Effect of microGARD on keeping quality of direct acidified Cottage cheese

S. Makhal · S. K. Kanawjia · Apurba Giri

Revised: 10 March 2013 / Accepted: 4 June 2013 / Published online: 18 June 2013
© Association of Food Scientists & Technologists (India) 2013

Abstract Direct acidified Cottage cheese could be kept well for only 10 to 12 days under refrigeration on account of its high moisture (~74 %) content and relatively high pH (~5.0). The study was, therefore, undertaken to improve the keeping quality of direct acidified Cottage cheese using MicroGARD. The effects of three different levels of MicroGARD 400 viz. 0.20, 0.35 and 0.50 % on the physico-chemical, microbiological and organoleptic properties were studied at four-day interval during storage under refrigeration (4–5 °C). Promising results were found using 0.50 % MicroGARD 400 for inhibiting psychrotrophs, yeasts and molds as well as retard the acidity development and proteolysis in Cottage cheese, thus extending its keeping quality. It was observed that addition of MicroGARD 400 at the level of 0.50 % considerably improved the flavour as well as the aesthetic quality of the product during storage and extended the shelf life of Cottage cheese from 12 to 26 days.

Keywords Cottage cheese · Shelf life extension · MicroGARD · Bacteriocin · Bio-preservative · Natural preservative

Introduction

Shelf life of direct acidified Cottage cheese is limited hardly to 10 to 12 days under refrigeration because of high moisture content (~74 %) and relatively high pH (~5.0), which is a vital problem in its widespread marketing and distribution. Commercial Cottage cheese remains acceptable hardly 7–days, while some, where stringent quality control is practiced, may exhibit a shelf life of 15 days (Luck et al. 1977;

Johnson 1979; Makhal and Kanawjia 2003a). Psychrophilic bacteria as well as yeasts and molds are considered the most serious among the organisms causing spoilage in Cottage cheese. Several factors have been reported to be the source of contamination in Cottage cheese, such as milk, wash water, rennet, starter culture, equipment, air, packaging materials, etc (Harmon and Smith 1956a, b; Scafer 1958; Angevine 1959; Emmons and Tuckey 1967). Spoilage of Cottage cheese is largely due to the microbial invasion, which brings about several physico-chemical and biochemical changes leading to the development of off-flavour and discolouration of the surface (Makhal and Kanawjia 2003a; Makhal et al. 2012).

During the past few decades, a number of chemical and physical treatments have been attempted to extend the shelf life of Cottage cheese. However, increased health consciousness of today's consumers has created a massive pressure on the food industry to reduce the use of chemical preservatives in foods. Consequently extensive research attention has focused on the application in foods of naturally occurring metabolites of some selected microorganisms to inhibit the growth of spoilage causing organisms. From the point of views of health and safety aspects, bacteriocins have exciting potential in preservation of food products (Makhal and Kanawjia 2003b). Such natural inhibitors can replace the use of chemical preservatives, such as sulfur dioxide, benzoic acid, sorbic acid, nitrate, nitrite etc. (Llyod and Drake 1975). Many other natural antimicrobial agents have good potential for food preservation in the near future. MicroGARD, a natural line of antimicrobial ingredients, has been developed to assist food manufacturers in meeting consumers' demands for fresh-testing foods with reliable shelf life (Makhal et al. 2003). The microbial metabolites and/or bacteriocins contained in MicroGARD retard the microbial outgrowth in a variety of foods including dairy products, thus extending their shelf life (Weber and Broich 1986; Salih et al. 1990; Makhal et al. 2003).

Fermentation of the skim milk produces natural metabolites that may retard microbial growth in numerous food

S. Makhal · S. K. Kanawjia · A. Giri (✉)
Cheese and Fermented Foods Lab., Dairy Technology Division,
National Dairy Research Institute, Karnal 132001, Haryana, India
e-mail: apurbandri@gmail.com

products (Makhal et al. 2003). MicroGARD is approved by the FDA (Daeschel 1989) and used extensively in preserving a number of food products. Al-Zoreky et al. (1991) reported that a heat-stable protease-sensitive polypeptide of 700 Da contributes significantly to the inhibitory effect of MicroGARD. The combination of inhibitor, organic acids and diacetyl probably contributes to the overall preservation effect of MicroGARD product (Salih et al. 1990; Al-Zoreky et al. 1991). MicroGARD is a potent inhibitor for Gram-negative bacteria, such as *Pseudomonas*, *Salmonella* and *Yersinia* (Al-Zoreky et al. 1991) and certain fungi (Weber and Broich 1986). The present study was, therefore, undertaken to improve the shelf life of direct acidified Cottage cheese using MicroGARD 400. The effect of three different levels viz. 0.20, 0.35 and 0.50 % of the final product, of MicroGARD 400 was studied on the sensoric properties, physico-chemical attributes and microbiological quality of Cottage cheese during storage under refrigeration (4–5 °C).

