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Original Article



Characteristics of Low-Fat White Soft Cheese Made with Different Ratios of *Bifidobacterium bifidum*

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Abstract

Low-fat cheese is considered a healthy dairy product that protects from the risks resulting from full-fat cheese, especially if it is made with probiotics, which have numerous health benefits. This study aimed to manufacture low-fat white soft cheese with different ratios of Bifidobacterium bifidum as a probiotic bacteria and study the chemical and the survival of this probiotic bacteria in the resultant cheese during 14 days of storage at 8 ± 2 °C. Pasteurized skim buffalo's milk was divided into six portions. The first portion of skim milk (control) was coagulated by adding 2% of Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus, while the other five portions of skim milk were converted into cheese by using the previous starter with the addition of 1, 2, 3, 4 and 5% of Bifidobacterium bifidum. Acidity, moisture, fat, salt and total protein (TP) contents were measured during storage of 14 days. Significant differences (p < 0.05) were recorded in the acidity, moisture, salt and TP contents of the resultant cheese between treatments, and during the storage period. Regarding the Bifidobacterium count in the examined treatments, proportional relationship could be established between the numbers detected in the examined treatment and the levels used of Bifidobacteria till 4%, which resulted in an increased numbers. However, by increasing the ratio up to 5%, resulted in negative effect by increasing the acidity which inhibits its activity and reduces its count.

Keywords: Probiotics; Bifidobacterium bifidum; Low-fat cheese; Chemical properties

Introduction

Full- and low-fat cheese are the main types of cheese based on their fat content (Hammam and Ahmed, 2019a). Due to its nutritious value (high protein and fewer fat or calories), low-fat white soft cheese is one of the most popular forms of cheese in Egypt and the Arab world. The incorporation of probiotic bacteria into this type of cheese is an important trend to get a healthier cheese. Keeping in mind that the bacteria must be able to survive while passing through the gastrointestinal tract, which includes exposure to harsh conditions (such as

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hydrochloric acid in the stomach and bile in the small intestine) and reaching sufficient amounts in the stomach to provide health benefits to the human body (Ross et al., 2003). Cheese offers a valuable career for the delivery of probiotics compared to fermented milk and yogurt, due to certain potential benefits (such as working as a buffer in acidic conditions against the very acidic environment of the gastrointestinal tract), which creates a more favorable environment for probiotic survival. Furthermore, the dense matrix of the cheese, as well as its fat content, can protect probiotic bacteria until it reaches the stomach (Ross et al., 2002; Bergamini et al., 2005 and Hammam and Ahmed, 2019b). Karish, Cheddar, Gouda, Ras, Cottage, white and fresh cheeses, and others have all been made with probiotic bacteria by many researchers (Dinakar and Mistry, 1994; Roy et al., 1997; Gardiner et al., 1998; Murad et al., 1998; Vinderola et al., 2000; Mc Brearty et al., 2001; Kasımoğlu et al., 2004; Buriti et al., 2005 and Hammam et al., 2018). Strains of probiotics should be carefully selected based on the type of cheese and production conditions (Gomes da Cruz et al., 2009 and Hammam and Ahmed, 2019b).

The addition of probiotic bacteria to low-fat cheese could affect the chemical properties (e.g., % acidity) of cheese. It has been reported that the addition of *Bifidobacterium bifidum* DI and *Lactobacillus rhamnosus* GR affected the chemical and physiological properties of Karish cheese, including acidity, soluble nitrogen, total protein, moisture content, texture and flavor (Mahmoud *et al.*, 2013). The moisture content and pH values were decreased during the storage period, while the acidity and soluble nitrogen contents increased.

The present study was aimed to manufacture low-fat white soft cheese with different ratios of *Bifidobacterium bifidum* and study the chemical properties and the *Bifidobacterium* count of this cheeses during 14 days of storage.

