Viability of commercial probiotic cultures in cottage cheese containing black cumin seed

Article *in* Journal of Food Measurement and Characterization · March 2018

DOI: 10.1007/s11694-018-9780-x

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Journal of Food Measurement and Characterization
https://doi.org/10.1007/s11694-018-9780-x

ORIGINAL PAPER

Viability of commercial probiotic cultures in cottage cheese containing black cumin seed

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Received: 16 July 2017 / Accepted: 14 March 2018

Abstract
In this study, the effect of black cumin seeds (*Nigella sativa*) was investigated on the viability of commercial probiotic cultures added to cottage cheese. Physicochemical and sensory properties of the probiotic cottage cheeses were also studied. The probiotic cottage cheeses were produced by incorporating of *Bifidobacterium animalis* or *Lactobacillus casei* and different levels of the black cumin seeds (0, 1, and 2%) and then stored at refrigerated temperature for 25 days. The population of the probiotic strains added to the flavored cottage cheeses was slightly increased during storage compared with the control cheeses. Enumeration results showed that the counts of *Lactobacillus casei* were higher than those of *B. animalis* during storage. Some of physicochemical properties of the samples changed by addition of the black cumin seeds, as significantly. Addition of black cumin seeds had a significant difference between flavored and control cheeses. Our results indicated that by addition of black cumin seed can produce a probiotic cottage cheese with improved taste and acceptability.

Keywords Black cumin seed · Cottage cheese · Probiotic cultures · Sensory evaluation · Viability

Introduction

Functional foods are one of the most exciting advances in the food industries that nowadays, have been strongly extended [1]. In general functional foods are defined as, foods that provide health benefit beyond normal nutrition [2]. Probiotic foods are one of the most important functional foods that the consumption of them is growing rapidly due to increased awareness of consumers. Probiotics are explained as live microorganisms when administered in sufficient amounts confer a health benefit on the host [3].

Cheese is one of the most versatile food products that consumed by everyone. Cheese seems to be a good carrier for probiotic delivery, due to creating a buffer against the high acidic medium in the gastrointestinal tract [4]. In the scientific literatures, populations of 10^6–10^7 CFU g^{-1} in the final product are required to be recognized as a probiotic food [5]. Cottage cheese is an unripened, soft, granular and slightly acidic cheese that produced by renting of pasteurized skim milk [6]. These curds can be optionally blended with a cream dressing with or without different flavoring agents. This dressing provides additional flavor and texture to the final product. The range of pH in cottage cheese is between 4.6 and 5.1 [5].

Overconsumption of high fat foods has been related to increasing human diseases, for instance obesity, coronary heart disease, etc. [7]. Besides that, the nutritional values, acceptability and sensory properties of cheese affect by reducing fat content. Hence, many researches around the world have been carried out to improve the quality of low-fat cheeses [8]. Cottage cheese is one of them and has been attempt to improve its taste, functionality and shelf life [6, 9, 10]. One of these attempts is using spices and plant extracts for taste improving of low-fat cheeses, e.g. cottage cheese [11, 12].

Spices are applied in many foods such as some cheeses to impart taste and health benefits [11]. Spices are also used as an antimicrobial and antioxidant agent in foods and beverages to extend the shelf life of products. Black cumin seed is one of them and almost used in coffee, tea, salads and breads as

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a flavoring agent [9]. The seed has a strong and hot peppery taste and is used as a natural remedy for some diseases such as asthma, hypertension and diabetes [9].

The objective of this work was to develop a probiotic cottage cheese containing black cumin seeds and *Lactobacillus casei* or *Bifidobacterium animalis (bb12)* and determine the survival of the probiotic strains in the cheeses during shelf life.

Materials and methods

Chemicals

Fresh skim milk and cream (approximately 40% fat) were obtained from Kalleh Dairy Co. (Amol, Iran). Probiotic cultures [*Lactobacillus casei* and *Bifidobacterium animalis* subsp. *lactis (BB12)*] were purchased from Chr-Hansen Co. (Horsholm, Denmark) in freeze-dried form. Other laboratory chemicals were purchased from Merck Company, Germany.

