

Effect of Casein Hydrolysates on Yogurt Fermentation and Texture Properties during Storage

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Received: November 9, 2005

Accepted: March 6, 2006

Summary

Effects of casein hydrolysates by papain on acidification of the yogurts and growth of probiotic bacteria during yogurt fermentation have been investigated. The viability of probiotic bacteria and texture characteristics of the yogurts during storage at 4 °C have been evaluated. The hydrolysates strongly decreased the fermentation and coagulation time of the yogurts. The post-fermentation acidification was retarded by the hydrolysates. The hydrolysates increased the probiotic counts during initial fermentation stage. The growth of the probiotic organisms decreased at the final stage. Survival of probiotic bacteria was improved by the hydrolysates. The hydrolysates significantly ($p < 0.05$) increased the adhesiveness of the yogurts except for 0.5 % of hydrolysate with degree of hydrolysis of 8.5 %. The sensory evaluation scores of the yogurts were significantly ($p < 0.05$) improved by the hydrolysates after the storage. The effect of casein hydrolysates on fermentation and texture properties was related to the molecular mass of the hydrolysates.

Key words: yogurt, casein hydrolysates, probiotic count, acidification, viability, texture properties, sensory evaluation

Introduction

Yogurt is a traditional fermented milk product in China. Since the renewed interest in probiotics, different types of products are proposed as carrier foods of probiotic microorganisms. Consumers can take in large amounts of probiotic cells through these carrier foods. Yogurt has long been recognized as a product with many desirable effects for consumers. In China, total output of yogurt products reached about 600 000 tonnes in 2002 (1). There has been an increasing interest in the functionality of these products because these products are rich in probiotic microorganisms. Maintenance of a minimal effective dose of living probiotic bacteria can regulate immune function (2) as well as improve lactose digestion

and the biological functions of the consumer (3). Fuller (4) elucidates systemically that probiotic bacteria have many beneficial effects in humans. Yogurt or yogurt-like products have been used as the most popular vehicle for incorporation of probiotic organisms. Unfortunately, most of the commercial products contain less probiotic bacteria than the minimum required, because these microorganisms grow slowly in milk and often show loss of viability during storage (5–7). In addition, the probiotic bacteria are sensitive to pH, lactic acid, hydrogen peroxide and dissolved oxygen in fermented milk (8–11).

A great number of efforts have been made to improve the growth and survival of these bacteria during storage. Some of the practices have been proved to be

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able to increase the survival of these bacteria in the yogurt products. The substances such as oligosaccharides (12), sugar sources (13) and non-protein nitrogen (13,14) can improve the growth of probiotic bacteria. Vitamins, dextrin and maltose stimulate the growth of bifidobacteria species in milk, while sucrose and iron salts have little effect. Ascorbic acid, an oxygen scavenger (7), does not improve viability of bifidobacteria in yogurt, but it can be applied to ensure better survival of *L. acidophilus* and *B. bifidum* in yogurt (15).

Some protein hydrolysates enhance the acidification rate of yogurt and reduce the fermentation time (8,16). However, these hydrolysates affect the texture and the physical properties of the yogurt by changing the fermentation time, starter culture metabolism, and interacting with milk proteins to form the building blocks of the gel network. Supplementation of milk with a combination of casein, casein hydrolysate and fructose stimulates the growth of *L. acidophilus* (8). The probiotic in the yogurt with cysteine is less firm and less viscous than yogurt without cysteine (17). Milk supplementation with peptides and amino acids may also increase the viability of probiotic organisms (14,18). Milk protein hydrolysate reduces fermentation time and increases the viability of two strains of probiotic bacteria in milk (19). The yogurts with casein and whey protein hydrolysates decrease the complex viscosity and fermentation time, and have a more open and less branched structure (20).

In the present study, the effects of casein hydrolysates with three different degrees of hydrolysis (DH=8.5, 14.6 and 26.7 %) by papain on acidification and probiotic counts in yogurt have been investigated. The viability of probiotic bacteria and texture characteristics of yogurts containing probiotic bacteria, and supplemented with casein hydrolysates during storage at 4 °C have been evaluated.

Materials and Methods

Strains and ingredients

The commercial bacterial strain YC-370 (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) was used (Chr. Hansen, Guangzhou, China). The strain was stored at -40 °C in a concentrate form and then it was thawed and diluted 10 times in sterilized skim milk just before inoculation. The food grade enzyme (papain) was provided by Guangzhou Enzyme Co. (Guangzhou, China). Sodium casein was also purchased from Chr. Hansen, Guangzhou, China.

