Angiotensin-converting enzyme inhibition and antioxidant activity of commercial dairy starter cultures

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Abstract In this study, skim milk fermented with 14 commercial dairy starters were evaluated for their proteolysis ability, angiotensin I-converting enzyme (ACE)-I, and antioxidant properties. The antioxidant activity was determined using DPPH radical scavenging, chelating of Fe^{2+} ions, and reducing power assays. The results showed that the coagulation time, pH, and titratable acidity varied among the used starters and appeared to be starter dependent. *Lactobacillus helveticus* Lh-B02 starter had the highest proteolytic and ACE-I activity. *Lactobacillus casei*-01, Yo-Fast 1, YC-281, MYE 96, and YO-MIX 205 starters had the highest DPPH radical scavenging activity. Chelating of Fe^{2+} ions of the *L. casei*-01 starter was the highest, whereas the O-114 starter was the greatest in reducing power. Regarding the yogurt starters, Yo-Fast 1 starter exhibited high values of proteolytic, ACE-I, and DPPH radical scavenging activity. A positive correlation was observed between the proteolytic and ACE-I or antioxidant activities of all starters.

Keywords: commercial dairy starter, proteolysis, ACE-I, antioxidant

Introduction

Commercial dairy starters contain one or more lactic acid bacteria (LAB) strains; these starters are produced by specialized companies. LAB have a potential role as protective cultures in food biopreservation and an influence on the organoleptic properties of final products (1). Moreover, some species of LAB play a large variety of health-promoting functions and have traditionally been used as probiotics in various food commodities (2,3).

The proteolytic activity of dairy starters plays a pivotal role in different cellular and physiological processes (4). LAB possess a very complex proteolytic system, which enables them to use milk casein as a source of amino acids and nitrogen in milk that is poor in these growth-promoting components (5,6). This proteolysis results in the formation of bioactive peptides, including antihypertensive, anti-thrombotic, antimicrobial, antioxidative, antidiabetic, opioid, immunomodulatory, mineral binding, and cholesterol-lowering peptides (7-11).

Hypertension is present when the resting blood pressure is above 140/90 mmHg, and it is considered to be a serious factor resulting in heart diseases (12). Angiotensin I-converting enzyme (ACE) is one of the factors responsible for increasing blood pressure wherein it converts the angiotensin-I (inactive) to angiotensin-II (vasoconstrictor) and degrades vasodilator bradykinin (13). ACE inhibition leads to reduced blood pressure, and it regulates the immune and nervous

systems activity (14). Several studies have been conducted on ACE-inhibitory peptides in fermented milk products (15-19).

In human beings, oxidation is one of the essential factors for the development of certain diseases, such as atherosclerosis, Alzheimer, inflammatory bowel, and cancer. Antioxidative peptides from foods contain 5-16 amino acid residues with molecular weight of less than 3,000 Da that are being considered as potential sources to control various oxidative processes in the human body as well as in food (20). Several studies reported that LAB exhibited antioxidant ability (21-23).

Consequently, selection of commercial starters for use in fermented dairy products is important owing to the significant variations in their ability to produce biopeptides (24). Few studies (25,26) have examined the ability of commercial dairy starters to produce bioactive peptides, although it is used in the production of commercial fermented dairy products. Therefore, our study aims to highlight the ability of some commercial dairy starters to produce ACE-inhibitory and antioxidant peptides.

Materials and Methods

Materials Cow milk, free from antibiotics, was obtained from an open nucleus herd belonging to the Cattle Information System of Egypt (CISE) and Technology Center of Agricultural Production,

Faculty of Agriculture, Cairo University, defatted, and sterilized at 121°C for 15 min. The chemicals, including O-phthaldialdehyde (OPA), 2-mercaptoethanol, hippuryl-L-histidyl-L-leucine (Hip-His-Leu), 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-*p*, *p*-disulfonic acid monosodium salt hydrate (ferrozine), 1,1-diphenyl-2-picrylhydrazyl (DPPH), sodium tetraborate buffer HPCE (pH 8.0), ferrous chloride (FeCl₂), and ferric chloride (FeCl₃), were obtained from Sigma-Aldrich (St. Louis, MO, USA). The 14 direct vat inoculation (DVI) commercial starters were obtained from different companies as shown in Table 1.