Production of probiotic cottage cheeses

Preparation of curds

Cottage cheese was produced according to the modified method of Blanchette et al. [13]. Pasteurised skim milk (100 kg) was fermented with mesophilic lactic bacteria R 704 (Chr-Hansen Co., Horsholm, Denmark) at 25 °C. The pH was monitored until 4.7 and after formation of the curds, the mass was cut into small cubes (approximately 1 cm^3). Following that, the curds were stewed (until 55 °C over a period of 1.5 h) and washed three times using cold water (5 °C). Finally, the cheese curds were mixed with different dressings.

Fermented dressing preparation

Calculated quantity of cream and skim milk were mixed to prepare cream dressing having 17% fat. The homogenized (150 bar) and pasteurized (75 °C for 15 s) cream was poured into sanitized containers and inoculated with *L. casei* or *BB12* at 32 and 37 °C, respectively. The end of fermentation was recognized by measuring of the pH (pH $4.8 \approx 10^7$ CFU g^{-1}).

Unfermented dressing preparation

The pasteurized and homogenized cream (produced in the previous stage) was mixed with different levels of black cumin seeds, potassium sorbate and salt (sodium chloride) and then pasteurized at 80 °C for 30 s into closed glasses. Finally, the probiotic cottage cheeses were produced by mixing of the curds, fermented dressing and unfermented dressing in the ratio of 70:20:10, respectively, and eventually were packed under hygienic conditions and stored at 5 °C for 25 days. The percentage of the salt and potassium sorbate in all of the cheeses was 0.5 and 0.1, respectively. Hereafter, the various probiotic cottage cheeses will be denoted in relation to type of culture [*Bifidobacterium (BB)* or *L. casei (LC)*] and the percentage of black cumin seed (C) in the final product; for example, the one produced with *Bifidobacterium* and 2% black cumin seed is termed BBC_2.

Enumeration of probiotic bacteria

The number of probiotic bacteria in the cheese samples enumerated at day 1, 8, 15 and 25. Ten grams of the cottage cheese samples were diluted in 90 g of water (containing 0.1% peptone) and homogenized by a stomacher 400 laboratory blender in the aseptic conditions. *L. casei* and *BB12* were counted according to the method of Abadía-García et al. [5] and Jesus et al. [14], respectively. Man, Rogosa and Sharpe-vancomycin (MRS-V) agar was prepared by addition of 5 mL of 0.1% (w/v) vancomycin solution to molten MRS agar to obtain 5 mg L^{-1} of final concentration. The counts of *Bifidobacterium animalis* subsp. *lactis (BB12)* were estimated using MRS agar with the addition of 100 mL of a 20% (w/v) glucose solution, 5 mL of a 0.01% (w/v) dicloxacillin solution (Sigma-Aldrich), 10 mL of a 11.11% (w/v) lithium chloride solution and 5 mL of a 10% (w/v) cysteine chloride solution. Finally, the inoculated plates were incubated in anaerobiosis jars at 37 °C ± 1 °C for 72 h.

Physicochemical assessments

The total solid content of the samples was carried out by oven drying method to constant weight. Determination of pH was measured as per the standard method [12]. Cottage cheese samples were firstly homogenized in water with a mixer (1:9 ratio) and then the pH of these samples was read using a digital pH meter (Mettler-Toledo, Switzerland). Protein and fat content were measured in duplicate using Kjeldahl and the Babcock method, respectively. NaCl content was also measured by Mohr method.

Viscosity measurements

Viscosity analysis was performed using a controlled stress rate rheometer (Anton Paar, RheolabQC, Austria) at 5 °C and 50 rpm. All tests were carried out in triplicate and the means are used.

Sensory evaluation

Sensory evaluation of probiotic cottage cheeses was carried out after 3 days of storage with 6 trained panelists to compare flavored and control cottage cheeses. It was performed an acceptance test using a nine-point hedonic scale with scores was anchored between one (extremely disliked) to nine (extremely liked). Evaluated features were included taste, overall acceptability, and texture as well as color and appearance.

Statistical analysis

Statistical analysis was conducted with SPSS 13 (SPSS Inc., Chicago, Illinois, USA) software. The analysis of variance and Duncan's mean comparison test was applied with a significance level of 0.05.

