

Full Length Research Paper

Effect of specialized combined strains on reconstituted milk reduced-fat cheese

Weijun Wang, Lanwei Zhang* and Yanhua Li

School of Food Science and Engineering, Harbin Institute of Technology, Harbin 150090, People's Republic of China.

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The reduced-fat cheese using reconstituted milk powder (CRMP) has the problems of texture and flavor because of heating and drying treatments of milk. The application of an exopolysaccharide (EPS)-producing strain, as well as an adjunct strain in CRMP was investigated to address these quality issues. Volatiles of these cheeses were determined by solid phase microextraction-gas chromatography-mass spectrometry and texture by the textural profile analysis. The EPS-producing strain could hold moisture and result in cheese with higher yield compared to the non EPS-producing strain. Meanwhile, the cheese made with the EPS-producing *Streptococcus thermophilus* TM11 had a lower hardness, springiness, chewiness, gumminess, resilience and a higher adhesiveness and cohesiveness than the cheese made with non EPS-producing *S. thermophilus* SP1.1. These changes were attributed to the differences of protein matrix according to observation of scanning electron microscopy. On the other hand, results of the main flavor compounds in CRMP indicated that combination of the EPS-producing *S. thermophilus* TM11 and an adjunct *Lactococcus lactis* ssp. *lactis* could provide CRMP with novel and proper flavor properties while improving the textural characteristics.

Key words: Reconstituted milk, reduced-fat cheese, *Streptococcus thermophilus*, texture, flavor.

INTRODUCTION

The consumption of low-fat products has grown due to consumer awareness about health concerns (Dabour et al., 2006). However, creating a cheese with less fat but not at the expense of flavor and texture is a challenge to dairy industry (Ritvanen et al., 2005). Beal and Mittal (2000) reported that hardness, gumminess and chewiness increased linearly, while cohesiveness and springiness decreased nonlinearly with the decrease in fat content in Cheddar cheese. Johnson (2011) also discussed technological problems and some possible ways for improving the quality of low fat cheese, for example, exopolysaccharide (EPS)-producing starter (Francois et al., 2004; Zisu and Shah, 2007) and adjunct cultures (Katsiari et al., 2002a; Manolaki et al., 2006).

The use of EPS-producing cultures could be alternative for increasing moisture retention in reduced-fat cheese. Perry et al. (1997) found that EPS-producing *Streptococcus thermophilus* could increase moisture retention in low-fat Mozzarella cheese. The bacterium EPS were responsible for the water-binding properties in the cheese (Low et al., 1998). It was also confirmed that encapsulated and ropy EPS-producing *S. thermophilus* strains can be utilized to increase the moisture level of cheese. However, only the encapsulated EPS can improve these properties without adversely affecting the viscosity of whey (Petersen et al., 2000; Broadbent et al., 2001). These studies devoted efforts on low-fat cheeses because such cheeses tend to become tough, rubbery, and have poor stretching properties (Mistry and Anderson, 1993). Similar characteristics also exist in the cheeses produced from reconstituted milk powder (CRMP). According to Tamime et al. (1990), the experimental processed cheeses (made from Cheddar cheese and CRMP) were markedly firmer than control cheeses (made from Cheddar cheese without CRMP). Reconstituted dried milk found applications in some soft cheese varieties. Leclercq-Perlat et al. (2000a, b) introduced

*Corresponding author. E-mail: wilkinghigh@yahoo.com.cn. Tel: +86 451 86282901. Fax: +86 451 86282906.

Abbreviations: CRMP, Cheese using reconstituted milk powder; EPS, exopolysaccharide.

a soft smear cheese from reconstituted skim milk powder using microorganisms to control the production.

Compared to fresh milk, reconstituted milk displays a changed attribute due to the heating and drying process and seems to adversely influence the metabolism of microorganism. However, there are only few studies about reduced-fat CRMP and the effect of EPS-producing strain or adjunct culture on the characteristics of reduced-fat CRMP. Because of the economic importance and recent development of reconstituted milk cheeses, the aims of the present study were to evaluate the application of specialized combined strains in CRMP by characteristics of cheeses and the effects of these strains on texture and flavor of reduced-fat CRMP.