Preparation of starter cultures The tested DVI commercial starters (Table 1) were first precultured (0.02%, w/v) in sterilized skim milk and incubated for 12 h at 42°C for thermophilic and 30°C for mesophilic starters. The stock cultures were used to inoculate the sterilized skim milk at 1% and were incubated at 42°C for thermophilic and at 30°C for mesophilic starters. After coagulation of milk, the proteolytic, ACE-I, and antioxidant activities were determined in the fermented skim milk.

Preparation of ACE from rabbit lung Angiotensin-converting enzyme extract was prepared according to Qian *et al.* (27). A fresh rabbit lung without connective tissues was cut into pieces, and the

blood was washed away with 10 mmol/L phosphate-buffered saline. The washed lung was homogenized in 10 mmol/L phosphate-buffered saline (pH 7.2) at a ratio of 1:5 (w/v). Then, the mixture was ultra-centrifuged at 40,000×*g* for 40 min at 4°C. The obtained supernatant containing the ACE was stored at –20°C until use.

Preparation of water-soluble extract from fermented milk The soluble nitrogen extract was prepared according to Shori *et al.* (28). Fermented milk (10 g) was added to distilled water (40 mL) in screw-cap tubes. The mixture was homogenized followed by holding at 40°C for 1 h. The homogenate was then centrifuged at 10,000×*g* for 30 min and the obtained supernatant (water-soluble nitrogen extract, WSE) was harvested and kept at –80°C until analysis.

Method of analysis

pH and titratable acidity: The pH values were measured using a digital pH meter with a glass electrode (Jenway 3305; Jenway, Stone, UK). Titratable acidity was determined by titration with 0.1 N NaOH using phenolphthalein as an indicator (29).

Proteolytic activity: The rapid, sensitive, and convenient OPA-based spectrophotometric method was used (28). The OPA solution was prepared from 25 mL of 100 mmol sodium tetraborate, 2.5 mL of

Table 1. The DVI commercial dairy starter cultures used in this study

Starter type	Species	Properties	Application
Thermophilic starters (42°C)			
La-5 ¹⁾	<i>Lb. acidophilus</i>	Medium acidification	Probiotic fermented milk
Lh-B02 ¹⁾	<i>Lb. helveticus</i>	Medium/fast acidification	Italian and Swiss cheese varieties
L. casei-01 ¹⁾	<i>Lb. casei</i>	Slow fermentation/mild acidic flavor	Probiotic fermented milk
Fresh Q ₄ ¹⁾	<i>Lb. paracasei</i> and <i>Lb. rhamnosus</i>	Inhibition of unwanted yeast and molds in the product	Fresh cheese, yogurt, and sour cream
Yo-Fast 1 ¹⁾		High viscosity, less flavor, and fast acidification	Yoghurt and hard and semi-hard high-cook cheeses
YC-281 ¹⁾	<i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> and <i>St. thermophilus</i>	High viscosity and mild flavor	
YC-380 ¹⁾		High viscosity and medium flavor	
YC-X11 ¹⁾		High viscosity, mild flavor, and low post-acidification	
MYE 96 ²⁾		High viscosity and medium flavor	
YC-180 ¹⁾	<i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> , <i>Lb. delbrueckii</i> subsp. <i>Lactis</i> , and <i>St. thermophilus</i>	High viscosity and mild flavor	Probiotic yogurt
YO-MIX 205 LYO ³⁾	<i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> , <i>St. thermophilus</i> , <i>Lb. acidophilus</i> , and <i>Bifidobacterium lactis</i>	Thick texture, yogurt taste, and low post-acidification	
Mesophilic starters (30°C)			
CHOOZIT MA 14 LYO ³⁾	<i>Lc. lactis</i> subsp. <i>lactis</i> and <i>Lc. lactis</i> subsp. <i>cremoris</i>	Closed texture	Many cheese types
O-114 ¹⁾	<i>Lc. cremoris</i> and <i>Lc. lactis</i>	Closed texture	Many cheese types
FI-Da N ¹⁾	<i>Lc. lactis</i> subsp. <i>lactis</i> , <i>Lc. lactis</i> subsp. <i>cremoris</i> , <i>Leu. mesentroides</i> subsp. <i>cremoris</i> , and <i>Lc. lactis</i> subsp. <i>diacetylactis</i>	High diacetyl, slow acidification, and high CO ₂	Cheese with small holes and lactic butter milk

