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ORIGINAL ARTICLE

Study of *Mucor spp.* in semi-hard cheese ripening

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Abstract A strain of *Mucor spp.* isolated from a fermented soybean food in central China was smeared on the surface of semi-hard cheese to investigate its role in cheese ripening. Cheese ripening indices showed increase by 3–4 fold upon ripening of cheese for 90 days. Electrophoretic studies revealed that caseins in the cheese were degraded gradually during ripening forming several low molecular weight peptides, especially of 14.4 kDa. Texture profile analysis and microstructure study of the cheese showed that the proteolytic activity of *Mucor* led to desirable texture development in ripened cheese. The study indicates that the strain of *Mucor* used in the experiment has desirable proteolytic capability for use in cheese system.

Keywords *Mucor* · Cheese · Ripening · Proteolysis · Texture · Microstructure

Introduction

Mould has an important role in cheese processing, especially for surface mould-ripened cheeses such as Brie (Fernández-Salguero 2004), Camermbert (Gaborit et al. 2001), Neufchatel (Fernández-Salguero 2004), Gamalost (Sienkiewicz-Szapka et al. 2009), and interior mould-ripened cheeses such as Stilton (Ercolini et al. 2003), Roquefort (Dall'Asta et al. 2008), Gorgonzola (Carminati et al. 2004) and Tulum cheeses (Cakmakci et al. 2008). Mould metabolism and degradation of milk constituents i.e. protein, fat by exogenous and endogenous enzymes lead to an improvement in texture, flavor and nutritional quality of cheeses. Specific texture and desirable flavor of surface mould-ripened cheese are correlated with proteolytic and lipolysis changes respectively. Moulds used in cheese include *Penicillium roqueforti*, *Geotrichum candidum/P. candidum*, *P. caseicolum* and *P. camemberti* and its mutant (Gripon 1993). Fernández-Salguero (2004) reported that gradual hydrolysis of proteins led to the formation of low molecular weight compounds in Blue cheese. Complete hydrolysis of both αs1- and β-caseins was reported in Blue cheese (Gripon 1993). O'Sullivan et al. (2005) studied the soluble nitrogen and amino acid content of cheese made and ripened with *P. camemberti* using animal and vegetable rennets.

Mao-tofu, a fermented soybean food covered with white fungus (*Mucor*) mycelia, is produced in central China. The processing of Mao-tofu is similar to that of mould-ripened cheeses but with a shorter ripening time (~5–6 days) (Zhao and Zheng 2009). It was envisaged that such *Mucor spp.* used in Mao-tofu may have potential application in surface smeared semi-hard cheese.

Materials and methods

Materials Fresh milk was obtained from a local dairy farm. Chymosin (Maxiren-180, 1:10000 strength) and casein were obtained from DSM Food Specialties, Holland and Sigma Chemical Co, St Louis, MO, USA respectively. Water used was purified by Milli-Q Plus (Millipore Corp, Bedford, MA, USA). The chemicals and reagents used in capillary zone electrophoresis (CZE) analysis were chromatogram reagents while other chemicals and reagents were of analytical grade.

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Strain and culture conditions A strain of *Mucor spp.* with proteolytic activity was isolated from local Mao-tofu product with casein plate medium by transparent ring method, and identified as *Mucor micheli ex fries*. The *Mucor spp.* was cultured in slope medium (in gL^{-1}); sodium nitrate 3, sucrose 30, potassium acid phosphate 1, magnesium sulfate 0.5, potassium chloride 0.5, ferrous sulfate 0.01, agar, 20. The culture was maintained at 28± 1 °C for 48 h in an incubator and then kept at 4 °C before use. The spore suspension of *Mucor* was prepared as per the method of Sparringa and Owens (1999).

Cheese manufacture and ripening Whole fresh milk was pasteurized in a vat at 63 °C for 30 min. After acidification of milk with lactic acid solution (10% w/w) to a pH of about 5.7, calcium chloride and potassium nitrate were added at a level of 0.016% (w/w) and 0.014% (w/w), respectively. Chymosin was added to the milk at 33 °C at a level of 0.0034% (w/w). The curd was cut into cubes (1 cm^3) and whey was drained three times at 15 min interval, after raising the temperature to 35 °C. The curd was pressed at 0.3 MPa for 2 h in a cylindrical mould (15 cm diameter, 30 cm height). It was then removed from mould and cut into cubes (8×4×4 cm). The cheese was smeared with *Mucor spp.* on the surface of cheese curd after salting in refrigerated brine (5% w/w) for 18 h, and cultured at 28±1 °C for 24 h. Later on, all the cheese samples were transferred to a ripening chamber maintained at a temperature of 4±1 °C and a relative humidity of 85%–90% until they were subjected to analysis.