MATERIALS AND METHODS

Cheese manufacturing

At each treatment, 3 L (12% (w/v) reconstituted milk powder was prepared. The milk fat was adjusted to protein-to-fat ratio of 1.6 with a medium-heat whole milk powder and a low-heat skim milk powder (Nestle, Heilongjiang, China) according to the content of milk components on their product descriptions. The milk was agitated for 30 min at ~600 rpm and then heated at 80°C for 5 min (excluding the time of heating and cooling) for sterilization in a stainless steel vat (20 × 25 cm with a height of 20 cm). The cheese manufacturing was performed according to Perry et al. (1997) with slight modifications. The strains used in the study were stored in a -80°C refrigerator in skim milk-based medium. *Lactococcus* was transferred to M17 broth supplemented with 0.5% (w/v) lactose (LM17), while *Lactobacillus* was transferred to MRS broth and then cultured (at 37°C for *S. thermophilus* TM11, *S. thermophilus* SP1.1 and *Lactobacillus delbrueckii* ssp. *bulgaricus* 34.5, and at 32°C for *L. lactis* ssp. *lactis* X9C2) for activation. The last inoculation step was performed in the reconstituted part-skim milk powder described above. These cultures were stored at 4°C for use the next day.

Three vats were used at each time with different treatments in a water bath of 32°C. Milk in vat 1 (cheese A) was inoculated with *S. thermophilus* SP1.1 and *L. delbrueckii* ssp. *bulgaricus* 34.5, vat 2 (cheese B) with *S. thermophilus* TM11 and *L. delbrueckii* ssp. *bulgaricus* 34.5, and vat 3 (cheese C) with *S. thermophilus* TM11, *L. delbrueckii* ssp. *bulgaricus* 34.5 and *L. lactis* ssp. *lactis* X9C2. Inoculation quantity of each strain was 200 ml in each vat. After pH reached 6.3 (40~50 min of ripening), rennet was added. After the final drain, the curd was salted by dry-stirring 5.0% (w/w) salt into each vat. The curd was hand-stretched for 5 min at 65°C. Three independent replicates of cheese manufacturing were performed on different days.

Chemical and microbiological analyses of CRMP

The yield of cheese was analyzed as the ratio (%) of the weight of cheese to the weight of cheese milk at first day of storage. Each cheese was divided into 6 parts and sealed in plastic bags. These were then stored at 6°C for analyses on different ripening days. Moisture content (%) was determined according to the weight loss by drying sample at 105 ± 1°C until constant weight (Fajardoa et al., 2010). The salt content was determined by the Volhard method (AOAC, 2000). Protein was determined by Kjeldahl method (AOAC, 2000) with conversion to protein content using a factor of 6.38. The pH was monitored with a combined electrode pH-meter (PB-10, Sartorius, Gottingen, Germany).

A sample of cheese (10 g) was aseptically transferred to a sterile

flask and homogenized with 90 ml of 0.9% (w/v) sterile saline water for 1 min at room temperature. Serial decimal dilutions were prepared with the sterile saline water for estimating the counts of Lactococci and Lactobacilli inoculated on LM17 and MRS media, respectively at 37°C for 72 h.

Texture of CRMP

The textural profile analysis (TPA) of cheese was based on the method described by Marshall and Rawson (1999). The compression testing was performed on the cheeses after different days of ripening using a texture analyzer (TA.XT.plus, Stable Micro Systems Ltd, UK) equipped with a 5-kg load cell. A plunger, 35 mm in diameter, was attached to the moving crosshead. Cubes (1 cm³) from each cheese were prepared and allowed to equilibrate to assay temperature (20 ± 1°C). The operating conditions were: test speed of 60 mm/min and two-compression mode. From each force-time curve obtained by compression of the sample to 75% of its original height, the following texture-profile parameters were calculated using TPA software: hardness, adhesiveness, cohesiveness, springiness, chewiness, gumminess and resilience. The average values of these textural variables for the three cheese-making trials were used for dimensionality reduction by principal component analysis (PCA).

