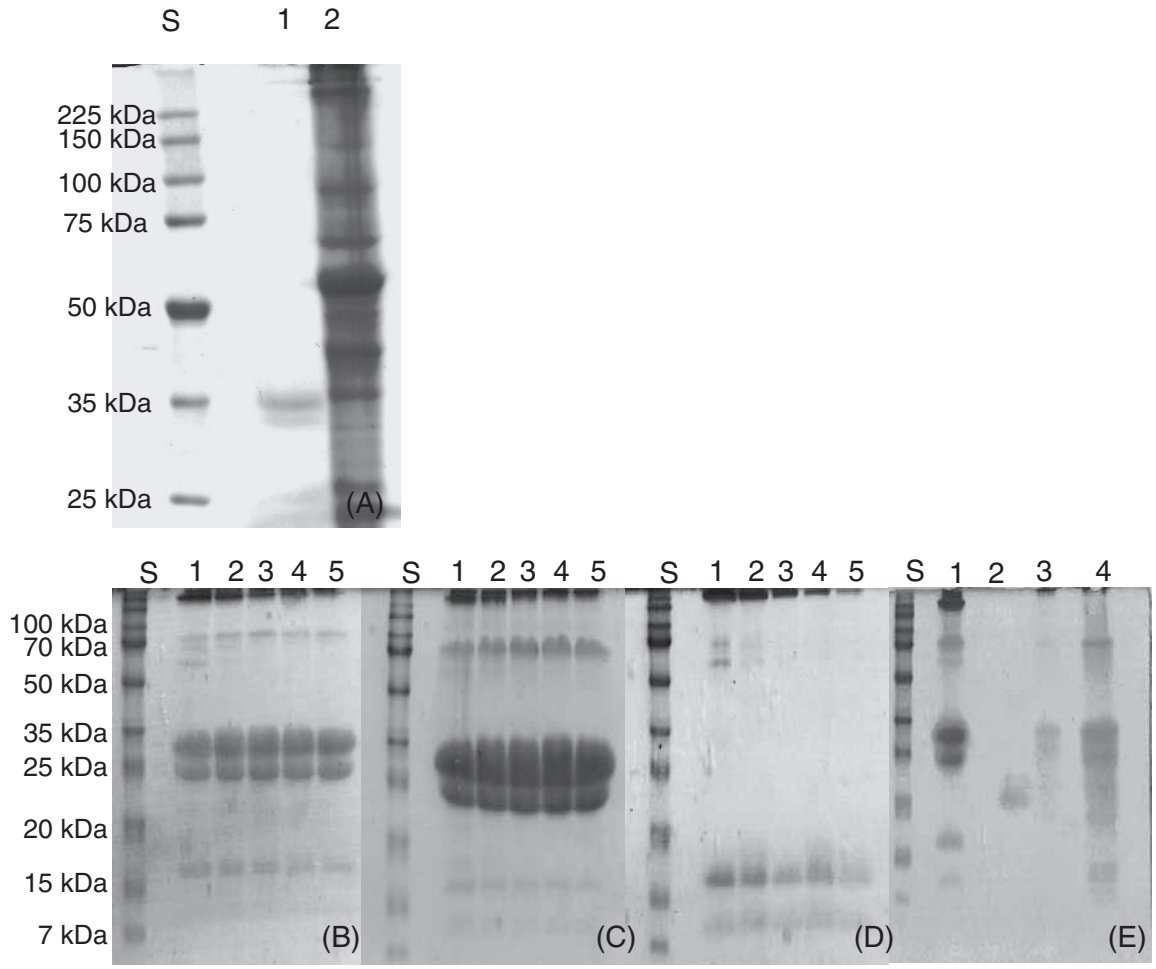

Figure 1. SDS-PAGE Patterns. (A) crude enzymes extracted from porcine small intestine, Lane S: standard, Lane 1: trypsin from SIGMA Chemical Co., USA, Lane 2: crude enzyme from porcine small-intestine; (B) skimmed colostrums, (C) caseins, and (D) whey, Lane S: standard, Lane 1-5: day 1-5 colostrum proteins, respectively; (E) hydrolysates of skimmed colostrums, Lane 1: skimmed colostrums, Lane 2: hydrolysates by alcalase, Lane 3: hydrolysates of flavourzyme, and Lane 4: hydrolysates of porcine small-intestinal enzymes.