¹⁾From Chr Hansen laboratories, Hoersholm, Denmark

²⁾From Rhodia Food, de Buxières, Dangé-Saint-Romain, France

³⁾From Danisco France SAS, rue de Clémencières-BP 32, 38360 Sassenage, France

20% (w/w) sodium dodecyl sulfate, 40 mg of OPA, and 100 μ L of β -mercaptoethanol. The volume was increased to 50 mL by adding distilled water. The OPA reagent was freshly prepared and used within 2 h of preparation. One milliliter of OPA reagent was added to 30 μ L of WSE in a cuvette. The solution was mixed by inversion and incubated for 2 min at room temperature. The absorbance was read at 340 nm using a spectrophotometer (Jenway® Genova Life Science Spectrophotometer UV/Visible, Bibby Scientific Ltd., Stone, UK).

ACE-I activity: The ACE-I activity of WSE was determined according to Cushman and Cheung (30) with some modifications by Donkor *et al.* (4) and Rodríguez-Figueroa *et al.* (17). The pH of the SNE was adjusted to 8.3 using 10 N NaOH. The buffered substrate solution was prepared by dissolving 5 mmol hippuryl-L-histidine-L-leucine in 100 mmol sodium borate buffer solution containing 300 mmol NaCl (pH 8.3). The four microtubes were prepared as follows: A: 100 μ L of the buffered substrate solution+40 μ L of distilled water+20 μ L of ACE; B: 100 μ L of buffered substrate solution+60 μ L of distilled water; C: 100 μ L of buffered substrate solution+40 μ L of WSE+20 μ L of ACE; and D: 100 μ L of buffered substrate solution+40 μ L of WSE+20 μ L of distilled water. All tubes were incubated at 37°C for 35 min. The reaction was stopped by adding 250 μ L of 1 N HCl. Ethyl acetate (1 mL) was added to every tube for the extraction of released hippuric acid. The tubes were stirred vigorously for 20 s and centrifuged at 1,500 $\times g$ for 10 min. An aliquot of the organic phase was evaporated at 80°C for 90 min. The residue was dissolved in 1 mL of distilled water and stirred vigorously. The absorbance was measured at 228 nm. The following equation was used to calculate the ACE-I activity:

$$\text{ACE-inhibition (\%)} = [1 - (C - D) / (A - B)] \times 100,$$

where A is the absorbance in the presence of ACE and the buffer only; B is the absorbance in the presence of the buffer only; C is the absorbance in the presence of ACE, WSE, and the buffer; and D is the absorbance in the presence of WSE and the buffer.

Antioxidant activity assay: The antioxidant activity was determined in three assay systems *in vitro* according to Li *et al.* (31) as follows:

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity: The WSE (1 mL) was mixed with 0.1 mmol/L DPPH (1 mL) dissolved in 95% ethanol. The mixture was shaken and kept at room temperature for 30 min. Absorbance of the resulting solution was measured at 517 nm. Distilled water was used as blank instead of the sample. Ascorbic acid (0.2 mmol/L) was used as a positive control. The scavenging activity was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = (A_0 - A_s) / A_0 \times 100,$$

where A_0 is the absorbance at 517 nm of blank; A_s is the absorbance at 517 nm of WSE.

Chelating of metal ions (Fe^{2+}) activity: The WSE (1 mL) was diluted with distilled water (2.7 mL). A solution of 2 mmol/L FeCl_2 (0.1 mL) was added. After 3 min, the reaction was inhibited by the addition of 5 mmol/L ferrozine (0.2 mL). The mixture was shaken vigorously and

kept at room temperature for 10 min. Absorbance of the resulting solution was measured at 562 nm. A blank without WSE was prepared in a similar manner. EDTA (0.1 mg/mL) was used as positive control. The chelating capacity was calculated as follows:

$$\text{Fe}^{2+} \text{ chelating activity (\%)} = (A_0 - A_s) / A_0 \times 100,$$

where A_0 is the absorbance at 526 nm of blank; A_s is the absorbance at 562 nm of WSE.