Microstructure of CRMP

Samples of cheese on day 45 of storage were cut into pieces (2 × 2 mm with a height of 0.5 mm) using a sterile blade, then immersed in 2% glutaraldehyde, and stored at 4°C. The cheese samples were then prepared for scanning electron microscopy using the method of Serrano et al. (2004).

Volatiles of CRMP

For the extraction of volatile compounds, 30 g of grated cheese and 30 ml NaH₂PO₄ (25%, w/v) were added in a 100-ml vial with a stirring bar. A 0.2-ml aqueous solution of 4-methyl-2-pentanone (50 mg/L) was added as an internal standard. The sealed vial was equilibrated for 30 min at 50°C in a thermostatic bath. A 75 µM CAR/PDMS fiber (Supelco, PA, USA) equipped with a SPME manual holder (Supelco, PA, USA) was desorbed at 250°C for 2 min and then inserted in the vial to extract the volatiles for 45 min. The samples were stirred with a magnetic stirrer (HJ-3, Guohua Inc., Jiangshu, China) during the equilibration and extraction.

The volatiles isolated by the fiber were desorbed in the injector port of a GC (Hewlett-Packard 6890, Delaware, USA) equipped with the HP 5973 mass selective detector (Hewlett-Packard Inc., Delaware, USA). A DB-5 capillary column with 60 m length, 0.25 mm internal diameter and 0.25 µm phase thickness (J&W Scientific, CA, USA) was used. The oven temperature was held at 40°C for 8 min, increased from 40 to 150°C at the rate of 4°C/min and from 150 to 250°C at 20°C/min, and then held at 250°C for 5 min. The temperatures of the injector and detector were 250 and 280°C, respectively. The flow rate of helium carrier gas was 1.0 ml/min. Electron impact ionization was used at a voltage of 70 eV. The mass range was m/z 30 - 500. The relative levels of individual compounds were assessed using the area ratio (AR) value treated as the area ratio of volatile to internal standard. The average AR values of these volatiles for the three cheese-making trials were used for dimensionality reduction by the PCA.

Statistical analysis

All the experiments mentioned above were performed in triplicate.

Table 1. Characteristics of CRMP made with different strains.

Parameter	Yield %	Protein %	Salt %	Moisture %	pH	Lactococci counts 10 ⁷ CFU/g	Lactobacilli counts 10 ⁷ CFU/g
Cheese A							
Day 1	21.4 ^a	27.1 ^a	3.54 ^a	62.2 ^a	5.19 ^a	37.7 ^a	5.2 ^a
Day 30	nt	nt	nt	59.5 ^b	5.06 ^b	56.3 ^a	2.9 ^a
Day 60	nt	nt	nt	56.7 ^c	4.97 ^c	63.5 ^a	3.7 ^a
Cheese B							
Day 1	22.9 ^b	25.7 ^b	3.73 ^a	63.4 ^a	5.22 ^a	7.2 ^c	2.8 ^a
Day 30	nt	nt	nt	62.3 ^{ab}	5.18 ^a	3.6 ^c	3.7 ^a
Day 60	nt	nt	nt	60.7 ^b	5.14 ^{ab}	9.8 ^{bc}	2.5 ^a
Cheese C							
Day 1	23.3 ^b	25.4 ^b	3.66 ^a	63.8 ^a	5.19 ^a	3.9 ^c	7.3 ^a
Day 30	nt	nt	nt	63.3 ^a	5.15 ^{ab}	5.3 ^c	6.2 ^a
Day 60	nt	nt	nt	62.7 ^{ab}	5.12 ^{ab}	15.4 ^b	4.9 ^a

^{a-c}Means in each column with different letters were significantly different ($P < 0.05$). CFU, Colony-forming unit; nt, not tested. Cheese A, inoculated with *S. thermophilus* SP1.1 and *L. delbrueckii* ssp. *bulgaricus* 34.5. Cheese B, inoculated with *S. thermophilus* TM11 and *L. delbrueckii* ssp. *bulgaricus* 34.5. Cheese C, inoculated with *S. thermophilus* TM11, *L. delbrueckii* ssp. *bulgaricus* 34.5 and *L. lactis* ssp. *lactis* X9C2.