Materials and methods

Manufacture of Cottage cheese The fresh skim milk (~0.03 % fat) from the Experimental Dairy, NDRI, Karnal was transferred to a pasteurizing vat. Cottage cheese was manufactured from 20 kg skim milk employing dual acidification technique following the procedure (Makhal et al. 2011; 2013) as outlined in Fig. 1.

Mixed strain multiple starter cultures (*Lc. lactis* subsp. *lactis* NCDC-91, *Lc. lactis* subsp. *cremoris* NCDC-86 and *Lc. lactis* subsp. *lactis* biovar. *diacetylactis* NCDC-60 in the ratio of 1:1:1 with a cell concentration of 10^7 cfu/ml) used for ripening of curd dressing were collected from the National Collection of Dairy Cultures (NCDC), NDRI, Karnal, India. Meito rennet (M/s Meito Sangyo and Co. Ltd., Tokyo, Japan) commercially produced from *Mucor pusillus* var. *lindt* was used in the experiment. MicroGARD 400 was supplied by Rhodia, Inc.-2802 Walton Commons West, Madison, WI 53718-6785 through their representative in India M/s Rhodia Chemicals India Pvt. Ltd., Mumbai. Procured MicroGARD 400 was in dried form and was produced by culture fermentation utilizing *Propionibacterium freudenreichii* subsp. *shermanii* (ATCC strain 9616) in skim milk. MicroGARD 400 was properly mixed with the curd dressing to attain the desired concentrations in the final product viz. 0.20, 0.35 and 0.50 %.

Preparation of curd dressing Calculated quantity of cream and skim milk were mixed to prepare 22 % fat curd dressing. The standardized cream, after homogenization at 176 kg/cm^2 and pasteurization at 75 °C for 15 s, was cooled to 22 °C and inoculated with the 3 % inoculum of mixed strain cultures and ripened for 14–16 h at 30 °C. The titratable acidity of the

Fig. 1 Flow diagram for manufacturing direct Cottage cheese employing dual acidification technique

ripened curd dressing was maintained at 0.75 to 0.80 %. Curd dressing was fermented before being admixed with MicroGARD 400 in order to avoid any possible antagonistic effect of MicroGARD 400 on the starter cultures.

Analysis

The samples were analyzed for sensory, biochemical as well as microbiological quality after each 4 days interval during the Study.

Sensory evaluation Sensory evaluation of Cottage cheese was carried out using the standard Cottage cheese scorecard recommended by the ADSA (2004). Descriptive sensory analysis method was performed using 10 trained panellists of the Institution, constituted based on their interest, performance, motivation, compliance and availability (ISO 8586-1: 1993E). Attributes evaluated were flavour, body and texture as well as colour and appearance of Cottage cheese. The panellists received preliminary training according to the methods recommended by the ISO (ISO 8586: 1993E). Before profiling, two sessions were used to train the assessors in the definition of attributes of a standard identity Cottage cheese. The panellists were allowed to use water and bland crackers for palate cleansing between the samples. All the samples (100 g), randomly coded using three digit numbers, were evaluated in each session and each

panellist was given the samples (25 °C) in white 100 ml plastic cup always 2 h before and after meals. The panellists expressed their judgments about the samples using the structured numeric scale as per the guidelines of ADSA (2004).