Casein hydrolysate preparation

A fraction of 10 % of aqueous dispersion of sodium casein was applied to enzymatic hydrolysis. The enzymatic hydrolysis was carried out at 60 °C, constant pH=6.8 with an enzyme to substrate ratio [E/S] of 380 U/g. The enzyme was inactivated by heat treatment at 90 °C for 10 min. The resulting mixture was rapidly cooled to ambient temperature in the ice-water bath and then centrifuged (4000 rpm) for 20 min. The resulting supernatant (hydrolysate) was freeze-dried and then stored for further application. The hydrolysates with DH of 8.5, 14.6

and 26.7 % were used as supplements to yogurt. These hydrolysates are expressed as CH1, CH2 and CH3.

Yogurt preparation

Whole powdered milk, sucrose and the hydrolysates were blended (model JB200-D, Japan). The protein content was standardized to $w=5$ % by the addition of powdered skim milk (Chr. Hansen, Guangzhou, China). The standardized milk was supplemented with CH1, CH2 and CH3 each at 0, 0.5, 1.0, 1.5 and 2.0 %. After being homogenized for 10 min at 20 MPa, it was heated (95 °C, 5 min), cooled to 4 °C in an ice-water bath, poured into 250-mL flasks and stored for 24 h before testing. Standardized milk that was not supplemented with hydrolysates was used as the control (CK). Standardized milk samples were inoculated at the fermentation temperature (42 °C) with $6.31 \cdot 10^5$ CFU/mL of bacterial strain YC 370 at inoculation rates of 0.005 % (m/V), according to the manufacturer's recommendation. Inoculated milk samples were incubated at 42 °C until pH=4.40. Fermentation was stopped by rapidly cooling the fermented milk to 4 °C in an ice-water bath. The cooled yogurt was poured into 100-mL cups and stored at 4 °C.

Chemical analysis

Contents of total nitrogen and non-protein nitrogen were measured by the Kjeldhal method (21). A conversion factor of 6.2 was used. All measurements were performed in duplicate.

Molecular mass distribution of the hydrolysates

The molecular mass distribution of the hydrolysates was determined by Amersham Protein Analytical and Purifying System equipped with Superdex-peptide-10/300-GI glass column. Eluting solution was phosphate buffer 0.25 M (pH=7.2) and flow rate was 0.5 mL/min. Globin III ($M_r=2512$), Globin II ($M_r=6214$), Globin I ($M_r=8519$), Globin I+III ($M_r=10700$), Globin ($M_r=16949$) were used as standard peptides (Amersham).

Acidification

The pH of the fermented milk was monitored at 17–20 °C by using the Cinac pH meter after calibrating it with fresh pH=4.0 and 7.0 standard buffers. The time taken for the pH to reach 4.4 was calculated as the fermentation time. Coagulation time expressed as the pH recorded after 35-day storage period at 4 °C and post-fermentation acidification were also recorded. This assay was performed in four replicates of each sample.

Probiotic counts

Probiotic cell numbers during fermentation and after storage for 30 days at 4 °C were recorded. Appropriate dilutions of the samples were prepared in 0.1 % (m/V) sterile water and subsequently plated in duplicate on selective media. Populations of *Lactobacillus delbrueckii* ssp. *bulgaricus* were enumerated on MRS agar plates at pH=5.4 and incubated at 37 °C for 72 h. Populations of *S. thermophilus* were enumerated on Elliker agar plates after a 72-hour incubation period at 37 °C.

Texture profile analysis

Texture profile analysis (TPA) was performed using a TA-XT2i texturometer (Stable Micro Systems, Surrey, UK) to determine the textures of the yogurts. The probe penetrated the samples to a depth of 15 mm at a speed of 1.0 mm/s and the force exerted on the probe was automatically recorded. The parameters recorded include hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness and resilience. Three yogurt samples were analyzed at (4±2) °C for each trial and average readings were taken.