Materials and Methods

Making of low-fat white soft cheese

The low-fat white soft cheese (LFWSC) was made from buffalo's skim milk. Fresh buffalo milk (24 liter), obtained from the herd of the Faculty of Agriculture, Assiut University, Assiut, Egypt) and separated at 20°C. The heating process was carried out at 73°C for 15 seconds followed by cooling at 40°C. Skim milk was divided into six portions, 3% of sodium chloride was added, and the starter cultures were then added. First portion was considered as control in which skim milk was incubated with 2% mix of starter (1:1) *S. thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Cairo Microbiological Resources Center, Faculty of Agriculture, Ain Shams University, Cairo, Egypt) was added into the first portion of skim milk without adding rennet. the second portion (T1): skim milk was incubated with 2% mix of starter (1:1) *S. thermophiles*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and 1% of *Bifidobacterium bifidum*. The third portion (T2): skim milk was incubated with 2% mix of starter (1:1) *S. thermophiles* and *Lactobacillus delbrueckii* subsp. *bulgaricus* and 2% *thermophiles* and *Lactobacillus delbrueckii* subsp. *bulgaricus* and 2%

Bifidobacterium bifidum. The fourth portion (T3): inoculated with a 2% mix of starter (1:1) S. thermophilus and Lactobacillus delbrueckii subsp. bulgaricus and 3% of Bifidobacterium bifidum. The fifth portion (T4): skim milk was incubated with a 2% mix of starter (1:1) S. thermophilus, Lactobacillus delbrueckii subsp. bulgaricus and 4% Bifidobacterium bifidum. the sixth portion (T5): skim milk was incubated with a 2% mix of starter (1:1) S. thermophilus, Lactobacillus delbrueckii subsp. bulgaricus and 5% of Bifidobacterium bifidum. The Starter cultures used in the experiment were obtained from the Cairo Microbiological Resources Center (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

The inoculated milk was left for 3 hours at 40°C until the coagulation was complete. Then the cheese was cut and packed in cheese cloth and left to dry for 24 hours at a suitable temperature at 5°C. The cheese was taken from the cheese cloth, cut into cubes, and stored at 8 ± 2 °C in clean glass containers. The cheese samples were examined when fresh and after 3, 7, 10 and 14 days of storage.

Chemical analysis

The % acidity, moisture, total protein, and salt content were estimated. Titratable acidity was measured by using sodium hydroxide 0.1 N and phenolphthalein as an indicator (Hooi *et al.*, 2004). The obtained results were calculated as the percentage of lactic acid. Fat content was determined by using Gerber method of Hooi *et al.* (2004). Total protein (TP) was determined by using the Kjeldahl method (Hooi *et al.*, 2004), by digesting and titrating the cheese samples to estimate the nitrogen content, and by multiplying the resultant concentration of nitrogen by 6.38 to obtain the protein content. The moisture content of LFWSC made with probiotics was determined by evaporating the moisture at 103-104°C/6 h until the weight was stable (Hooi *et al.*, 2004) using a forced-air oven and the differences in the weight referred to the moisture content. The salt content of cheese was determined by using Mohr method (Hooi *et al.*, 2004). Total solids (TS) in cheese samples were calculated as follow: %TS= 100 - % moisture.

Preparation of starter cultures

Skim milk medium was prepared according to Harrigan (1998). Skim milk powder was reconstituted to 12% total solids in distilled water and sterilized at 121°C for 10 min, subsequently cooled to the incubation temperature, then the starter cultures were inoculated at level of 2%. The incubation temperature was 37°C for 16 hours.

Count of Bifidobacterium

Bifidobacterium count was enumerated in suitable dilutions (10⁴-10⁵) in duplicates using MRS-modified agar medium by using pouring plate method. The plates were anaerobically incubated at 37°C for 48 h. The small and white colonies were counted as CFU. MRS-modified agar medium was prepared as described in Brewer (1940).

Statistical analysis

The impact of treatments and storage on the chemical characteristics of LFWSC was studied by the statistical analysis. An ANOVA was done to obtain the mean squares and P-values using the GLM procedure available by R software (R x64 3.3.3 using R studio). Differences were tested using the least significant difference (LSD) comparison test when a significant difference (P < 0.05) was detected between treatments, time, or their interaction.

Results and Discussions

Acidity

Results in Table 1 illustrate the acidity (%) of the LFWSC made with *Bifidobacterium bifidum*. The lowest acidity (%) was detected in the control sample, compared to other cheese treatments. An increase from 0.33% to 0.68% was detected during 14 days of storage at 4°C. However, the acidity of T5 was the highest, compared to others, which increased from 0.78% to 1.28% during the storage period. Slight variations of the acidity were found in T1, T2, T3, and T4, when fresh ranged from 0.58 to 0.66% and ranged from 0.90 to 1.0% after 14 days of ripening. Significant differences (p < 0.05) were found in the acidity between treatments and between the storage periods. Nevertheless, there was no significant difference (p > 0.05) in the interaction between treatments and the ripening time.

Table 1. Mean acidity (%) of the LFWSC during 14 days of storage.