Results and discussion

The mean values of pH, total solids, fat, protein, and chloride content in the probiotic cottage cheeses during storage at 5 °C are shown in Table 1. The results showed that there were no significant differences ($p > 0.05$) among the two groups of probiotic cheeses (LCC and BCC) with respect to their total solid, fat, NaCl and protein contents. There also was a significant difference ($p < 0.05$) among the samples containing or without cumin in each group with respect to their total solids; this can be due to the addition of cumin seeds. Most of the obtained results in the current study are similar to those reported by Blanchette et al. [13] when *Bifidobacterium* was added to cottage cheese.

Figure 1 shows changes in the pH of the cheese samples during storage. As can be seen, the pH of all the samples was decreased during storage, irrespective of the culture type or cumin seed levels. Probiotic cottage cheeses without any black cumin seed (control cheeses) presented the minimum changes in the pH. The results also showed that as the black cumin seed contents increased, the pH dropping of the samples increased. During the storage, significant differences were observed between flavored and unflavored probiotic cheeses ($p < 0.05$). Over the 25 day storage period, the pH dropped from 5.1 to 4 in the probiotic cheeses containing *BB12*, whereas in the other ones decreased to 3.9. This reduction in the pH can be attributed to the growth of the probiotic culture and production of different organic acids or metabolic activity of microbiological contamination [12, 15, 16]. These results are compatible with the finding of the viable counts that presented in Fig. 2. It also can be observed from Fig. 1 that as the storage time increased, the pH dropping of the samples partially decreased; this may be due to that the reduction in pH is an inhibitory factor for the growth and survival of the probiotic bacteria [14].

Figure 2 shows the viable counts of the probiotic cultures during storage. As can be seen, the counts of *Lactobacillus casei* were higher than those of *BB12* during shelf life. *Bifidobacterium animalis* is more sensitive to the stress factors such as pH, oxygen, and temperature rather than *Lactobacillus casei* [1] and as can be observed in Fig. 2, it grew slower than *L. casei* in the refrigerated temperatures. The slope of the viability curves decreased during the storage, this can be due to the importance of the pH on the stability of probiotic cultures. High losses in the viability of LCC2 after 15 days of storage might partially be because of the more diminution of nutritional matters resulted in a fast growth of *L. casei*. It is interesting that the addition of black cumin seed (level of 2%) slightly increased the population of *BB12* and *L. casei*. These results may be due to that the black cumin seed can effect on the counts of different microorganisms as selective. Some researchers have reported that black cumin seeds have a negative effect on the growth of many microorganisms in low concentrations, but in these levels, the cumin seeds have no effect on the growth of lactic acid bacteria [17–19]. Therefore, the growth of LAB may increase during shelf life, resulting in the reduction of antagonistic effects among LAB and other bacteria. Similar results were reported by Arici et al. [19]; they reported that black cumin seed oil had no effect on *Lactobacillus casei* in low concentrations but it had an adverse effect on the other microorganisms.

Figure 3 shows the sensory evaluation of the different probiotic cottage cheeses. As can be observed, the overall acceptability of the probiotic cottage cheeses produced

Table 1 Physiochemical characteristics of different probiotic cottage cheeses

Components	Probiotic cottage cheese					
	LCC_0	LCC_1	LCC_2	BBC_0	BBC_1	BBC_2
Fat (%)	5.0^a	4.9^a	5.0^a	4.9^a	4.8^a	5.1^a
TS (%)	20.7^b	21.5^{ab}	22.2^b	21.4^b	21.6^{ab}	22.8^a
pH	5.18^a	5.15^a	5.12^a	5.15^a	5.17^a	5.09^a
Protein	12.8a	12.9^a	13.8^a	13.2^a	13.0^a	13.9^a
NaCl	0.71^a	0.73^a	0.76^a	0.74^a	0.74^a	0.75^a

In each row, mean values bearing similar superscript letters are not significantly different at 5% probability