Experimental data were statistically analyzed using PASWStatistics18.0 Software (SPSS Inc, USA). One-way analysis of variance (ANOVA) using the Tukey's test ($P < 0.05$) was applied to examine the effect of different treatments. The PCA was used for dimensionality reduction of the textural variables and volatiles of CRMP.

RESULTS AND DISCUSSION

Cheese analyses

Table 1 shows the main characteristics of CRMP made with different strains. The reduced-fat cheeses made with EPS-producing *S. thermophilus* TM11 (cheeses B and C) increased the yield by 7.0 and 8.9%, respectively compared to that of non EPS-producing strains (cheese A), which could be attributed mainly to the increased moisture retention in these cheeses. Moisture content of cheese A significantly decreased during storage ($P < 0.05$). On days 30 and 60, moisture content of cheese A decreased by 4.3 and 8.8%, respectively compared to day 1. Meanwhile, cheese B showed a slight decrease in moisture content (Table 1). In addition, the change in cheese C had no statistical significance ($P > 0.05$). The results therefore suggested that EPS-producing *S. thermophilus* TM11 had a strong water-holding capacity and could be used in CRMP to solve the problem of whey leakage.

Although, cheeses produced by *S. thermophilus* TM11 had slightly lower protein content than cheese A ($P < 0.05$), the mean protein contents for the reduced-fat CRMP are similar to those of previous studies (Olabi and Barbano, 2002; Dabour et al., 2006). Moreover, *S.*

thermophilus SP1.1 has a higher capacity to proliferate and acidify than *S. thermophilus* TM11. The CRMP made with *S. thermophilus* SP1.1 had higher Lactococci counts, while it had lower pH value at day 60 than those made with *S. thermophilus* TM11 ($P < 0.05$). Furthermore, the pH of cheese made with *S. thermophilus* SP1.1 decreased during storage ($P < 0.05$).

Effect of strains on the texture of CRMP

The biplot of PCA for the textural variables of CRMP is shown in Figure 1. Since the dimensionality reduction normalizes data without losing the information of original data, the differences among the different treatments can be observed from each dimensionality. The use of *S. thermophilus* TM11 generally resulted in cheeses with lower hardness, springiness, chewiness, gumminess and resilience and higher adhesiveness and cohesiveness than strains of non EPS-producing, regardless of the storage time. The textural differences between the EPS cheese and the non-EPS cheese could be seen clearly with the new variable PC1 (81.7%). By PC 2 (15.2%), the differences from the storage of cheeses could also be found. The later periods of storage had low values of PC 2 in cheeses A and B, while the opposite result was found in cheese C. This finding might be due to mild degradation of proteins. The textural changes during cheese ripening could be attributed to the proteolysis of cheeses (Pappa et al., 2007), which led to decrease in hardness of the cheese (Fox et al., 1996). The hardness of cheese C slightly decreased in later periods of storage. However, the