Reducing power The WSE (1 mL) was mixed with 1 mL of 0.2 M phosphate buffer (pH 6.6) and 1 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min followed by the addition of 1 mL 10% TCA. An aliquot (2 mL) from the incubation mixture was mixed with 2 mL of distilled water and 0.4 mL of 0.1% ferric chloride in test tubes. After 10 min, the absorbance of the resulting solution was measured at 700 nm. Ascorbic acid (0.2 mmol/L) was used as a positive control. An increased absorbance of the reaction mixture indicates an increased reducing power.

Statistical analysis Data are expressed as the mean $\pm$ standard deviation. All data were analyzed in three replications for each parameter. One-way analysis of variance and correlation analysis were performed on the data using SAS 9.2. The mean of the treatments were compared using the least significant difference approach at the significance level of $p < 0.05$.

Results and Discussion

Fermentation characteristics The pH, titratable acidity, and coagulation time of each starter are shown in Table 2. In both thermophilic or mesophilic starters, the pH ranged from 4.25 to 4.61 and the titratable acidity ranged between 0.67 and 0.82%. The coagulation time was the lowest (4.00 h) for the YC-180 starter, whereas it was the highest (17.30 h) for *L. casei*-01, and these variations appeared to be starter dependent.

Proteolytic activity The proteolytic activity of used commercial dairy starters growing in sterilized skim milk was estimated by determination of free amino groups and peptides concentration in the water-soluble extract using the OPA method and the results are represented in Table 3. The results showed that the extent of proteolysis varied among the used starters. *L. helveticus* Lh-B02 and Fl-Da N starters had the significant highest, whereas YO-MIX 205, YC-281, YC-380, and Fresh Q4 starters had the significant lowest of proteolytic activity. Among the seven yogurt starters, the Yo-Fast 1 starter had the significant highest proteolytic activity. Our results are in agreement with Kilpi *et al.* (32) and Nielsen *et al.* (33), who found that *L. helveticus* strains have higher proteolytic activity than other commercial LAB. Moreover, Lahtinen *et al.* (34) reported that LAB strains belonging to *Lactobacillus* species showed low proteolytic activity as compared with *Lactococcus* species. Donkor *et al.* (4)

Table 2. The fermentation characteristics of commercial dairy starters

Starter	Time (h)	pH	Titrateable acidity (%)
Thermophilic starters (42°C)			
L-a5	9.00	4.61	0.67
Lh-02	7.30	4.51	0.73
L. casei-01	17.30	4.40	0.77
Yo-Fast 1	4.15	4.50	0.72
YC-180	4.00	4.59	0.68
YC-281	6.00	4.50	0.73
YC-380	4.00	4.60	0.67
YC-X11	4.00	4.59	0.67
MYE 96	4.00	4.50	0.71
YO-mix 205	4.00	4.50	0.71
Fresh Q4	17.00	4.50	0.71
Mesophilic starters (30°C)			
FI DaN	9.30	4.33	0.77
CHOOZIT MA 14 LYO	8.30	4.25	0.82
O-114	8.30	4.29	0.77

Table 3. The proteolytic and ACE-I activities of commercial dairy starters

Starter	Proteolytic activity (A ₃₄₀)	ACE-I (%)
Thermophilic starters (42°C)		
L-a5	0.237 ^{de} ±0.011 ¹⁾	36.80 ^f ±1.10
Lh- B 02	0.487 ^a ±0.01	75.87 ^a ±0.76
L. casei-01	0.219 ^{ef} ±0.013	32.10 ^{gh} ±1.51
Yo-Fast 1	0.279 ^c ±0.01	42.09 ^d ±0.86
YC-180	0.209 ^{ef} ±0.011	32.31 ^g ±0.96
YC-281	0.172 ^g ±0.022	26.31 ⁱ ±0.88
YC-380	0.189 ^{fg} ±0.025	30.11 ^j ±1.10
YC-X11	0.238 ^{de} ±0.03	37.10 ^f ±0.70
MYE 96	0.270 ^{cd} ±0.031	39.00 ^e ±0.98
YO-MIX 205	0.168 ^g ±0.04	25.09 ^j ±0.82
Fresh Q4	0.187 ^{fg} ±0.021	30.61 ^{hi} ±0.78
Mesophilic starters (30°C)		
FI-Da N	0.480 ^a ±0.01	70.60 ^b ±0.43
CHOOZIT MA 14 LYO	0.288 ^c ±0.015	42.77 ^d ±0.55
O-114	0.344 ^b ±0.012	51.34 ^c ±0.66