Sensory analysis

A number of 10 to 15 trained panelists who consume yogurts regularly in their diets and have previous experience in taste evaluation were selected to rate sensory properties of yogurt. Firstly, the panelists were trained in 2-hour sessions prior to evaluation to be familiar with the attributes and scaling procedures of yogurt samples under study. Yogurt samples were organoleptically examined according to the method modified from Bodyfelt *et al.* (22) with maximum scores of 10, 5, and 5 for flavour; body and texture; and appearance and colour, respectively, the highest number indicating extreme liking and the lowest extreme dislike. Panelists were also asked to note any acetaldehyde, bitter, cooked, foreign, acid (too high and too low), oxidized or unnatural flavour, and other perceived attributes for flavour; gel-like, grainy, ropy, too firm, too weak, and other perceived attributes for body and texture; and whey-off, lumpy, shrunken, dark colour, partial colour, and other perceived attributes for appearance and colour. All yogurt samples (in 100-mL yogurt cups) were coded with three-digit random numbers and presented to panelists on a tray in individual booths. Order of serving was completely randomized. Panelists were instructed to cleanse their palate with plain crackers and water before tasting each sample. Panelists evaluated all yogurt samples after storage for 1, 7, and 14 days at 4 °C.

Statistical analysis

Unless otherwise stated, all the tests were performed in triplicate and data were averaged. Standard deviation was also calculated. Duncan's multiple-range test (23) was used to evaluate significant difference ($p < 0.05$) between means.

Results and Discussion

Nitrogen content and peptide mass distribution of hydrolysates

Total nitrogen mass fraction (w) of the three hydrolysates ranged from 86.5–87.3 %, without any significant difference ($p < 0.05$). However, difference among their non-protein nitrogen contents was observed from $w = 68.4$ –83.1 %. Hydrolysate CH3 had the highest non-protein nitrogen content. The difference was possibly caused by their different degrees of hydrolysis. Enzymatic hydrolysis produced the peptides with low molecular mass. Moreover, the hydrolysis resulted in the conversion of some peptides into amino acids (non-protein nitrogen).

Most of the peptides in CH1 with the lowest DH (8.5 %) were the ones with high molecular mass (>1.0 kDa) and they made 86.3 % of total peptides in CH1, while CH3 with the highest DH (26.7 %) mainly contained the peptides with low molecular mass (<1.0 kDa).

Acidification

Fig. 1 presents changes in fermentation times of yogurts supplemented with casein hydrolysates. Addition of hydrolysates to yogurts has a strong effect on fermentation time. The fermentation times of the yogurts with added hydrolysates were shorter than the control. This is consistent with the previous reports (16,18,19). It means that the hydrolysates promote the growth of probiotic bacteria and increase the acidifying activity of these microorganisms. In addition, yogurts with CH3 with the highest DH decreased the fermentation time more significantly ($p < 0.05$) than yogurts with CH1 and CH2. CH3 hydrolysates probably contained higher levels of the peptides with low molecular mass. These small peptides were known to be bioactive peptides and can enhance the growth and acidifying activity of the probiotic organisms.

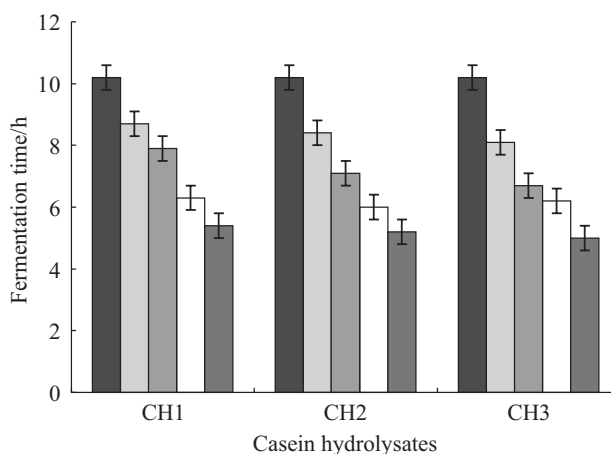


Fig. 1. Fermentation times of yogurt with added hydrolysates. CH1, CH2 and CH3, the casein hydrolysate with degree of hydrolysis of 8.5, 14.6 and 26.7 %, respectively. ■ CK, ■ 0.5 %, ■ 1.0 %, □ 1.5 %, ■ 2.0 %

Supplements of casein hydrolysates decrease significantly ($p < 0.05$) the coagulation time of yogurts (Fig. 2). The greatest decrease in coagulation time occurs in the CH3-treated yogurts. Coagulation time is closely associated with the viability of probiotic bacteria. The shorter the coagulation time, the better viability of these microorganisms. Short coagulation time can improve the growth and survival of the probiotic bacteria.