Composition	Twootmont*		Maan				
Composition	Treatment [*]	Fresh	3	7	10	14	Mean
	Control	0.33	0.35	0.50	0.58	0.68	0.49^{d}
	T1	0.58	0.60	0.73	0.81	1.11	0.77^{c}
	T2	0.59	0.59	0.75	0.94	1.12	0.80^{bc}
Acidity (%)	T3	0.64	0.73	0.82	0.94	1.17	0.86^{b}
	T4	0.66	0.76	0.86	0.93	1.07	0.86^{b}
	T5	0.78	0.84	0.97	1.14	1.28	1.00^{a}
	Mean	0.60^{E}	0.65^{D}	$0.78^{\rm C}$	0.89^{B}	1.07^{A}	

*Control= skim milk incubated with 2% *S. thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, T1= as control+1 % of *B. bifidum*, T2= as control+2 % of *B. bifidum*; T3= as control+3 % of *B. bifidum*; T4= as control+4% of *B. bifidum*; T5= as control+5% of *B. bifidum*

Means within the same columns and rows with different subscriptions are significantly different ($P \le 0.05$).

As was expected, the control of low-fat white soft cheese characterized with low acidity (Figure 1), compared to other treatments during the storage period. This could be due to the lowest level of the starter free from Bifidobacterium culture used in making it, After 14 days of storage, T5 with 2% (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus*) and 5% Bifidobacterium starter resulted in the highest acidic values.

It could also be observed the highest acidity in Karish cheese made with starter cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Hussein and Shalaby, 2014). It could Mahmoud *et al.*, 2013 acidity, as it well be known that *Bifidobacterium* produces slight levels of acidity (Blanchette *et al.*, 1996 and Hamdy *et al.*, 2021). These results came in harmony with those obtained by, who found that *Bifidobacteria* produced levels of acidity in white brined cheese during the storage period. Similar results by could also be observed by Oliveira *et al.*, 2012; Mahmoud *et al.*, 2013 and Yerlikaya and Ozer, 2014. Approximately 1% acidity was detected in Karish cheese made with probiotic bacteria, Mahmoud *et al.*, 2013. An increase in the acidity from 0.5% to approximately 1% after 14 days of storage was observed. The acidity content also increased from 0.5% to approximately 1% after 14 days of storage of Brazilian semi-hard goat cheese made with probiotic bacteria (Oliveira *et al.*, 2012), which is similar to the present results.

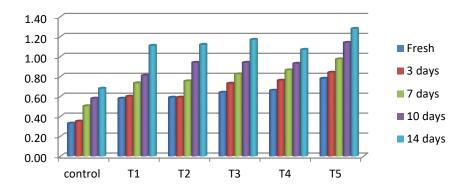


Figure 1. The acidity values of LFWSC during 14 days of storage.

Moisture

Data presented in Table 2 show the moisture content (%) of the LFWSC made with *Bifidobacterium bifidum*. The highest content of moisture was 83 and decreased to 82% to 77.05% during the 2 weeks of storage period. The moisture content of T1, T2, T3, T4, and T5 ranged from 82.41% to 80.15% when fresh, and ranged from 77.03% to 69.88% after 14 days of ripening. There was a significant difference (p < 0.05) in the moisture content between the examined treatments and between the ripening period of probiotic white soft cheese. The detected loss of the moisture content in the form of whey was observed in all treatments as the pickling period proceeds, which could be attributed to the curd shrinkage as a reaction of the development of acidity during the pickling period, which helps to eject the whey from the curd. Similar results were reported by Kebary and Youssef (2015).

Table 2. Mean mo	oisture (%)	of the LFWS	SC during 14	days of storage.

Commonition	Tuestment*		Maan				
Composition	Treatment*	Fresh	3	7	10	14	- Mean
	Control	83.82	81.98	80.30	79.21	78.05	80.67 ^a
	T1	82.41	81.37	80.40	79.15	77.03	80.07^{b}
	T2	82.31	81.25	80.05	78.49	76.14	79.65°
Moisture (%)	T3	82.25	81.01	80.02	78.11	76.02	79.48°
	T4	82.21	80.27	78.71	77.51	75.72	78.88^{d}
	T5	80.15	76.46	74.44	73.45	69.88	74.88 ^e
	Mean	82.19^{A}	80.39^{B}	78.99 ^C	77.65^{D}	75.47^{E}	

^{*} To clarify the treatments, see Table 1. Means within the same columns and rows with different subscriptions are significantly different ($P \le 0.05$).