Physico-chemical analysis Moisture content as well as titratable acidity of Cottage cheese was determined as per the AOAC method (AOAC 2000). Moisture content in Cottage cheese curd was determined by oven drying to constant weight. Titratable acidity of the samples was expressed as per cent of lactic acid (w/v). Determination of pH was carried out as per the standard method (Shakeel-ur-Rehman et al. 2003). For this, 10 g of grated Cottage cheese was mixed with 10 ml distilled water and slurry was prepared thereof. The pH of the slurry was determined using microprocessor controlled pH Analyser (version I; Labindia, New Delhi) with combined glass electrode. In order to measure the degree of lipolysis in the samples, total free fatty acid (FFAs) of the cheese sample was estimated using the standard method (Deeth and Fitzgerald 1976). In a stoppered test tube, 5 g of sample was mixed with 5 ml of distilled water, 10 ml of extraction mixture (Isopropanol: Petroleum ether: 4N H₂SO₄::40:10:1) and 6 ml additional petroleum ether. The test tube was stoppered and tempered at 40 °C for 10 min followed by vigorous shaking for 20 s and keeping undisturbed for 5–10 min to separate two layers. A 5 ml of the upper layer was titrated with 0.02 N methanolic KOH after addition of 2 drops of α -naphtholphthalein indicator (1 g α -naphtholphthalein in 100 ml methanol) and the free fatty acid percentage of the cheese was calculated as $0.1128VT/W$, where V = net titration volume (ml), T = total volume of upper layer (ml), W = weight of the sample (g). The extent of proteolysis was measured by determining soluble nitrogen following the procedure delineated by Kosikowski (1982).

Microbiological analysis The psychrotrophs, yeasts and molds as well as coliforms counts of Cottage cheese was determined according to the method described by Houghtby et al. (1993). For psychrotrophic count, incubation was done at 5 °C for 7 days.

Statistical analysis All experiments were in triplicate. The data were statistically analyzed as per the methods described by Evanston (1990) with applying one way analysis of variance (ANOVA) using IBM SPSS Statistics 20 software package. When significant (5 % levels) differences were observed, individual means were compared using Tucky Post Hock multiple comparison test. Graphical representation of the results has been made using GraphPad Prism® Software, Version 3.02, GraphPad Software Inc, 5755 Oberlin Dr #110, San Diego, CA 92121. All results are expressed as mean $\pm$ standard error of the mean (SEM).

Results and discussion

Changes in sensory quality

Flavour The T₁, T₂ and T₃ imply samples treated with 0.20, 0.35 and 0.50 % MicroGARD 400, respectively. Addition of MicroGARD 400 improved flavour of the fresh samples depending upon the level of addition, because of its own characteristic mild cultured flavour. Figure 2 highlights that despite the treatment, flavour score gradually deteriorated with progressing storage period. On 8 day, control sample attained somewhat poor ($P<0.05$) flavour score compared to the sample T₃; however, the treated samples showed no marked variation among them in terms of flavour. With advancing storage period upto 16 days, the same observations were made; although the control sample exhibited sharp deterioration ($P<0.05$) compared to the sample T₂ and T₃. Further on 20 days, sample T₁ too experienced reasonably higher ($P<0.05$) flavour deterioration in comparison to the sample T₂ and T₃. Subsequently on 24 and 26 days, the sample T₂ and T₃ also exhibited the similar observations. Sensory evaluation showed that upon storage beyond 12, 20, 24 and 26 days, control sample as well as the sample T₁, T₂ and T₃, respectively were judged unacceptable. It was also observed that with elevating the level of MicroGARD 400, deterioration in flavour quality occurred relatively at a slow rate.

Body and texture Addition of MicroGARD 400 was found to exert significant effect on the body and texture during storage (Fig. 2). The deterioration in body and texture of the control sample started on 8 day and it achieved significantly a poor ($P<0.05$) score compared to the sample T₂ and T₃; however, the treated samples exhibited no marked variation among themselves. Upon storage for 12 days, similar observations were made. With further progress in storage period to 16 days, the control sample exhibited sharp deterioration ($P<0.05$) in body and texture as compared to the sample T₂ and T₃; even sample T₁ also showed significantly poor body and texture score compared with the sample T₂ and T₃. Thereafter, the treated samples regardless of the levels of MicroGARD 400 added too experienced moderately rapid deterioration in body and texture score (Fig. 2). On 20 days, though the sample T₁ and T₂ exhibited no marked variation in body and texture, sample T₁ achieved significantly ($P<0.05$) poor score compared to the sample T₃. With further increasing in storage period upto 24 days, body and texture of the sample T₁ severely deteriorated ($P<0.05$) as compared to the sample T₂ and T₃. Subsequent storage upto 26 and 28 days exhibited similar observations for the T₂ and T₃ samples, respectively. It was observed that after a certain period of storage, body and texture quality sharply deteriorated depending upon the levels of MicroGARD 400