Post-acidification

The hydrolysates greatly inhibited post-fermentation acidification of the yogurts (Fig. 3). The pH of the yogurts declined after storage for 30 days at 4 °C. However, slow decrease in pH of the yogurts with added hydrolysates was found, compared to the control. The control had a pH value of 4.16 compared to pH=4.18–4.37 of the yogurts with CH1, CH2 and CH3 at storage pe-

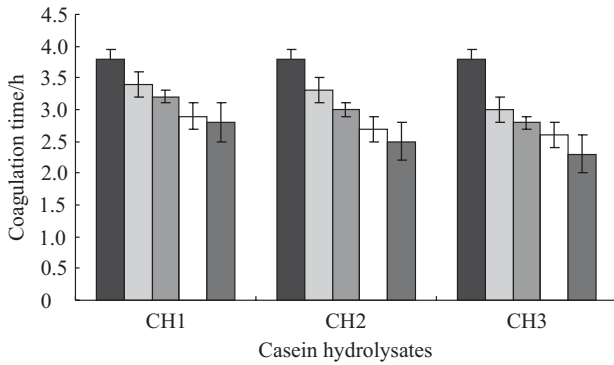


Fig. 2. Coagulation time of yogurt with added casein hydrolysates. CH1, CH2, CH3 and the columns represent the same as in Fig. 1

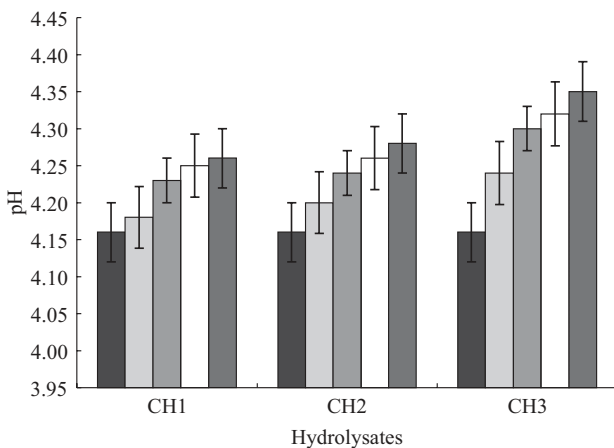


Fig. 3. Change in pH of yogurts with added hydrolysates after storage for 30 days. CH1, CH2, CH3 and the columns represent the same as in Fig. 1

riod of 30 days at low temperature. Post-fermentation acidification had a negative effect on the quality, and shortened the shelf life. It is closely associated with the persistent metabolic activity of lactobacilli during cooling at 4 °C (24). Decrease in post-fermentation acidification was favourable for sensory improvement and consumers' preference.

Change in growth and viability of probiotic bacteria during fermentation and storage

The growth of probiotic organisms during fermentation is presented in Fig. 4. The hydrolysates increased the probiotic counts at initial stage compared to the control. It indicated that the hydrolysates enhanced the growth of probiotic organisms. However, the growth of the probiotic organisms decreased at the final stage when the hydrolysates were added. The growth of these bacteria decreased with the increase in the addition of hydrolysates. This was related to the decrease in fermentation time.

L. acidophilus and *B. bifidum* have to retain viability and activity in the food system to meet the requirements for consumption (25). Viability of probiotic bacteria in products during long shelf life at refrigeration tempera-

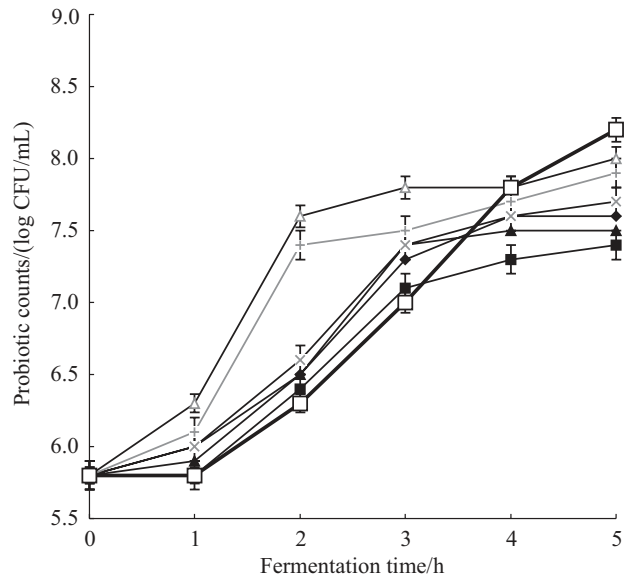


Fig. 4. Effect of the supplement of hydrolysates on growth of probiotic bacteria in yogurt. □ CK, ■ CH1 (0.5%), ◆ CH1 (1.5%), ▲ CH2 (0.5%), × CH2 (1.5%), + CH3 (0.5%), △ CH3 (1.5%). CH1, CH2 and CH3 are the same as in Fig. 1

ture is generally unsatisfactory (8,26) because the low survival of probiotic organisms in the fermented dairy products is the greatest obstacle encountered in processing, and especially during storage (27).