¹⁾Values are means±standard deviation; values in the same column with different superscript letters differ significantly.

reported that cultures of *L. acidophilus* (L10 and La 4962) and *L. casei* (L26 and Lc 279) have different degrees of proteolysis with intra and extracellular specific peptides. These variations in the proteolytic activity of starters may be because of the differences in their proteolytic system. This system involves a cascade of processes with a number of steps, including the exploitation of casein by a cell-envelope proteinase that degrades the protein into oligopeptides that are subsequently taken up by the cells via specific peptide transport systems for further degradation into shorter peptides and amino acids by a concerted action of various intracellular peptidases (35).

ACE-inhibitory activity The ACE-I activity of used commercial dairy starters are shown in Table 3. The results indicate that all used starters have ACE-I activity and there are significant differences in ACE-I values between all of them. *L. helveticus* Lh-B02 and FI-Da N starters showed the significant highest ACE-I activity at 75.87 and 70.60%, respectively, whereas the YC-281 and YO-MIX 205 starters showed the significant lowest ACE-I activity. The Yo-Fast 1 starter, one of the yogurt starters, had the significant highest ACE-I activity. Our findings are in agreement with Chen *et al.* (15), who found that 37 strains of *L. helveticus* have an ACE-I activity of over 50%. Extracellular proteinases from *L. helveticus* CP790 have been reported to generate large β -casein fragments with ACE-inhibitory activity (36). Ueno *et al.* (37) extracted an endopeptidase from *L. helveticus* CM4 and confirmed that this enzyme can release tripeptides IPP and VPP, which have ACE-I activity. Fuglsang *et al.* (38) reported that most LAB produced different amounts of ACE-I peptides during milk fermentation, which varied with the strains. Moreover, Ong *et al.* (25) reported that LAB starters generate a variety of bioactive peptides, including ACE-I peptides, via enzymatic proteolysis of milk protein by more than 40% of the peptide bonds of β -CN or α_{s1} -CN, resulting in the formation of more than 100 different oligopeptides, which are in turn actively degraded by the complex peptidase system.

The correlation between the ACE-I and proteolytic activities of used starters was calculated. High positive correlations ($r=1$) were found between the ACE-I and proteolytic activities for most of the starters, whereas the ACE-I activity correlated ($r=0.5$) with proteolytic activity for YO-Fast 1 and YC-180 starters was low. Pihlanto *et al.* (39) observed a high correlation ($r=0.88-0.99$) between the ACE-I activity and degree of proteolysis for all used *Lactobacillus* strains.

Antioxidant ability The antioxidant ability of used commercial starters was determined *in vitro* by three assay systems, including DPPH radical scavenging activity, chelating metal ions (Fe^{2+}), and reducing power. Ascorbic acid and EDTA were used as a positive control.

DPPH radical scavenging activity DPPH is a stable free radical that shows maximum absorbance at 517 nm. The absorbance decreases because of a color change from purple to yellow as the radical is scavenged by antioxidants (40). The results (Fig. 1) indicate that DPPH radical scavenging activity ranges from 42.78 to 83.57% for all starters. The starters of *L. casei*-01, YO- Fast 1, MYE 96, and YO-MIX 205 had significant higher values than ascorbic acid, whereas the other starters had significant lower values. As for yogurt starters, the Yo-Fast 1 starter has the significant highest DPPH radical scavenging activity. Virtanen *et al.* (22) found that *L. helveticus* and *L. acidophilus* have radical scavenging activity. Moreover, Lin and Chang (41) reported that *L. acidophilus* has the ability to scavenge radical DPPH at 43.2-52.1%. Aloglu and Öner (42) observed that the DPPH antioxidant activity is higher in traditional yogurt than in commercial

Fig. 1. DPPH radical scavenging activity (%) of the commercial dairy starters. Different letters indicate significant differences among starters. Ascorbic acid serves as a positive control.