Fig. 2 Effect of addition of different concentrations of MicroGARD 400 on sensory quality attributes (Flavour, Body and texture and Colour and appearance, $n=10$ panelists) and chemical parameters (Moisture, Acidity, pH, FFAs, Soluble nitrogen, $n=3$) of direct acidified cottage cheese

packaged in polystyrene cup and stored at 4–5 °C. T₁, T₂ and T₃ imply samples treated with 0.20, 0.35 and 0.50 % MicroGARD 400, respectively. The vertical bars indicate the errors of means. FFAs: Total free fatty acid

resulting in a product with the distinct mark of gelatinized curd particles accompanied by pasty, compact and mealy curd characteristics. Use of MicroGARD 400 was found to protect against the deterioration in body and texture compared to the control sample. The sample added with higher level of MicroGARD 400 was observed to produce fairly a slow rate of deterioration because of the strong preservative effect of MicroGARD 400 against the growth of spoilage microorganisms in Cottage cheese.

Colour and appearance Sensory evaluation shows that use of MicroGARDTM 400 exhibited no noticeable effect on the initial colour and appearance. With the progress in storage, colour and appearance slowly and steadily deteriorated regardless of the treatment (Fig. 2). Though storage for 8 days produced no significant variation in colour and appearance among the different samples; on 12 days, the control sample attained significantly ($P<0.05$) poor score compared to the treated samples. On 16 days, colour and appearance of the control sample sharply deteriorated ($P<0.05$) in contrast to the samples treated with MicroGARD 400. However upon storage for 20 days, no noticeable variation was observed among the treated samples in respect of colour and appearance. With further advances in storage period to 24 and 26 days, sample T₁ and T₂ experienced a faster deterioration compared to the sample T₂ and T₃, respectively. Subsequently, sample T₃ too exhibited a sharp deterioration in colour and appearance upon storage onwards 26 days.

Towards the end of storage period, colour and appearance sharply deteriorated irrespective of the treatment because of the faster microbial growth. Depending upon the levels of MicroGARD 400 used, the treated samples experienced relatively a slow rate of deterioration compared to the control sample. The preservative effect of MicroGARD 400 was found effective to delay the deterioration in colour and appearance, because of its strong antimicrobial activity against a number of psychrotrophs as well as yeasts and molds, which, in turn, could result in deterioration through their characteristic metabolic activities.

Changes in physico-chemical properties

Moisture It is evident from the Fig. 2 that moisture content showed a slow and gradual reduction upon storage of Cottage cheese irrespective of the treatment. It was observed that in contrary to the early phase of storage, moisture loss occurred relatively slowly towards the end of storage period. Moisture content of the fresh samples varied from 73.0 to 73.15 %, which gradually declined to 71.64–71.80 % at the end of storage period depending upon the length of storage, which exhibited a loss of moisture by 1.64 to 2.06 %, respectively. Loss of moisture from Cottage cheese upon storage was found unaffected by the addition of MicroGARD

400; rather moisture loss was directly related to the length of storage period.

Acidity Initially acidity of the fresh Cottage cheese ranged from 0.61 % to 0.62 %. With progressing storage, acidity increased slowly upto a certain period regardless of the levels of MicroGARD 400 used; thereafter it followed a sharp increase upon further advance in storage period (Fig. 2). Storage upto 12 days produced no marked variation in acidity among the samples. However on 16 days, acidity of the control sample sharply increased, but storage upto 20 days exhibited no major variation amongst the treated samples. Upon further increase in storage period to 24 days, sample T₁ showed sharp acid development ($P<0.05$) compared to the sample T₂ and T₃ and on subsequent storage upto 26 and 28 days, similar trend was found for the sample T₂ and T₃, respectively. Development of acidity occurred relatively at an elevated rate in control sample compared with the treated samples and increasing the level of MicroGARD 400 was found to exhibit comparatively slow acid development in course of storage. This effect is attributed to the MicroGARD 400, which specifically inhibits the growth of spoilage causing Gram-negative psychrotrophs and selected yeast and molds, but not against Gram-positive bacteria (Al-Zoreky et al. 1991). Though the mixed strain starter cultures used in ripening of curd dressing viz. the strains of different *Lactococcus lactis*, which are Gram positive in nature and unaffected by MicroGARD 400 possibly contributed to the comparatively slow rate of acid development in sample T₂ and T₃.