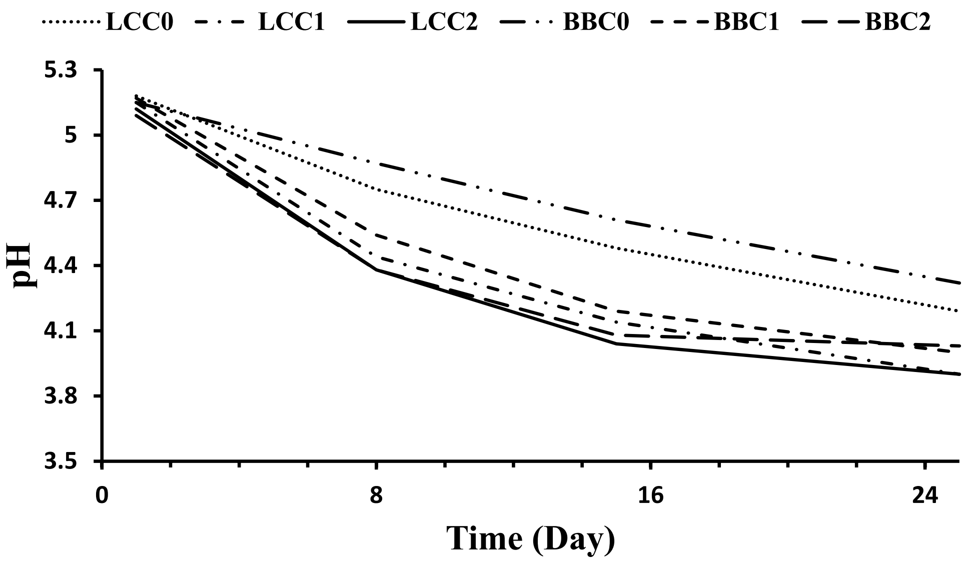

Fig. 1 Changes in the pH of the cheese samples during storage. LCC_0 (cottage cheese containing *L. casei* without any cumin), LCC_1 (cottage cheese containing *L. casei* and 1% cumin), LCC_2 (cottage cheese containing *L. casei* and 2% cumin), BBC_0 (cottage cheese containing *BB12* without any cumin), BBC_1 (cottage cheese containing *BB12* and 1% cumin), BBC_2 (cottage cheese containing *BB12* and 2% cumin)

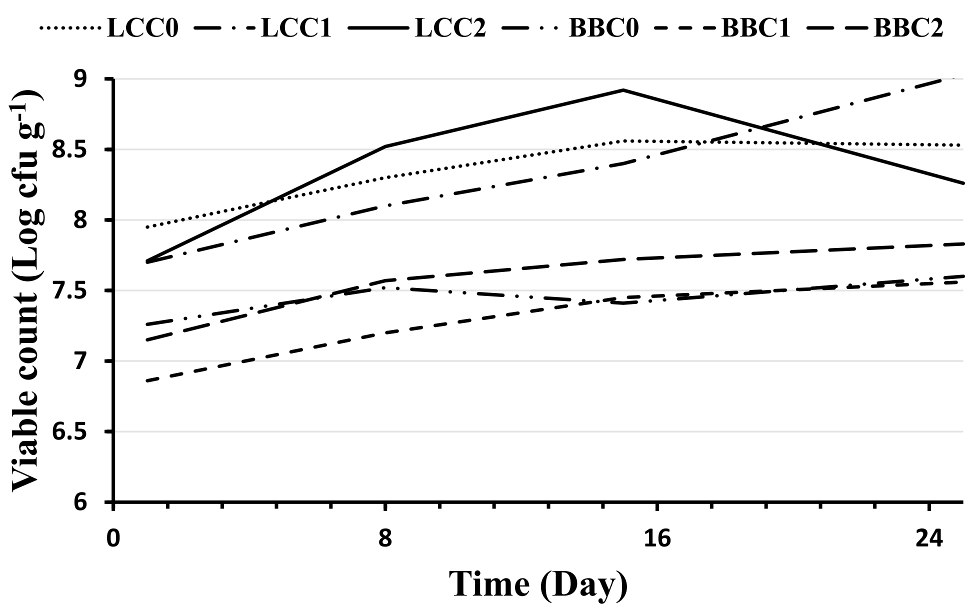

Fig. 2 The viable counts of the probiotic cultures during storage

by *BB12* was more than that of the other probiotic cottage cheese produced by *L. casei*. In the view of the panelists, the probiotic cheeses produced by *BB12* had a significant difference ($p < 0.05$) in the taste rather than the other ones and were more delicious. In this study, *BB12* produced a high viscosity dressing rather than *L. casei* (Table 2) and this may affect the taste and overall acceptability of final products. In general, the overall acceptability and taste of the probiotic cheeses were improved by addition of black cumin seeds until the level of 1 percent and then deteriorated at higher levels (up to 2%). All of the samples showed no significant difference ($p > 0.05$) on the texture. The color of the produced cottage cheeses without any cumin seed was whiter than the others and well-liked among the panelists. As the cumin seed content increased, the dressing part of the samples showed a more viscous appearance. This increased viscosity may be attributed to the absorption of dressing serum using the cumin seeds.