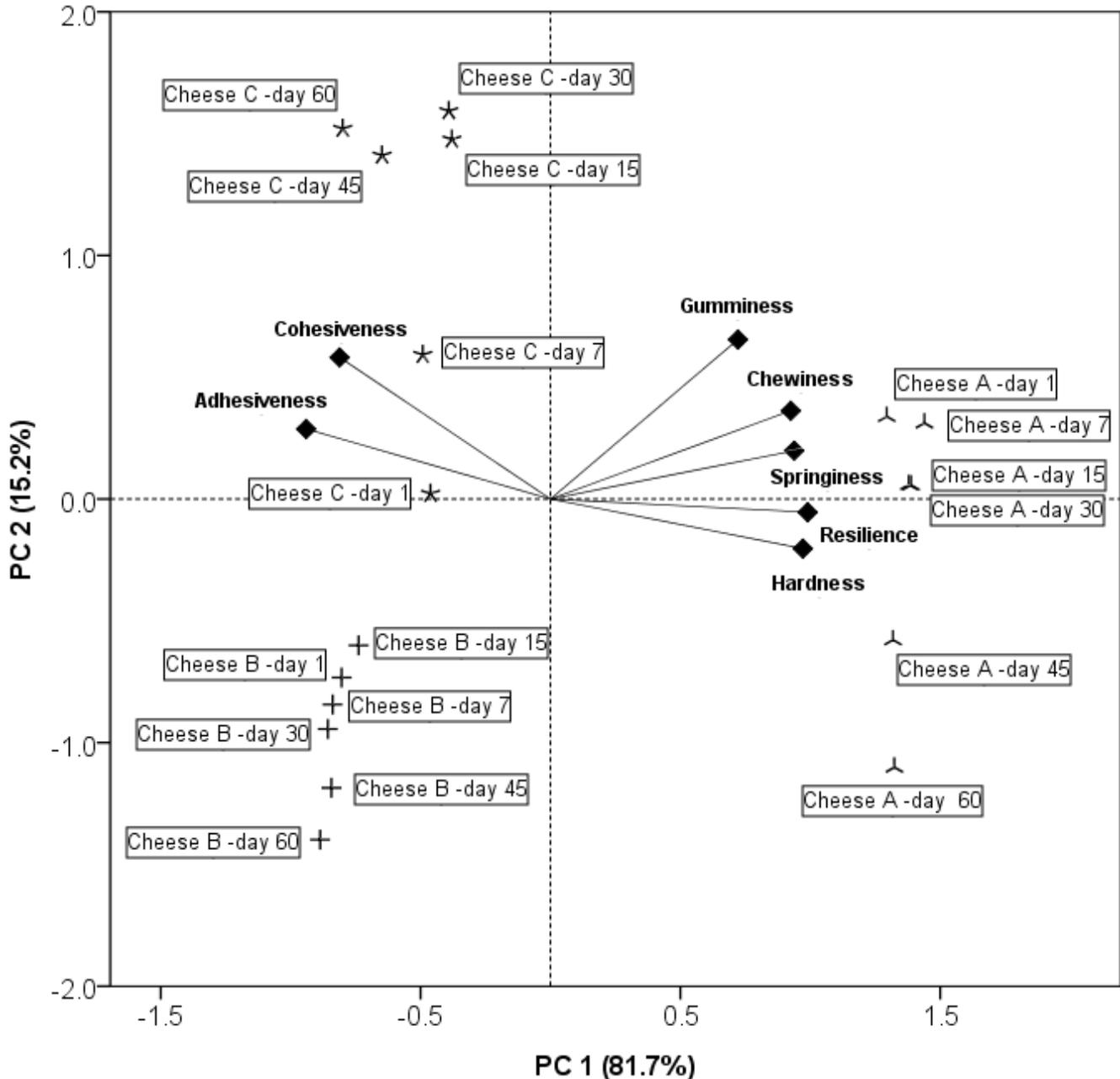


Figure 1. Biplot of PCA on the textural variables of CRMP.

hardness of cheeses A and B increased slightly in the corresponding periods.

Texture is one of the most important characteristics of cheese that determines identity and acceptability (Pappa et al., 2007). Low-fat cheese has a rubbery, dry and hard body, and texture (Mistry and Anderson, 1993). The use of some special cultures can improve the body and texture of the low-fat cheese due to the high proteolysis levels (Katsiari et al., 2002b). In the research, the texture of CRMP was improved by the increased moisture retention using the EPS-producing *S. thermophilus* TM11, as well

as the proper proteolysis of *L. delbrueckii* ssp. *bulgaricus* 34.5 and *L. lactis* ssp. *lactis* X9C2.