pH The effect of using MicroGARD 400 in Cottage cheese on the rate of change in pH of Cottage cheese upon storage is depicted in Fig. 2. With an initial pH ranging from 4.98 to 5.0, all samples experienced a slow and gradual decline in pH with progressing storage because of the slow development in acidity. On 12 days, pH of the control sample swiftly declined ($P<0.05$) compared to the sample T₂ and T₃; while no marked variation in pH was observed between the treated samples. Upon storage upto 16 days, the control sample exhibited an increase in pH; while the treated samples still experienced a gradual decline in pH and the sample T₁ had significantly ($P<0.05$) a lower pH than the sample T₂ and T₃. It was further observed that pH of the sample T₁, T₂ and T₃ continued to decline upon storage upto 20, 24 and 26 days, respectively, and thereafter all treated samples showed a rise in pH.

The study showed that use of MicroGARD 400 was proved effective to setback the rate of change in pH upon storage as compared to the control sample. Increasing the level of MicroGARD 400 was also observed to cause relatively a slow rate of change in pH. This was attributed to the antimicrobial effect of the metabolites contained in the preparation of MicroGARD 400 against numerous organisms

including yeasts and molds, causing degradation of lactose into lactic acid as well as break down of proteins into several small peptides that overrides the mere effect of lactic acid development to cause a decrease in pH. The steady decline in pH during the storage was due to the slow rate development of acidity. Subsequently, the increase in pH might be attributed to the facts that microbial activity causes break down of citrate and hydrolysis of protein resulting in the formation of more protein bound residues having high pKa ($-\log k_a$) values and the medium with higher pKa value is likely to have high pH values (Walstra and Jenness 1984). Direct acidified Cottage cheese contains ~1.10 % lactose and hence, the effect of protein bound residues formed and the release of basic amino acids and ammonia because of hydrolysis of some amino acids towards the end of storage due to higher rate of proteolysis seems to override the mere effect of lactic acid causing a decrease in the pH (Fox et al. 2000). It has been reported that some proteolytic yeast species are able to raise the pH through their excessive casein hydrolysis capabilities (Suriyarachchi and Fleet 1981).

Free fatty acids Cottage cheese was manufactured from skim milk (~0.03 % fat), however the final product contained around 4 % fat as maintained using fermented cream dressing. Consequently with increasing storage period, there was a slow and steady increase in FFAs content regardless of the treatment. There was no marked difference in FFAs content of Cottage cheese during storage upto 8 days (Fig. 2). However on 12 days, FFAs content was significantly ($P<0.05$) higher for the control cheese compared to the treated samples. Upon further progress in storage period upto 16 days, control sample experienced a fast ($P<0.05$) lipolysis in comparison to the treated samples, which while showed no significant difference amongst themselves in respect of FFAs content during storage still 20 days. With advancing storage to 24 days, sample T₁ too experienced a sharp lipolysis as compared to the sample T₂ and T₃. Subsequently on 26 and 28 days, similar trend was observed for the sample T₂ and T₃. Figure 2 clearly depicts that the control sample showed comparatively rapid lipolysis upon storage than the treated samples and the use of MicroGARD 400 at higher level was observed more effective in controlling the lipolysis.

Soluble nitrogen It was observed that with advancing storage period, soluble nitrogen content steadily increased irrespective of the treatment with MicroGARD 400, which was followed relatively by a faster proteolysis towards the end of storage period (Fig. 2). Storage for 12 days exhibited no significant difference in soluble nitrogen content among the samples. However, storage exceeding 12 days showed that soluble nitrogen content of the control sample rapidly increased ($P<0.05$) compared to the treated sample. Although the treated samples also experienced a steady increase in

soluble nitrogen, they showed no marked difference among themselves upon storage for 20 days. With progressing storage period to 24 days, sample T₁ exhibited significantly ($P<0.05$) a sharp rise in soluble nitrogen content as compared to the sample T₂ and T₃ on 26 and 28 days, respectively. It was observed that the control sample experienced a rapid proteolysis and thereby a faster rate of change in soluble nitrogen content compared to the treated samples. It was also investigated that with the elevation in level of MicroGARD 400, the rate of proteolysis significantly declined, on account of the preservative effect of MicroGARD 400 against several spoilage organisms, which otherwise might cause break down of proteins in Cottage cheese and consequently increased the soluble nitrogen content.