The highest moisture content was found cheese samples of control (Figure 2), which might be due to its lowest acidity, compared to other treatments. On the other hand, the samples of T5 had the highest moisture loss, which might be due to increasing of the dose of the *Bifidobacteria* culture percentage to 5%, which results in high acidity levels in T5. With increasing acidity, the moisture content in cheese samples decreases during the storage period (Hammam *et al.*, 2018). Close moisture contents of T1, T2, T3, and T4 were observed during the storage period. Similar results were found by Korish and Abd Elhamid (2012), who reported that using of different bacterial strains resulted in a minor changes in the moisture content of Karish cheese. Also, Mahmoud *et al.* (2013) reported that the moisture content of Karish cheese made with probiotic bacteria was 74.0% after 14 days of storage, which is similar to our study.

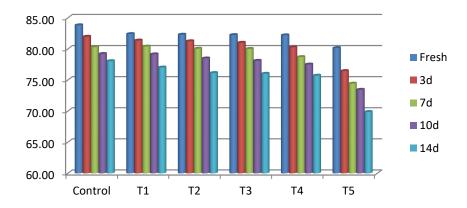


Figure 2. The moisture % of LFWSC during 14 days of storage.

Salt content %

Result presented in Table 3 illustrate the salt content (%) of the LFWSC made with *Bifidobacterium bifidum*. The salt % of control cheese, T1, T2, T3, T4, and T5 ranged from 2.71% to 2.91%, when fresh, and ranged from 3.19% to 3.57% after 2 weeks of ripping. There was a significant difference (p < 0.05) in the salt content between treatments, which might be due to differences in water loss during storage, resulting in differences in salt content in the treatments.. A

significant difference (p < 0.05) was also found during the ripening of the white soft cheese.

Table 3.	Mean sal	t of the	e LFWSC	during	14 days	s of storage

Composition	Treatment*		Mean				
Composition	Treatment	Fresh	3	7	10	14	Miean
	Control	2.91	2.92	3.01	3.11	3.21	3.03 ^b
	T1	2.71	2.82	3.05	3.14	3.19	2.98^{c}
	T2	2.89	2.92	3.30	3.51	3.57	3.24^{a}
Salt (%)	T3	2.90	2.91	3.08	3.28	3.45	3.12a ^b
()	T4	2.81	2.89	3.07	3.16	3.27	3.04^{b}
	T5	2.81	2.71	3.24	3.45	3.48	3.14a ^b
	Mean	2.84 ^C	2.86 ^C	3.13 ^B	3.28 ^{AB}	3.36 ^A	

^{*} To clarify the treatments, see Table 1. Means within the same columns and rows with different subscriptions are significantly different ($P \le 0.05$).

Nevertheless, there was no significant difference (p > 0.05) in the interaction between treatments during 14 d of ripening on the salt content of *Bifidobacterium bifidum* in white soft cheese. It was expected that there is a small increase (Figure 3) in the salt content of cheeses during the storage period due to the loss of some moisture at different stages. It could also be reported an increase in the salt content in hard and soft cheese by Ahmed *et al.*, (2005); Hammam *et al.*, (2018) and Moneeb and El-Derwy (2021) during storage.

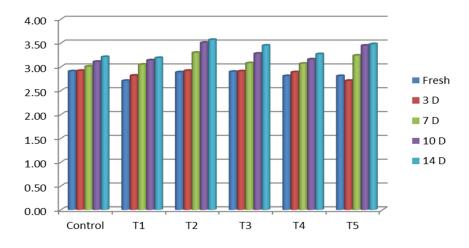


Figure 3. The salt content of the LFWSC during 14 days of storage

Total protein

Results in Table (4) show the TP values of LFWSC made with *Bifidobacterium bifidum*. The highest TP content was found in T5, which increased from 17.61 % to 21.26 % during the 14-day of storage; however, the lowest TP% was detected in T1, which increased from 15.90 to 17.96% throughout the 14 day storage period. On the other hand, similar results in the

TP% in T1, T2, T3, and control treatments, when fresh (ranged from 15.65% to 17.61) and after 14 days of ripening (ranged from 17.96% to 21.26%).