Porcine small-intestinal enzyme preparation is inexpensive and can be produced in large amounts. Figure 2 plots the changes in the degree of hydrolysis (DH) of skimmed colostrums hydrolyzed by different enzymes, indicating that the DHs of the hydrolysates increased with hydrolysis time, results similar to those obtained by Chiang and Chang[33]. The DHs measured on day 2 sample were the lowest among the five days of samples, thereby revealing that the structure and the compositions of the proteins of day 2 colostrums significantly differ from those observed on the other days.

IV. *Effect of Bovine Colostrums on U937 Cell Growth*

There are two ways to evaluate the effects of natural substances on the growth of leukemic cells, either by direct inhibition on the proliferation of the cells or by stimulating the secretion of differentiation-inducing factors from immune cells to act indirectly on the leukemic cells. In this study the effects of different protein hydrolysates on the growth of U937 cells were studied via a direct model.

The results in Table 1 show that the days on which the colostrums were collected are significantly related to cell growth. As the number of collection days increased, the inhibition of U937 cell growth decreased, and the inhibition rates of the colostrum hydrolysates were higher than those of the colostrums. When the cells were exposed for 120 hrs to the hydrolysates obtained from porcine small-intestinal enzymes (PIS) of colostrums collected on the 1st - 5th days, the inhibition rates of cell growth were 56.20, 55.27, 52.71, 43.93, and 35.65, respecively. The low inhibition rate of the day 5 colostrum hydrolysates accounts for bovine colostrums collected on the fifth day postpartum being almost the same as for mature milk. Kelly[6] observed that the difference between bovine colostrums and mature milk is that there are higher levels of immunoglobulins, proteins, vitamins A and D, iron, calcium, as well as other vitamins and minerals, in colostrums than in mature milk. The change in the inhibition rates of U937 cell growth can be attributed to the respective protein compositions. Meisel[8] reported that milk proteins were precursors of many