Effect of strains on the microstructure of CRMP

The microscopic studies of CRMP on day 45 of storage were demonstrated in Figure 2. The cheeses exhibited their protein matrix in which few fat globules and substantially larger whey pockets were dispersed compared to full-fat cheese. The reduced-fat cheese displays a

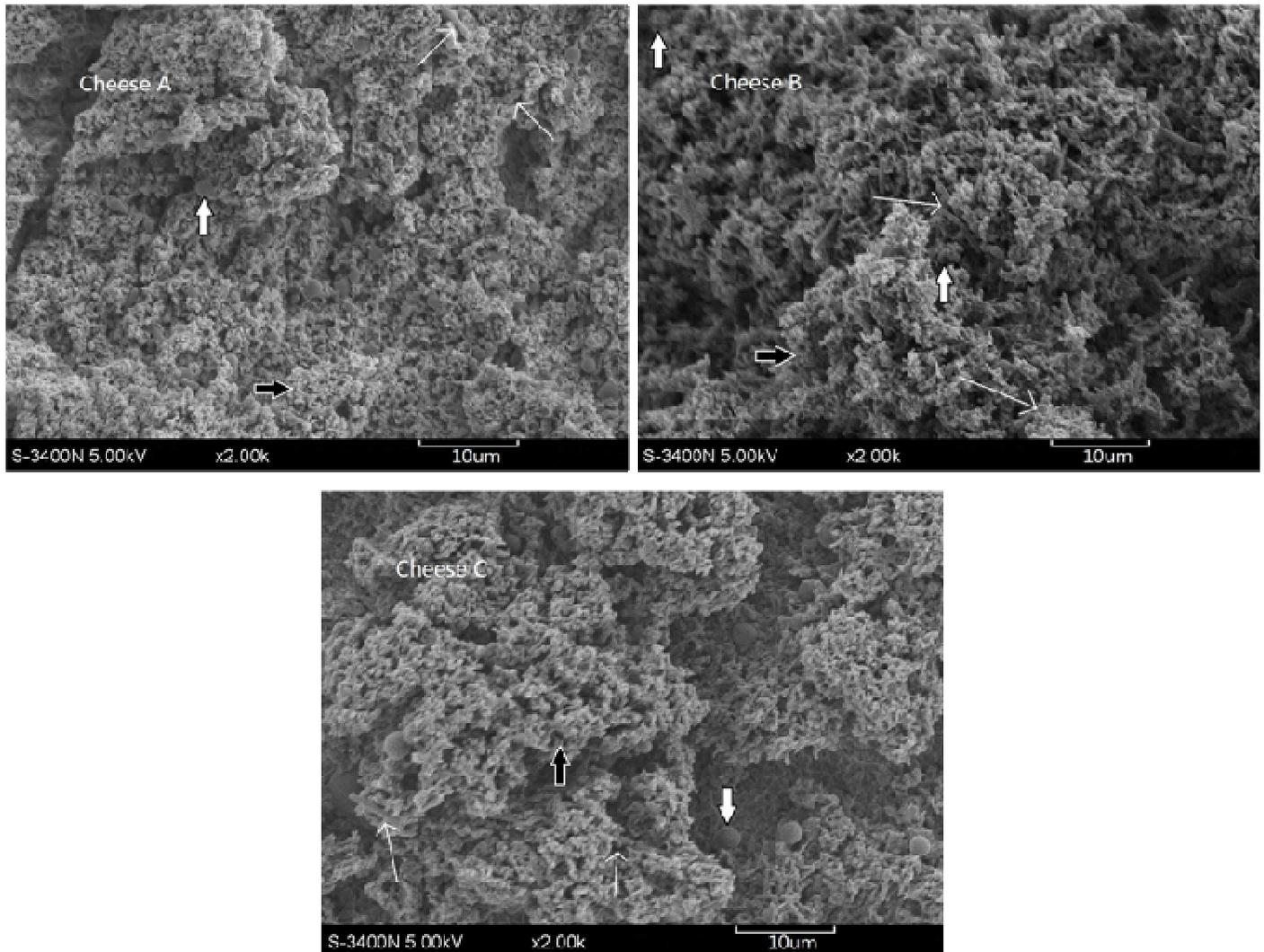


Figure 2. Microstructure of CRMP on day 45 of storage. The white thick arrow points to fat globule. The black thick arrow points to protein matrix. The thin arrow points to the bacteria.