Ripening indices of cheese The cheese samples were selected at random after fixed interval of time of ripening, and divided into central and external zones as described by Sihufe et al. (2007). Water-soluble nitrogen (WSN) fraction of the samples was prepared (Durrieu et al. 2006). Further, pH 4.6-soluble nitrogen (SN) and pH 4.6 insoluble nitrogen (ISN) fractions were prepared (Kuchroo and Fox 1982). A 12% trichloroacetic acid-soluble nitrogen (TCA-SN) fraction of the samples was also prepared (Polychroniadou et al. 1999).

Chemical analyses All chemical analyzes were carried out in triplicate. Moisture was determined by standard method (IDF 1982). For pH determination, the grated cheese sample (~3 g) was mixed with 10 ml of distilled water at room temperature and then homogenized using a pulp refiner (Baoli Scientific Research and Appliance Co, Jiangyin, China) for 3 min. The pH of homogenate was measured using pH meter (pH-3C, Mettler Toledo Delta 320, Shanghai, China). Total nitrogen (TN), WSN and TCA-SN were determined by Kjeldahl method (IDF 2001).

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) Two grams of pH 4.6-ISN fraction separated from pH 4.6-SN was washed three times with 6 ml of 1 $molL^{-1}$ sodium acetate buffer (pH 4.6). The residual fat was eliminated by washing with 4 ml of diethyl ether, twice. The residue (~0.2 g) was mixed with 0.2 ml Tris-buffer (0.06 $molL^{-1}$, pH 6.8) containing glycerin (25% w/v), β-mercaptoethanol (5% v/v), SDS (2% w/v) and bromophenol blue (0.1% w/v). After boiling and centrifuging at 10,000 g for 10 min, the supernatant was separated for analysis. SDS-PAGE analysis was carried out as per Laemmli (1970) using gradient gel (12% w/v). The gel was 1.5 mm thick, consisting of a 2 cm stacking gel and 10 cm running gel. Ten μL prepared sample was applied in the sample slots. The electrophoresis was carried out for 4 h at 120 V. At the end of electrophoresis, the gel was separated. Protein bands were stained with 0.25% Coomassie Brilliant Blue R-250 in methanol-water-acetic acid (5:5:1 v/v), and then destained in the same solvent. The standard protein markers and their molecular weights (kDa) used were as follows: egg albumin lysozyme 14.4, trypsin inhibitor 20.1, bovine carbonic anhydrase 31.0, rabbitactin 43.0 and bovine serum albumin 66.2. The images were photographed using PhotoDoc-It Imaging System (UVP Inc., San Gabriel, USA).

Capillary zone electrophoresis (CZE) The running buffer (50 $mmolL^{-1}$) was prepared by mixing 14.7 $molL^{-1}$ H_3PO_4, 6 $molL^{-1}$ urea solution and 0.05% (w/v) hydroxypropyl methyl cellulose. The pH of buffer was adjusted to 2.5 with 2 $molL^{-1}$ NaOH. Sample buffer (pH 8.0) comprised of 10 $mmolL^{-1}$ H_3PO_4, 8 $molL^{-1}$ urea and 10 $mmolL^{-1}$ dithiothreitol. Both buffers were filtered through 0.22 μm filter before CZE analysis. Standard casein and samples of pH 4.6-ISN fractions prepared at 35, 60 and 90 days of ripening, were dissolved in buffer with a concentration of 10 $mgml^{-1}$, then left for at least 1 h at room temperature before filtration and analysis. CZE analysis was carried out using a Beckman P/ACE™ system MDQ (Beckman Instruments Inc, USA), equipped with an UV detector, a temperature-controlled capillary compartment and an auto-sampler. Separations were performed using an uncoated fused-silica capillary column of 60 cm× 50 μm internal diameter (50 cm to the detector window). All experiments were carried out in the cationic mode (anode at the inlet and cathode at the outlet). The sample of 50 μl was introduced by pressure injection at 24 kPa for 5 s. The separation was conducted at 18.5 kV and temperature was kept constant at 30 °C. UV detection was performed at 214 nm (data collection rate 5 Hz). The capillary was washed with 0.1 $molL^{-1}$ NaOH (3 min), deionised water (5 min), 0.1 $molL^{-1}$ HCl (3 min) and equilibrated with the running buffer (5 min), before each injection. The image

obtained was analyzed by 32-Karat software (Beckman Instruments Inc, USA).