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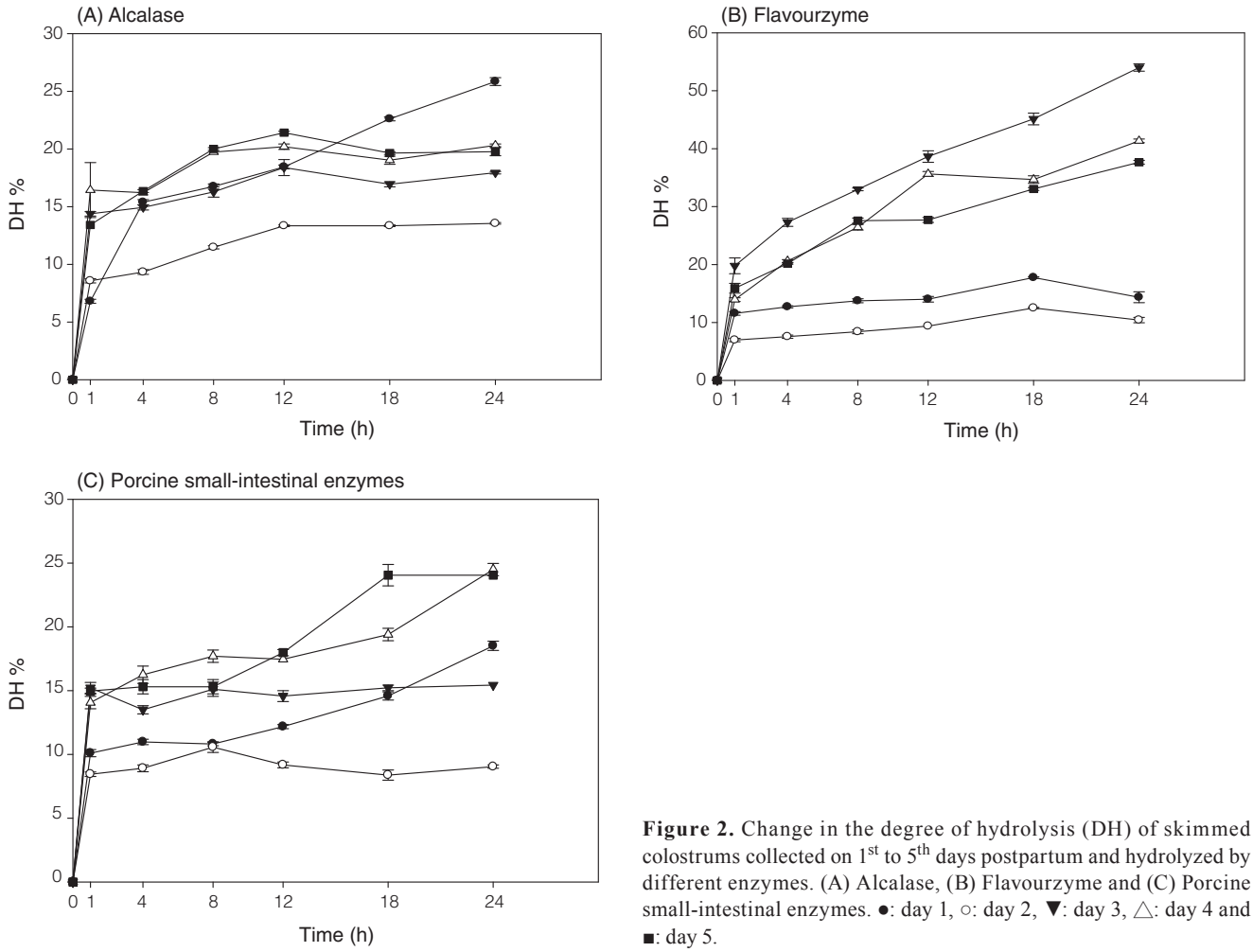

Figure 2. Change in the degree of hydrolysis (DH) of skimmed colostrums collected on 1st to 5th days postpartum and hydrolyzed by different enzymes. (A) Alcalase, (B) Flavourzyme and (C) Porcine small-intestinal enzymes. ●: day 1, ○: day 2, ▼: day 3, △: day 4 and ■: day 5.

Table 1. Growth inhibition of U937 cells treated with skimmed colostrum hydrolysates (1000 μg/mL) collected on different days postpartum

	Growth inhibition (%)				
Sample	Day 1	Day 2	Day 3	Day 4	Day 5
Skimmed colostrums	25.88 ± 1.25[b]	27.23 ± 0.46[b]	20.40 ± 0.96[b]	17.24 ± 1.18[b]	18.92 ± 0.90[b]
PIS	56.20 ± 0.51[a]	55.27 ± 1.75[a]	52.71 ± 0.77[a]	43.93 ± 0.89[a]	35.65 ± 0.61[a]
ALS	52.54 ± 1.20[a]	53.15 ± 1.52[a]	51.56 ± 0.52[a]	42.01 ± 0.90[a]	34.10 ± 0.41[a]
FLS	55.37 ± 1.23[a]	55.92 ± 1.04[a]	51.6 ± 0.58[a]	42.71 ± 0.38[a]	37.87 ± 1.50[a]
PBS	3.96 ± 0.45				
Medium	4.30 ± 0.28				

Means with identical letters in same column are not significantly different ($p > 0.05$).

different biologically active peptides. The peptides are inactive within the sequence of the precursor proteins but active while being released by enzymatic proteolysis. Although the day 2 colostrums have the lowest degree of hydrolysis, the highest inhibition rates of their hydrolysates on the U937 cell growth reveal that the function of the peptides released by enzymatic hydrolysis is not directly related to the degree of hydrolysis, but significantly dependent on the hydrolysis site and peptide sequence.