The counts of probiotic bacteria in yogurts decrease throughout storage. However, decline in the counts of probiotic organisms during storage can be retarded by hydrolysates (Fig. 5). After 30 days of storage, the yogurts with the hydrolysates have more probiotic bacteria than the control. The yogurt with 1.5 % of CH3 has about 3-fold more probiotic bacteria than the control. The hydrolysates significantly ($p < 0.05$) improve the viability. This is in accordance with the reports by other authors (8,19).

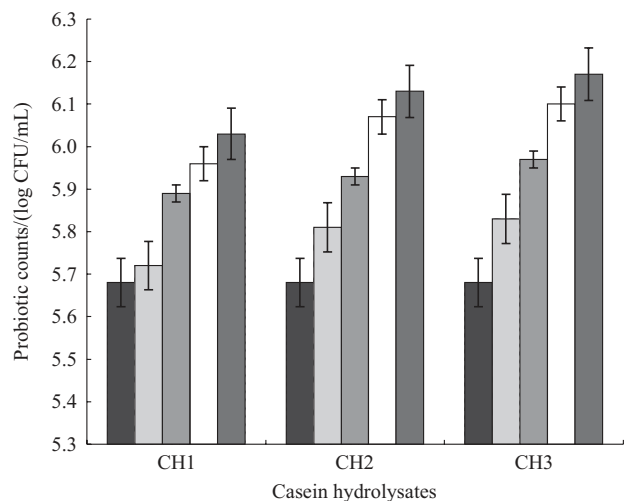


Fig. 5. Effect of the supplement of casein hydrolysates on viability of probiotic bacteria in yogurts after storage for 30 days at 4 °C. CH1, CH2, CH3 and the columns represent the same as in Fig. 1

Texture characteristics

Table 1 shows changes in texture properties of the yogurts determined by TA-XT2i texturometer. The treatments and control had no significant ($p < 0.05$) difference of the texture properties except for adhesiveness. The hydrolysates significantly ($p < 0.05$) increased the adhesiveness of the yogurts, except for CH1 (0.5 % sample). The adhesiveness had a positive effect on the thickness of the yogurts, and was an important factor governing the stability of the products. This resulted in the good

mouth-feel, improved the texture characteristics and stability of the yogurts during storage.

Sensory evaluation

Table 2 presents the sensory evaluation values of the yogurts. Average flavour scores of the yogurts with the hydrolysates were higher than the control during storage. There were different scores, but without significant difference ($p < 0.05$) after storage for 1 day. Storage time had a negative impact on the flavour scores, all flavour

Table 1. Effect of the supplement of casein hydrolysates on texture properties of yogurts*

	Hardness	Adhesiveness	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
CK	26.4±1.3c	69.6±4.8c	0.98±0.02b	0.52±0.01ac	13.6±1.1bd	13.4±0.1ab	0.04±0.005cd
CH1							
0.5 %	25.7±0.7ac	72.7±2.5cd	0.98±0.01ab	0.54±0.01bc	13.7±1.3ab	13.5±0.3be	0.04±0.002ac
1.5 %	25.9±0.2bc	76.3±2.0ab	0.96±0.01b	0.57±0.01ad	13.9±0.7ab	13.5±0.9ae	0.03±0.001bc
CH2							
0.5 %	25.4±0.1acd	75.5±1.8b	0.97±0bc	0.53±0ac	13.9±0.5ac	13.6±0.2ab	0.05±0.001ce
1.5 %	24.7±0.4ac	86.9±5.1e	0.98±0.02bd	0.58±0bc	14.2±0.2bc	13.8±0.6ac	0.04±0ac
CH3							
0.5 %	25.1±0.9cd	82.4±3.8ae	0.96±0b	0.57±0.002ad	13.9±0.4ab	13.5±0.6bd	0.03±0ad
1.5 %	23.6±0.7c	89.4±1.9ab	0.98±0b	0.58±0ab	13.9±1.4ad	13.7±1.0bc	0.05±0bc