Fig. 2. Reducing power (A₇₀₀) of the commercial dairy starters. Different letters indicate significant differences among starters. Ascorbic acid serves as a positive control.

yogurt and this may be because of their hydrogen-donating ability. Sarmadi and Ismail (20) reviewed that antioxidative properties of the peptides are more related to their composition, structure, and hydrophobicity. Amino acids with aromatic residues can donate protons to electron deficient radicals. This property improves the radical-scavenging properties of the amino acid residues.

Reducing power The reducing power of different starters is illustrated in Fig. 2. From the obtained results, the O-114 starter shows the significant highest reducing power compared with the other starters and ascorbic acid (positive control). However, the *L. helveticus* Lh-B02 starter exhibits the significant lowest reducing power. In contrast, Li *et al.* (31) found that *L. helveticus* shows high reducing activity, which is equivalent to 60-67% of the reducing power of positive control. Among the yogurt starters, the YO-MIX

205 starter has the significant highest reducing power. The high reducing power of a starter could be attributed to the reducing ability of amino acids present in the sequence of peptides that are exposed by protein hydrolysis during fermentation and may react with free radicals to stabilize and block the chain reactions (31).

Chelating of metal ions (Fe²⁺) Ferrous ions contribute to produce the free radicals that lead to heart diseases, cancer, and arthritis (21). Therefore, the ability of starters to chelate metal ion (Fe²⁺) was studied and the results are shown in Fig. 3. Our findings show significant differences in Fe²⁺ chelating activity among all starters; this difference ranges between 4.53% (*L. helveticus* Lh-B02) and 72.12% (*L. casei*-01). Regarding the yogurt starters, the YC-380 starter has the significant highest Fe²⁺ chelating activity. Moreover, from the obtained results, all starters have a significant lower ability

Fig. 3. Fe²⁺ chelating activity (%) of the commercial dairy starters. Different letters indicate significant differences among starters. EDTA serves as a positive control.

to chelate ferrous ions compared with EDTA (positive control). Accordingly, Li *et al.* (31) found that peptides from fermented milk with tested LAB strains exhibit chelating Fe²⁺ ions at a level between 23.20% (*L. helveticus*) and 42.34% (*St. thermophilus*). Zhang *et al.* (43) reported that *L. casei* subsp. *casei* SY13 has the ability to chelate Fe²⁺ ions. Rajapakse *et al.* (44) proposed that the antioxidant activity of His-containing peptides is in relation with the metal ions chelating ability of the imidazole group.

The correlation between the antioxidant activity of the three system assays and the proteolytic activity of used starters was calculated. High positive correlations ($r=1$) were observed between the antioxidant and proteolytic activities for all starters except the YC-180 starter, which exhibited low positive correlation ($r=0.5$). In this respect, Ramesh *et al.* (23) found a good correlation ($r=0.5-0.99$) between the proteolytic and antioxidant activities among the selected strains of *Lactobacillus* assessed, indicating that the production of antioxidant peptides with proteolysis contributes toward the development antioxidant activity.

In conclusion, all used commercial starters had varying degree of proteolytic, ACE-I, and antioxidant activity. The *L. helveticus* Lh-B02 starter exhibited the highest activity for proteolysis and ACE inhibition. All commercial starters can exhibit antioxidant activity but using different mechanisms. The *L. casei*-01 starter showed the highest DPPH radical scavenging and chelating Fe²⁺ ions activity, whereas the O-114 starter showed the highest reducing power. Regarding the yogurt starters, the Yo-Fast 1 starter had the highest activity of proteolytic, ACE-I, and DPPH radical scavenging, whereas the YC-380 and YO-MIX 205 starters had the highest ability to chelate Fe²⁺ ions and reduce power, respectively. The ACE-I and antioxidant activities of all commercial starters were closely connected to proteolysis. Based on our findings, it could be select the commercial

starters with a great impact on the ACE-I and antioxidant activities to be used for manufacturing fermented dairy products relevant to cardiovascular disease prevention. Furthermore, this study may encourage the companies that manufacture commercial starters to involve the ability of bioactive peptide production in the starter properties criteria for increasing the industrial application of bioactive peptides.

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