After obtaining the aforementioned results, we then used the hydrolysates of the day 2 colostrums as samples to investigate their effects on the growth of U937 cells.

To determine the effects of different colostrum protein hydrolysates on the inhibition of U937 cell growth, the U937 cells were all exposed to PIS, ALS and FLS at a concentration of 1000 μg/mL for 24, 72 and 120 h. The graphs

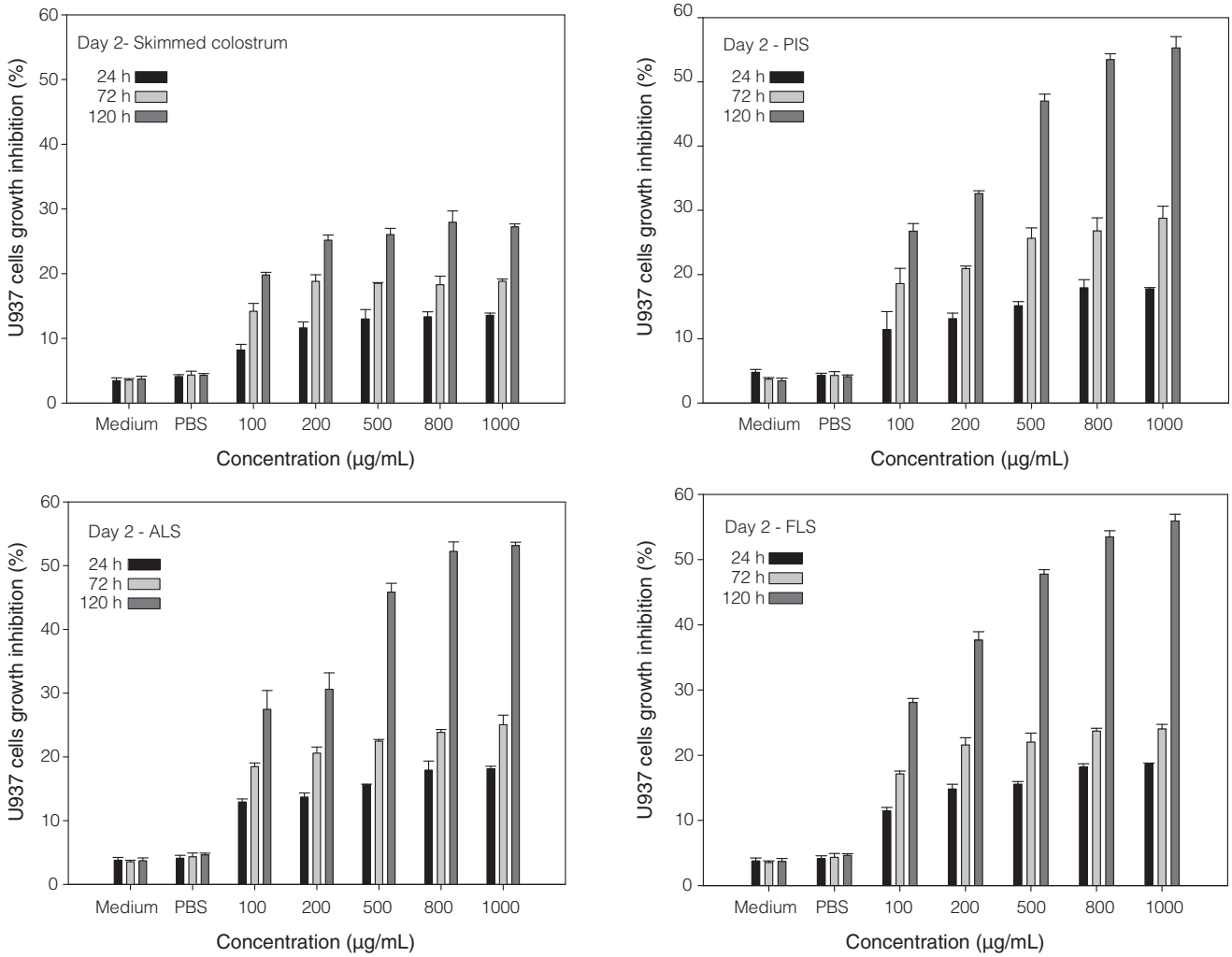

Figure 3. Growth inhibition of U937 cells treated with hydrolysates of day 2 skimmed colostrums at concentration of 100, 200, 500, 800 and 1000 μg/mL.

in Figure 3 indicate that the growth of cells was slightly inhibited under treatments for 24 and 72 h. The inhibition rates were 17.76 - 28.76% for PIS, 18.13 - 25.05% for ALS and 18.58 - 24.02% for FLS. However, when the U937 cells were treated for 120 h, all the hydrolyslate samples inhibited U937 cell growth by more than 50% (the inhibition rate being 55.27% for PIS, 53.15% for ALS and 55.92% for FLS). Skimmed colostrums inhibited the growth of U937 cells by only 13.57 - 27.24% during the testing period. When the cells were exposed to various concentrations of the samples for various periods, the cell growth was significantly inhibited in a dose-and-time-dependent manner. Similarly, the growth inhibition of U937 cells treated with berberine at 75 μg/mL for 24 h was 80%[34]; U937 cells treated with Chlorpyrifos at 285 μM for 2, 4 and 6 h were 57, 78 and 80%, respectively[35]; these inhibition rates were higher than those in our experiments. The growth inhibition of human lung carcinoma A549 epithelial cells treated with *Tremella mesenterica* extract at 1000 μg/mL for 24, 48 and 120 h were 25 - 31.7%[36], which were lower than those in our experiments.