Table 4. I	Mean total	protein (%]) of the l	LFWSC	during	14 d	lays of	storage

Composition	Treatment*		Mean				
	1 reatment	Fresh	3	7	10	14	
	Control	15.96	17.17	17.74	18.26	18.85	17.59 ^{bc}
	T1	15.90	16.63	17.41	17.20	17.96	17.02^{c}
Total mustain	T2	16.77	17.30	18.23	18.39	19.70	18.08^{bc}
Total protein (%)	T3	16.71	17.32	18.08	19.60	19.74	18.29 ^{ab}
(70)	T4	15.65	17.41	16.82	19.84	19.37	17.82 ^{bc}
	T5	17.61	18.11	18.95	20.43	21.26	19.27^{a}
	Mean	16.44 ^C	17.45 ^{BC}	17.74 ^B	18.95 ^A	19.48 ^A	

^{*}To clarify the treatments, see Table 1. Means within the same columns and rows with different subscriptions are significantly different ($P \le 0.05$).

There was a significant difference in TP between treatments (p < 0.05). Also, During the cheese storage period, a significant differences (p < 0.05) was discovered. Nevertheless, there was no significant difference (p > 0.05) in the interaction between treatments and the ripening time of probiotic white soft cheese. We found an increase (Figure 4) in the TP Due to moisture loss during storage period and thereby, increasing the TS (Effat *et al.*, 2001).

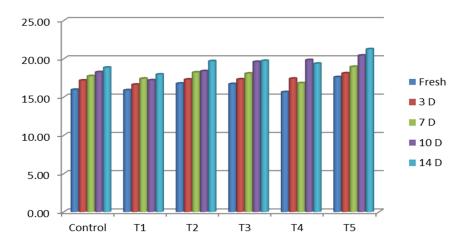


Figure 4. The total protein (TP) content of the LFWSC during 14 days of storage

Fat

Data presented in Table (5) illustrate fat (%) of LFWSC made with *Bifidobacterium bifidum*. The lowest fat content was observed in control, and increased from 2.69% to 3.19% during the storage period at 4°C. Whereas the highest was detected in T5, which increased from 3.06% to 3.53% after the 14 days of ripening. T1, T2, T3, and T4 were nearly similar in the fat when fresh, which ranged from 2.75 % to 3.03%, and after 14 days of ripening, which ranged from 3.30% to 3.33%).

tuble 3. Weath 1 at (70) of the E1 West during 11 days of storage										
Composition	Tuestment*		Storage period (days)							
	Treatment [*]	Fresh	3	7	10	14	Mean			
	Control	2.69	2.72	2.83	2.99	3.19	2.88 ^d			
	T1	2.75	2.89	2.94	3.13	3.30	$3.00^{\rm c}$			
	T2	2.88	2.93	3.03	3.13	3.27	3.05^{c}			
Fat (%)	T3	3.03	3.06	3.00	3.24	3.31	3.13^{b}			
()	T4	2.96	3.02	3.16	3.30	3.33	3.15^{b}			
	T5	3.06	3.17	3.47	3.43	3.53	3.33^{a}			
	Mean	2.90^{D}	$2.97^{\rm C}$	$3.07^{\rm C}$	3.20^{B}	3.32^{A}				

Table 5. Mean Fat (%) of the LFWSC during 14 days of storage

There was a significant difference (p < 0.05) in the fat content between treatments. A significant difference (p < 0.05) was also found during the ripening period of probiotic white soft cheese. Nevertheless, there was no significant difference (p > 0.05) in the interaction between treatments and 14 d of ripening time of the white soft cheese. An increase was found (Figure 5) in the fat content due to moisture loss during storage period; as a result, increasing the fat with increasing the solids (Mahmoud *et al.*, 2013).

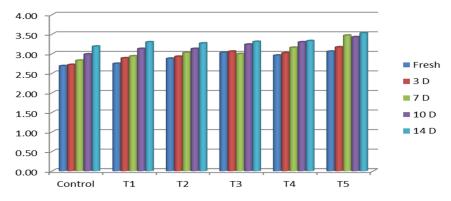


Figure 5. The fat content of the LFWSC during 14 days of storage.

Bifidobacteria count

Data illustrated in Table 6 show the viable growth of *Bifidobacterium bifidum*. No colonies of *Bifidobacterium bifidum* were detected in the control, because the starter used in the cheese manufacture is free from *Bifidobacteria* culture. The highest *Bifidobacterium bifidum* counts was detected in T4, as compared to other treatments during 14 days of storage (7.53 when fresh and 7.96 at the end of storage period), which may be due to using the high dose of 4% of *Bifidobacteria*. Increased numbers of *Bifidobacterium* in the examined treatments was expected by increasing the Bifidobacterium concentration in the used starter (Ahmed *et al.*, 2021), However, decreased numbers of the tested bacteria was observed in T5, which could be due to high acidity levels in this treatment. Bifidobacteria showed low resistance under extended acidic conditions

^{*} To clarify the treatments, see Table 1. Means within the same columns and rows with different subscriptions are significantly different ($P \le 0.05$).