*The values in this table are the means of a triplicate. Means followed by the same letter in the same column are not significantly different ($p < 0.05$) according to Duncan's multiple range test

Table 2. Effect of casein hydrolysates on the sensory evaluation values of yogurts during storage*

Samples	Storage time/day		
	1	7	14
Flavour values			
CK	7.84±0.9ac	5.72±0.3bd	4.38±0.1a
CH1-0.5 %	8.01±0.6ac	6.35±0.7ab	5.84±0.5ac
CH1-1.5 %	7.96±0.4ac	6.69±0.5ab	5.90±0.3c
CH2-0.5 %	8.05±1.0ab	7.83±0.1ce	6.31±0.8bd
CH2-1.5 %	8.12±0.7ac	7.91±0.5cf	6.57±0.2be
CH3-0.5 %	7.82±0.8bc	7.85±0.2ce	6.38±0.7b
CH3-1.5 %	8.19±1.1ad	7.83±0.4e	6.52±0.4bf
Body and texture values			
CK	4.07±0.6d	3.24±0.1cd	3.38±0.3bf
CH1-0.5 %	4.23±0.1ad	4.05±0.4ae	3.64±0b
CH1-1.5 %	4.28±0.3cd	4.12±0.1ab	4.07±0.6ad
CH2-0.5 %	4.25±0.4cde	4.09±0.5a	3.83±0.1bc
CH2-1.5 %	4.35±0.9ad	4.16±0.2af	4.11±0.4d
CH3-0.5 %	4.39±0.7b	4.25±0.3abe	4.08±0.5ad
CH3-1.5 %	4.41±0.1bf	4.18±0.1af	4.13±0.2ad
Colour and appearance values			
CK	4.10±0.7bc	3.28±0.1a	3.41±0.3ac
CH1-0.5 %	4.18±0.3bd	3.67±0.3bc	3.53±0.1bc
CH1-1.5 %	4.31±0.1ae	4.15±0.6ad	4.22±0.5a
CH2-0.5 %	4.29±0.6e	4.21±0.7ae	4.08±0.8ab
CH2-1.5 %	4.45±0.8bf	4.29±0.3ac	3.86±0.1d
CH3-0.5 %	4.43±0.4f	4.17±0.5df	4.04±0.3ad
CH3-1.5 %	4.68±0.5fg	4.42±0.7abf	4.37±0.6ab

*The values in this table are the means of a triplicate. Means followed by the same letter in the same column are not significantly different ($p < 0.05$) according to Duncan's multiple range test

scores significantly ($p < 0.05$) decreased throughout the storage. The most obvious drawback according to the panelists was high acidity and the other was rancid flavour for all samples at the end of the storage period. About 28 % of the panelists evaluated control, CH1 (0.5 %) and CH1 (1.5 %) as having more rancid flavour, while 31 % of them evaluated control and CH1 (0.5 %) samples as having higher acidity. The body and texture values of all samples decreased during storage. The body and texture values of the yogurts with and without CH1 and CH2 had no significant difference after storage of 1 day ($p < 0.05$). The yogurts with CH3 had significantly ($p < 0.05$) higher body and texture values than control and those with CH1 and CH2 after storage of 1 day. During the 7- and 14-day storage period, there was no obvious difference in the body and texture values between the control and CH1 (0.5 %). The yogurts with CH1 (1.5 %), CH2 and CH3 showed significantly ($p < 0.05$) different values of the body and texture at these two periods. The same trend as in the body and texture scores occurred in the appearance and colour scores when the amount of hydrolysate and storage time increased. About 20 % of the panelists observed a negative change in appearance because of the whey-off attributes of the control and CH1 (0.5 %) yogurts.

Conclusion

Casein hydrolysates significantly ($p < 0.05$) shorten the fermentation time of the yogurts and inhibit the post-fermentation acidification. The hydrolysates increased the probiotic counts during the initial stage of fermentation. However, the growth of the probiotic organisms decreased at the final period. The counts of probiotic bacteria in the yogurts decreased during storage. Moreover, the decline of the probiotic organism counts during storage could be retarded by the hydrolysates. The hydrolysates significantly ($p < 0.05$) increased the adhesiveness of the yogurts, except for CH1 (0.5 %) sample. The sensory evaluation scores of the yogurts were significantly improved by the hydrolysates after storage for 1, 7 and 14 days.

Acknowledgements

The authors thank the financial support of National Science Foundation of China (20276022) and the Doctorate Foundation of South China University of Technology.

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