Changes in microbiological quality

Psychrotrophs Table 1 depicts that with advancing storage, psychrotrophic count steadily increased irrespective of the treatment. Upon storage ahead of 12, 20, 24 and 26 days, control sample as well as the sample T₁, T₂ and T₃, respectively exhibited a prompt rise in psychrotrophic load. It was also observed that the control sample showed a rapid deterioration in microbiological quality in terms of psychrotrophic count upon storage in comparison to the treated sample. Use of elevated level of MicroGARD 400 was significantly more efficient in restraining the growth of psychrotrophs in Cottage cheese upon storage.

Coliforms Fresh control Cottage cheese showed coliforms count of not exceeding 1 log cfu/g. Addition of 0.20 % of MicroGARD 400 produced no beneficial effect in respect of reducing initial load of coliforms. However, Cottage cheese added with 0.35 and 0.50 % MicroGARD 400 was found helpful to maintain the coliforms count below 1 log cfu/g upon storage for 8 and 12 days, respectively. With advancing storage, there was a slow and gradual increase in coliforms count regardless of the levels of MicroGARDTM 400, which was subsequently followed by a sharp increase, particularly in the last phase of the storage period. It was observed that on 16 days, control sample experienced a higher load of coliforms as compared to the treated samples and upon further increase in storage ahead of 20, 24 and 26 days, the sample T₁, T₂ and T₃, respectively exhibited the similar observations. Fresh cultured Cottage cheese should not show coliforms count more than 1 log cfu/g (Robinson 1981). Initially the fresh direct acidified Cottage cheese exhibited coliforms count not exceeding the limit of 1 log cfu/g; upon further storage, coliforms count steadily increased.

Yeasts and molds Fresh control samples showed a yeasts and molds count of zero to 0.60 log cfu/g, whereas the sample T₁ and T₂ as well as the sample T₃ exhibited no occurrence of

Table 1 Effect of different levels of MicroGARD 400 on (A) Psychrotrophs, (B) Coliforms and (C) Yeasts and molds count (log cfu/g) of direct acidified Cottage cheese during storage at 4–5 °C

Days	Control	Levels of MicroGARD 400 (%)		
		T ₁	T ₂	T ₃
(A) Psychrotrophs				
0	3.9±0.04 ^{dp}	3.8±0.01 ^{cp}	3.7±0.01 ^{bp}	3.6±0.01 ^{ap}
4	4.4±0.03 ^{cq}	3.8±0.02 ^{bp}	3.8±0.01 ^{bpq}	3.5±0.01 ^{ap}
8	5.0±0.05 ^{cr}	4.0±0.01 ^{bq}	3.9±0.02 ^{abq}	3.8±0.01 ^{aq}
12	5.6±0.02 ^{ds}	4.6±0.01 ^{cr}	4.1±0.02 ^{br}	4.0±0.02 ^{ar}
16	8.7±0.02 ^{ds}	4.9±0.02 ^{cs}	4.8±0.02 ^{bs}	4.1±0.02 ^{ar}
20	NP	5.6±0.04 ^{ct}	5.1±0.03 ^{bt}	4.9±0.02 ^{as}
24	NP	8.7±0.04 ^{cu}	5.6±0.04 ^{bu}	5.0±0.03 ^{as}
26	NP	NP	8.6±0.04 ^{bv}	5.5±0.03 ^{at}
28	NP	NP	NP	7.7±0.05 ^u
(B) Coliforms				
0	0.90±0.000 ^{dp}	0.81±0.010 ^{cp}	0.60±0.001 ^{bp}	0.50±0.001 ^{ap}
4	1.3±0.00 ^{dq}	1.2±0.00 ^{cq}	0.81±0.002 ^{bq}	0.71±0.001 ^{aq}
8	1.8±0.00 ^{dr}	1.4±0.01 ^{cr}	0.90±0.001 ^{bq}	0.82±0.010 ^{aqr}
12	2.2±0.02 ^{ds}	1.7±0.01 ^{cs}	1.1±0.00 ^{br}	0.91±0.010 ^{ar}
16	3.7±0.02 ^{dt}	1.9±0.02 ^{ct}	1.7±0.02 ^{bs}	1.2±0.01 ^{as}
20	NP	2.3±0.10 ^{cu}	2.0±0.02 ^{bt}	1.7±0.02 ^{at}
24	NP	3.6±0.07 ^{cv}	2.4±0.04 ^{bu}	2.0±0.03 ^{au}
26	NP	NP	3.2±0.05 ^{bv}	2.3±0.06 ^{av}
28	NP	NP	NP	3.2±0.07 ^w
(C) Yeasts and molds				
0	0.60±0.010 ^p	ND	ND	ND
4	0.90±0.040 ^q	ND	ND	ND
8	1.2±0.05 ^r	ND	ND	ND
12	2.0±0.03 ^{cs}	0.85±0.000 ^{bp}	0.30±0.000 ^{ap}	ND
16	2.7±0.02 ^{dt}	1.3±0.02 ^{cq}	0.80±0.021 ^{bq}	0.60±0.000 ^{ap}
20	NP	2.1±0.02 ^{cr}	1.2±0.02 ^{br}	1.1±0.02 ^{aq}
24	NP	2.6±0.03 ^{cs}	2.0±0.03 ^{bs}	1.6±0.03 ^{ar}
26	NP	NP	2.6±0.03 ^{bt}	2.1±0.02 ^{as}
28	NP	NP	NP	2.7±0.05 ^t