stretched protein matrix with few fat globules scattered in between, which might be responsible for firmer characteristics compared with full-fat cheese (Drake et al., 1996). The overall structure of cheese A was more compact and dense compared with cheeses B and C. However, the water in large pockets would lose its stability under pressure or long storage causing the dehydration of cheese A (Table 1). On the contrary, the use of *S. thermophilus* TM11 in CRMP greatly changed the protein matrix with more open cavities. These results agreed with the reported studies in which a more open structure was observed in Mozzarella (Perry et al., 1997), Feta (Hassan et al., 2002) and Panela (Jimenez-Guzman et al., 2009) cheese made with the EPS-producing culture of *S. thermophilus*. Accordingly, these cavities could contain more water, which was in agreement with the increasing moisture and yield.

Effect of strains on the volatiles of CRMP

Eighteen volatiles were selected and compared among cheeses using PCA (Figure 3). These flavor impact compounds determined in CRMP on days 7 and 60 of storage belong to different chemical families including aldehydes, ketones, esters and acids. Butyl acetate detected only in cheese C, and ethyl acetate detected in all cheese on day 7, but not on day 60, was not included.

For many cheeses, methyl ketones are by far the most abundant compounds. The two major methyl ketones are 2-heptanone and 2-nonanone, considering the quantity of octanoic and decanoic acids present in milk fat (Molimard and Spinnler, 1996). In our research, all ketones detected were methyl ketones including acetoin (3-hydroxy-2-butanone), acetophenone and 3,5-octadien-2-one in addition to 3 alkan-2-ones and 2