Table 2 lists the growth inhibition data for U937 cells treated with different protein hydrolysates (1000 μg/mL) of colostrums collected on the second day postpartum. When the cells were exposed to these samples at a concentration of 1000 μg/mL for 120 h, it was found that the growth was significantly inhibited. The cell growth inhibition rates were 55.27% for PIS, 53.17% for ALS, 55.92% for FLS; 45.13% for PIC, 41.56% for ALC, 43.12% for FLC; and 40.08% for PIW, 37.12% for ALW, 40.11% for FLW. An examination of the results presented in Table 2 reveals that the inhibition rate of the cells treated with skimmed colostrum hydrolysates is higher than those treated with the caseins and the whey hydrolysates. The manner in which the peptide sequences of the casein hydrolysates obtained by trypsin affect the inhibition of cell growth has been studied by several researchers[37-41]. In this study, the samples hydrolyzed by porcine small-intestinal enzymes had a more profound effect on cell growth than the samples hydrolyzed by other enzymes.

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Table 2. Growth inhibition of U937 cells treated with different protein hydrolysates (1000 μg/mL) of colostrums collected on the second day postpartum

Growth inhibition (%)					
Sample		Sample		Sample	
PIS	55.27 ± 1.75^{aA}	ALS	53.17 ± 0.37^{aA}	FLS	55.92 ± 1.04^{aA}
PIC	45.13 ± 0.37^{bA}	ALC	41.56 ± 0.71^{bC}	FLC	43.12 ± 0.51^{bB}
PIW	40.08 ± 0.41^{cA}	ALW	37.12 ± 0.33^{cB}	FLW	40.11 ± 0.62^{cA}
PBS		3.96 ± 0.45			
Medium		4.30 ± 0.28			

A-C Means with identical letters in the same row are not significantly different ($p > 0.05$); a-c Means with identical letters in the same column are not significantly different ($p > 0.05$).