Table 2 shows the viscosity of the fermented dressing containing *L. casei* or *BB12*. It can be observed that the viscosity of cream dressing fermented by *BB12* is higher than the one fermented by *L. casei*; it may be related to the type and content of exopolysaccharides produced by these strains. Thermophilic strains can produce high amounts of exopolysaccharides under optimal conditions rather than mesophilic strains [20].

Fig. 3 Sensory evaluation of different probiotic cottage cheese samples

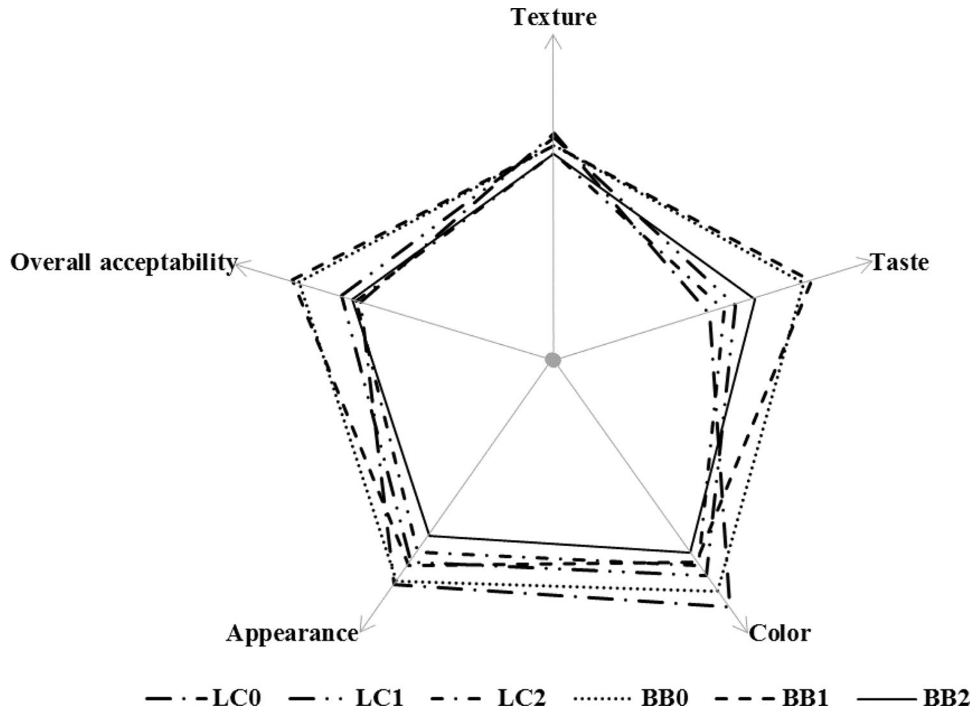

Table 2 Viscosity of fermented dressing containing probiotic bacteria

Sample	Viscosity (cP) at 50 rpm
Fermented dressing containing *L. Casei*	1253
Fermented dressing containing *BB12*	1432

Conclusions

In summary, the addition of black cumin seeds didn't effect on the NaCl, protein and fat content, as significantly, whereas it can dramatically effect on the pH and sensorial properties. *Lactobacillus casei* grew more than *Bifidobacterium animalis* into the samples at refrigerated temperature during storage. Addition of black cumin seeds, until a special level, can increase the counts of the probiotic cultures. Sensorial properties of the probiotic cottage cheese can significantly affect the amounts of black cumin seeds and the overall acceptability of final product decreased in the higher levels. In general, the cottage cheese produced by 1% black cumin seed and *Bifidobacterium animalis* had a more delicious taste and high overall acceptability compared with the other samples.

Acknowledgements The authors gratefully acknowledge the financial support received from Baqiyatallah University of Medical Science (Tehran, Iran) and Kalleh Dairy Company (Amol, Iran).

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