T₁, T₂ and T₃ imply samples treated with 0.20, 0.35 and 0.50 % MicroGARD 400, respectively. *n*=3; Mean ± Standard error; ^{abcd} Means within each row followed by different superscripts differ significantly (*p*<0.05); ^{pqrstuvw} Means within each column followed by different superscripts differ significantly (*p*<0.05); *ND* Not detected; *NP* Not performed

yeasts and molds upon storage for 8 and 12 days, respectively. Sample T₁ and T₂ first exhibited the appearance of yeasts and molds on 12 days, while the sample T₃ showed the incidence of yeasts and molds on 16 days. Once yeasts and molds appeared, there was a steady increase in the count, which followed a sharp rise towards the end of storage period depending upon the concentration of MicroGARD 400 used. On account of the antifungal effect, use of MicroGARD 400 was proven to be efficient in controlling the prevalence of yeasts and molds in Cottage cheese in course of storage period. MicroGARD 400 at higher levels was also found more effective in checking the incidence of yeasts and molds. Yeast and molds count of the fresh cultured Cottage cheese should not exceed 1 log cfu/g (Robinson 1981). However, the yeast and molds count of the fresh samples were below this limit; during storage period, it gradually increased causing spoilage of the product.

Conclusion

Cottage cheese preserved with different levels of MicroGARD 400 underwent extensively slower rate of storage changes in quality attributes in respect of sensory quality, physico-chemical attributes as well as microbiological parameters compared to the control sample. Use of 0.50 % MicroGARD 400 instead of using 0.20 and 0.35 % produced additional favorable effect in extending the storage life of Cottage cheese exclusive of any adverse effect on the quality. Considering the cost of the preservative as well as its likely unfavorable effect, especially on the body and textural properties of the product, exercise of further increasing its levels was discontinued. Addition of 0.50 % MicroGARD 400 was observed substantially useful to extend the quality decay of Cottage cheese from 12 to 26 days with an additional shelf life of 14 days.