V. *Bovine Colostrum Proteins and Their Hydrolysates Affecting the Growth of MNC*

Table 3 illustrates the effect of Bovine colostrum proteins and their hydrolysates on MNC growth. The MNC treated with bovine colostrum proteins and their hydrolysates had more effect on the growth of MNC than did the PBS-treated control group. The greatest growth index, up to 1.43, was achieved by a treatment of PIS at 300 μg/mL for 3 days. MNC treated with the positive control (PHA; 5 μg/mL) growth index was 1.54. From the results, one may deduce that the bovine colostrum protein and its hydrolysates are able to stimulate MNC growth.

Table 3. The growth index of human mononuclear cells incubated with colostrum proteins and their hydrolysates for 24 and 72 h

Sample (300 μg/ mL)	Human mononuclear cells growth index incubation time 24 h	72 h
Skimmed colostrums	1.18 ± 0.09^{abA}	1.20 ± 0.09^{aA}
Casein	1.13 ± 0.08^{abA}	1.17 ± 0.06^{aA}
Whey	$1.04 \pm 0.05b^{cA}$	1.08 ± 0.08^{aA}
PIS	1.27 ± 0.04^{aB}	1.43 ± 0.20^{aA}
PIC	1.26 ± 0.04^{aB}	1.37 ± 0.05^{aA}
PIW	1.11 ± 0.03^{abB}	1.27 ± 0.01^{aA}
ALS	1.16 ± 0.04^{abB}	1.36 ± 0.06^{aA}
ALC	1.17 ± 0.04^{abB}	1.26 ± 0.07^{aA}
ALW	1.13 ± 0.03^{abB}	1.26 ± 0.02^{aA}
FLS	1.29 ± 0.08^{aA}	1.33 ± 0.15^{aA}
FLC	1.19 ± 0.07^{abA}	1.21 ± 0.03^{aA}
FLW	1.12 ± 0.04^{abB}	1.21 ± 0.02^{aA}
PBS	1.07 ± 0.35	1.09 ± 0.43
PHA(5 μg/mL)	1.43 ± 0.05	1.54 ± 0.59

A-B: Data with identical letter in the same row are not significantly different ($p > 0.05$). a-c: Data with identical letter in the same column are not significantly different ($p > 0.05$).

CONCLUSIONS

In this study, it was observed that the proteins hydrolyzed by porcine small-intestinal enzymes had the greatest ability to inhibit cell growth; moreover, the skimmed colostrum hydrolysates had higher inhibition rates than caseins and whey hydrolysates. The most likely explanation is that the hydrolysate samples had different peptide compositions and different amino acid sequences, due to the different enzymes used for hydrolysis. Several articles[37-42] have reported that milk protein hydrolysates are directly related to cellular reactions. Goldsby *et al.*[43] proposed a possible mechanism as that the small peptide molecules could penetrate the cancer cell wall, inhibit protein synthesis, thereby affect the cell RNA and DNA synthesis, and ultimately change cell shape as well as function to make it quick death. Another possible mechanism is that the cell membrane receptor is combined with the peptides, resulting in that the cells can not get sufficient nutrients, ultimately leading to death.

This research also demonstrated that the days on which the colostrums were collected were significantly correlated with cell growth (Table 1). Gauthier *et al.*[42] reported that bovine colostrums contain a number of growth factors, hormones and cytokines; however, the distinction between these molecules is not clear, since they all can be involved in cell proliferation and differentiation. It may be stated from the above results that bovine colostrum proteins and their hydrolysates not only inhibit the growth of U937 cells but also stimulate the growth of MNC. The bioactivities of protein hydrolysates are most likely related to peptides and/or free amino acids liberated during digestion. However, the possibility of the unknown compounds should also be kept in mind.

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