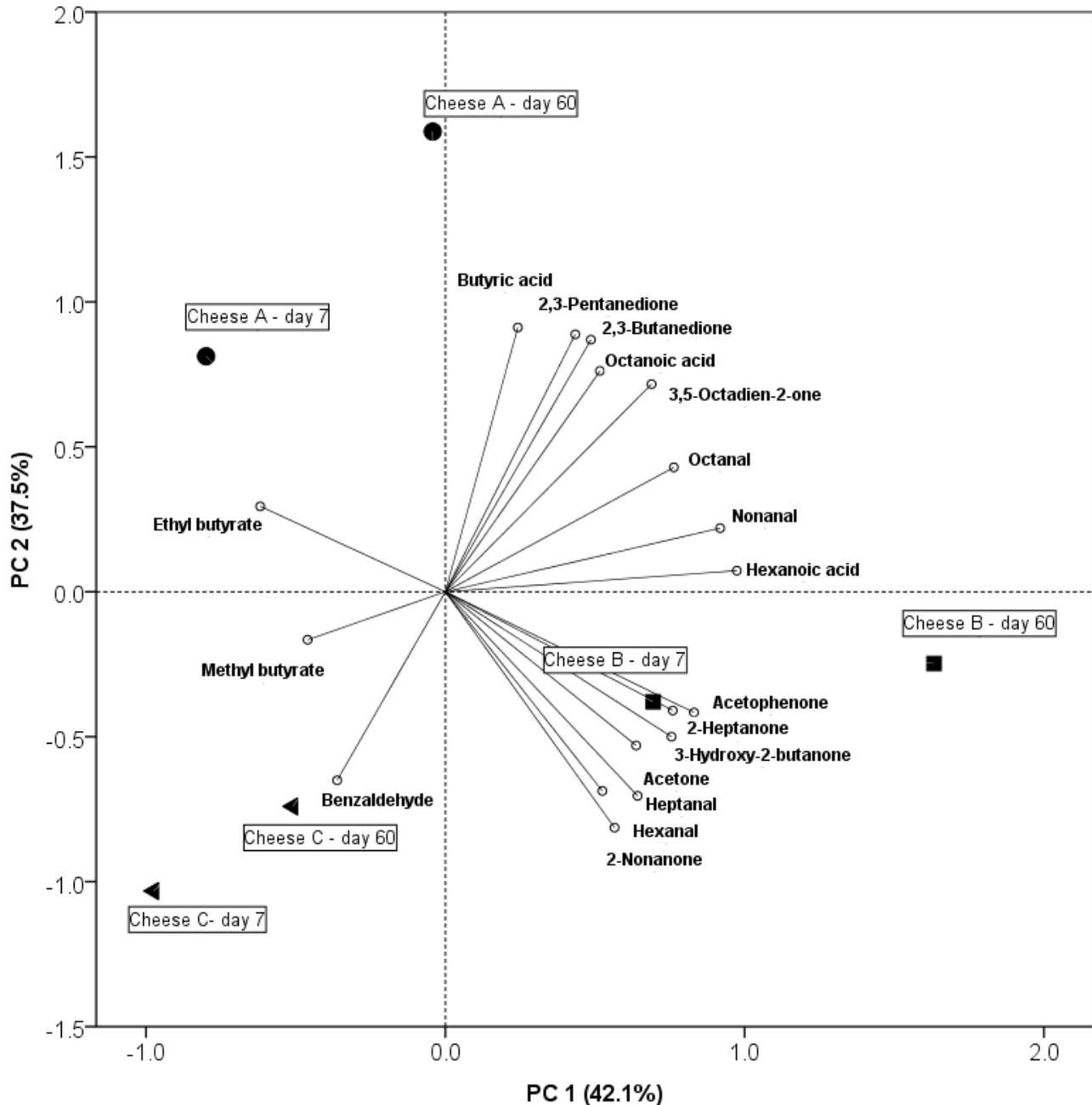


Figure 3. Biplot of PCA on the main volatiles of CRMP.

diketones. The cheeses A and C showed lower contents of 2-heptanone and 2-nonanone than cheese B ($P < 0.05$). The results suggest that the strains had different abilities of synthesis and degradation for 2-heptanone and 2-nonanone. Two metabolism pathways of methyl ketones have been identified: The degradation of corresponding fatty acid and the successive β -oxidation of long-chain fatty acid (Dartey and Kinsella, 1973), both of which can be controlled by the enzymes from bacteria and indigenous enzymes from milk. The production of diacetyl and acetoin mainly depends on the activity of lactic acid bacteria.

Moreover, heat treatment of milk powder might slightly produce diacetyl and acetoin (Yuceer et al., 2009). In this research, the contents of diacetyl in cheese C (AR, 1.13 and 1.45 on days 7 and 60, respectively) were lower than those of cheeses A (AR, 1.91 and 2.43 on days 7 and 60, respectively) or B (AR, 1.76 and 2.06 on days 7 and 60, respectively) ($P < 0.05$).

Among the cheeses, cheese B showed the highest contents of hexanal and heptanal, while cheese C showed the highest content of benzaldehyde and the lowest contents of octanal and nonanal (Figure 3). Butyric and

hexanoic acids are common odor-active free fatty acids contained in cheeses (Carpino et al., 2004). Lower contents of butyric, hexanoic and octanoic acids were found in cheese C than the other two cheeses in our research. Besides ethyl acetate and butyl acetate, ethyl butyrate and methyl butyrate in cheese C displayed higher contents than other cheeses, thus suggesting that esters were produced by the organism, *Lactococcus lactis* strain. This finding agreed with the study of Centeno et al. (2002). The combination of *L. lactis* ssp. *lactis* X9C2 with EPS-producing *S. thermophilus* TM11 resulted in providing a novel and proper flavor to CRMP.

Conclusion

In this study, the adaptability of specialized combined strains on reduced-fat CRMP was analyzed. The use of EPS-producing *S. thermophilus* TM11 could slightly increase the yield and hold the moisture of cheese during the storage. The strain could improve the texture due to the change in microstructure of CRMP. It was suggested that the EPS-producing strain could solve the textural problem of CRMP originated from the reduction of fat and use of the reconstituted milk. On the other hand, the major volatiles in CRMP such as aldehydes, ketones, acids and esters were greatly influenced by the strains employed. Overall, the screening and combination of specialized strains in CRMP could be alternatives for improving the textural and flavor attributes. It is therefore recommended that the research about specialized strains should be further performed on the other attributes of CRMP.

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