(Hammam and Ahmed, 2019b). These results came in the same trend with those obtained by (Vinderola *et al.*, 2000), who reported that using *Bifidobacterium bifidum* in the manufacture of Fresco cheese (soft cheese) led to increasing the number of *Bifidobacterium bifidum* from 5.9–7.3 log cfu/g at the beginning of the storage period to 6.4–7.6 log cfu/g after 9 weeks of storage period. By the end of ripening, the highest total *Bifidobacteria* count was 7.96 log cfu/g for T4. This number of *Bifidobacteria* is enough to provide its health benefits (Hammam and Ahmed 2019b).

Table 6. Mean (n= 3) total Bifidobacterium count of the LFWSC during 14 days of

	Treatment*		Maan				
	i reatment	Fresh	3	7	10	14	Mean
	Control	ND	ND	ND	ND	ND	ND
	T1	5.3	5.78	5.91	6.12	6.25	5.872
Bifidobacterium	T2	5.59	5.89	6.09	6.29	6.47	6.066
count	Т3	6.35	6.69	6.87	6.98	7.05	6.788
(log cfu/g)	T4	7.53	7.91	7.92	8.12	7.96	7.888
	T5	7.21	6.35	6.68	6.54	6.11	6.578
	Mean	6.396	6.524	6.694	6.81	6.768	

To clarify the treatments, see Table 1

Conclusion

The low-fat probiotic white soft cheese was made from skim milk by using different ratios of *Bifidobacterium bifidum*. A significant differences (p< 0.05) were found in the acidity, moisture, salt, TP, and ash contents between treatments and during 14 days of storage period. The high acidity in T5 could be due to the high activity of started cultures (p< 0.05) compared to other treatments, while control treatment presented lower moisture content (p< 0.05) compared to other cheeses. For *Bifidobacteria* count it was found that using ratios of *Bifidobacteria* till 4% give positive effect in increasing *Bifidobacteria* count but by increasing ratio to 5% it led to increasing acidity which had a negative effect on *Bifidobacteria* activity. In general, probiotic bacteria could impact on the chemical characteristics of LFWSC. Also, the use of 4% of *Bifidobacteria* starter is enough to provide its health benefits.

Conflicts of Interest: The authors declare no competing financial interests

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خصائص الجبن الأبيض الطري منخفض الدهن المصنع باستخدام نسب مختلفة من Bifidobacterium bifidum

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الملخص

تعتبر الجبن منخفض الدهن منتجات الألبان الصحية التي تحمي من المخاطر الناتجة عن الجبن كامل الدهن ؛ خاصة إذا كانت مصنوعة باستخدام البكتريا الداعمة للحيوية، والتي لها فوائد صحية عديدة. تهدف هذه الدراسة إلى تصنيع جبن أبيض طري منخفض الدهن بإضافة نسب مختلفة من بكتريا Bifidobacterium bifidum كبكتريا داعمة حيوية ودراسة التركيب الكيميائي ومدى بقاء هذه البكتريا في الجبن خلال ٤ أيومًا من التخزين. تم تقسيم اللبن الجاموس الفرز المبستر إلى ستة أجزاء. تم تجبين الجزء الأول من الحليب الخالي من الدهن (المجموعة الكنترول). بإضافة ٢٪ من Streptococcus thermophilus و Streptococcus اللبن الفرز المنتخدام بادئ الكنترول و Bifidobacterium bifidum با و ٢ و ٣ و ٤ و ٥٪. تم تقدير الحموضة والرطوبة والبروتين الكلي (TP). ومحتوى الدهون خلال ١٤ يومًا من التخزين. كانت هناك فروق معنوية في محتوى الحموضة والرطوبة والملح و البروتين للجبن الأبيض الطري منخفض الدهن بين المعاملات وخلال ١٤ يوم من فترة التخزين. بالنسبة لعدد النبيض الطري منخفض الدهن بين المعاملات وخلال ١٤ يوم من فترة التخزين. بالنسبة لعدد النسبة إلى ٥٪ أدى إلى تأثير سلبي عن طريق زيادة الحموضة مما يثبط نشاطها.