References

- ADSA (2004) Cottage cheese score card, 2003 Rules. In: 83rd Annual collegiate dairy products evaluation contest. Committee on Dairy Product Evaluation, American Dairy Science Association. 1111 N. Dunlap Avenue Savoy, IL 61874, USA. p 16
- Al-Zoreky N, Ayres JW, Sandine WE (1991) Antimicrobial activity of MicroGARD™ against food spoilage and pathogenic microorganisms. *J Dairy Sci* 74:758–763
- Angevine NC (1959) Keeping quality of cottage cheese. *J Dairy Sci* 42:2015–2019
- AOAC (2000) Association of official analytical chemists. In: Horwitz W (ed) Official methods of analysis, Vol. II, 17th edn. Assoc Official Agric Chem, Washington DC
- Daeschel AM (1989) Antimicrobial substances from lactic acid bacteria for use as food preservatives. *Food Technol* 43:91–94
- Deeth HC, Fitz-Gerald CH (1976) Lipolysis in dairy products: a review. *Aust J Dairy Technol* 31:53–64
- Emmons DB, Tuckey SL (1967) Cottage cheese and other cultured milk products. Has Pfizer & Co, Inc, New York
- Evanston IL (1990) System of Statistics (SYSTAT). Evanston IL (ed), Wilkinson
- Fox PF, Guinee TP, Cogan TM, McSweeney PLH (2000) Fundamental of cheese science. Aspen Publishers, Inc, Gaithersburg, pp 236–279
- Harmon LG, Smith CL (1956a) Spoilage organisms in cottage cheese. *J Dairy Sci* 39:9–15
- Harmon LG, Smith CL (1956b) The influence of environment and processing in spoilage organisms in Cottage cheese. *J Dairy Sci* 19:252–255
- Houghtby GA, Maturin LJ, Koenig EK (1993) In: Marshall RT (ed) Microbiological count methods, 16th edn. American Public Health Association (APHA), Washington DC, pp 213–246
- ISO 8586:1993E. Sensory analysis-methodology-general guidance. International Organization for Standardization, Geneva, Switzerland
- ISO 8586-1:1993E. Sensory analysis-general guidance for the selection, training and monitoring of assessors. International Organization for Standardization, Geneva, Switzerland
- Johnson P (1979) Cottage cheese: a primer for high quality and long shelf life. *Am Dairy Rev* 41:40O–40P, 40T–40V, 40X–40Z
- Kosikowski FV (1982) Cottage cheese. In: Kosikowski FV (ed) Cheese and fermented milk foods, 2nd edn. F. V. Kosikowski and Associates, New York, pp 109–143
- Llyod AG, Drake JJP (1975) Problems posed by essential food preservatives. *Br Med Bull* 31:214–219
- Luck H, Mosteret JF, Husmann RA (1977) Shelf life of perishable dairy products. *S Afr J Dairy Technol* 9:25–27
- Makhal S, Kanawjia SK (2003a) Preservation of Cottage cheese: a review. *Indian J Dairy Sci* 56(10):1–12
- Makhal S, Kanawjia SK (2003b) Microbial metabolites in preserving dairy products—an overview. *J Food Dairy Home Sci* 23:157–171
- Makhal S, Mandal S, Kanawjia SK (2003) MicroGARD™ in food preservation: a shifted paradigm towards natural preservatives. *Indian J Dairy Biosci* 14:70–78
- Makhal S, Giri A, Kanawjia SK (2011) Effect of κ -carrageenan and tetrasodium pyrophosphate on the yield of direct acidified cottage cheese. *J Food Sci Technol*. doi:10.1007/s13197-011-0438-5
- Makhal S, Kanawjia SK, Giri A (2012) Effectiveness of thymol in extending keeping quality of cottage cheese. *J Food Sci Technol*. doi:10.1007/s13197-012-0715-y
- Makhal S, Kanawjia SK, Giri A (2013) Role of calcium chloride and heat treatment singly and in combination on improvement of the yield of direct acidified Cottage cheese. *J Food Sci Technol*. doi 10.1007/s13197-013-1028-5
- Robinson RK (1981) Dairy microbiology, vol 2. Elsevier Science Publishers Ltd, London
- Salih MA, Sandine WE, Ayres JW (1990) Inhibitory effects of MicroGARD™ on yoghurt and Cottage cheese spoilage organisms. *J Dairy Sci* 73:887–893
- Scafer JG (1958) Cottage cheese sanitation practice. *Am Milk Rev* 20:42–44
- Shakeel-Ur-Rehman, Farkye NY, Drake MA (2003) Reduced fat Cheddar cheese from a mixture of cream and liquid milk protein concentrate. *Int J Dairy Technol* 56:94–98
- Suriyarachchi RV, Fleet HG (1981) Occurrence and growth of yeast in yogurts. *Appl Environ Microbiol* 42:574–577
- Walstra P, Jenness R (1984) Dairy chemistry and physics. John Wiley & Sons, New York
- Weber GH, Broich WA (1986) Shelf life extension of cultured dairy foods. *Cult Dairy Prod J* 21:19–23