Texture profile analysis Textural properties of cheese were measured with a TA.XT2 Texture Analyzer (Stable Micro Systems Ltd, Godalming, UK) with Texture Expert for Windows (Texture exponent 32 software), equipped with a cylinder probe (type *p*/0.5). Cheese samples subjected to different ripening periods were sealed in plastic bags and tempered at 23 °C for 2 h prior to analysis. Test was carried out in three different locates of each cheese sample. The speed of the crosshead before and after analysis was kept at 2.0 mms^{-1}, and the analysis was carried out at a crosshead speed of 0.5 mms^{-1}. The measuring run was 5.0 mm and the initial force was 0N. The uniaxial compression test was performed in two successive cycles, and textural parameters were calculated as described by Bárcenas et al. (2007).

Cheese microstructure Sample preparation was done according to the method of Anderson and Mistry (1994) with some modifications by rapid refrigerating specimen in liquid nitrogen. Cheese samples were cut into flakes (~3× 3×3 mm) and fixed in 2.5% glutaraldehyde (Sigma, MO, USA) for 3 h at 4 °C. After refrigerated storage, the specimens were processed according to Oberg et al. (1993). The specimens were then mounted on a specimen holder using silver paint, gold coated and examined with scanning electron microscope (S-3400N, Hitachi, Japan) operated at 10 kV. Micrographs were obtained at magnification of 1,500 and compared visually.

Statistical analysis All the data are expressed as means ± standard deviation (SD) from at least three independent experiments. One-way analysis of variance (ANOVA) was used to evaluate the significance levels of the data. SPSS 15.0 software for Windows (SPSS Inc., Chicago, IL, USA) was used to analyze the data. The level of confidence required for significance was set at $P<0.05$.

Results and discussion

Chemical changes in cheese during ripening The moisture content in external and central zones of the cheese decreased steadily during ripening (42.8%–43.3% in fresh cheese vs. 36.8%–39.5% at 90 days), while pH increased significantly as a result of proteolysis, leading to formation of amine alkaline compounds (~5.7 in fresh vs. 6.7–6.8 in cheese at 90 days) (Table 1).

Proteolysis in cheese leads to an increase in the soluble nitrogen fractions (Sousa et al. 2001), which is considered as an index of proteolysis. The proteolysis indices viz., WSN/TN increased 4-fold, while pH 4.6-SN/TN and TCA-SN/TN exhibited 3-fold increase during ripening (Table 1). The WSN/TN increased from 4.54 (external) or 4.55 (central) to 23.68 (external) or 21.45% (central); pH 4.6-SN/TN increased from 4.59 (external) or 4.16 (central) to 19.38 (external) or 18.55% (central), while TCA-SN/TN increased from 1.91 (external) or 1.96 (central) to 8.61 (external) or 8.08 (central) upon ripening. Such an increase in proteolysis indices indicated protein degradation during cheese ripening. Such proteolysis was found to be greater in external than in central zone of cheese. This could be attributed to the growth of *Mucor* at the surface of the cheese. Majority of the proteolysis took place during the initial 35 days of ripening; further ripening (55 days) contributed to only 10%–30% rise in soluble nitrogen fraction.

The pH 4.6-insoluble nitrogen fractions separated from the cheese were subjected to SDS-PAGE and CZE, the profiles of which are presented in Figs. 1 and 2. In Fig. 1,

Table 1 Moisture, pH and proteolytic indices of cheese during ripening[a]

Parameters	Location	Ripening times			
		1	35	60	90
Moisture (%)	External	42.83±0.48[a]	39.23±1. 29[ab]	36.79±1.59[b]	36.78±1.14[b]
	Central	43.28±0.40[a]	41.30±0.59[ab]	39.81±0.84[b]	39.51±0.85[b]
pH	External	5.71±0.08[a]	6.27±0.45[b]	6.73±0.17[bc]	6.82±0.24[c]
	Central	5.70±0.16[a]	6.21±0.68[b]	6.54±0.22[bc]	6.68±0.29[c]
WSN/TN (%)	External	4.54±0.93[a]	20.61±1.67[b]	22.04±1.94[bc]	23.68±1.07[c]
	Central	4.55±0.73[a]	18.64±2.12[b]	20.62±0.55[bc]	21.45±0.93[c]
pH 4.6 SN/TN (%)	External	4.59±0.66[a]	17.65±0.67[b]	17.90±0.73[bc]	19.38±1.31[c]
	Central	4.16±0.34[a]	16.53±1.21[b]	16.90±0.92[bc]	18.55±0.93[c]
12%TCASN/TN (%)	External	1.91±0.78[a]	6.82±0.23[b]	7.71±0.74[bc]	8.61±0.85[c]
	Central	1.96±0.72[a]	6.07±0.55[b]	7.21±1.16[bc]	8.08±0.56[c]

[a] Each value was expressed as mean ± standard deviation. Number of trials was three. Different alphabets as superscripts in same row indicates data differ significantly ($P<0.05$)

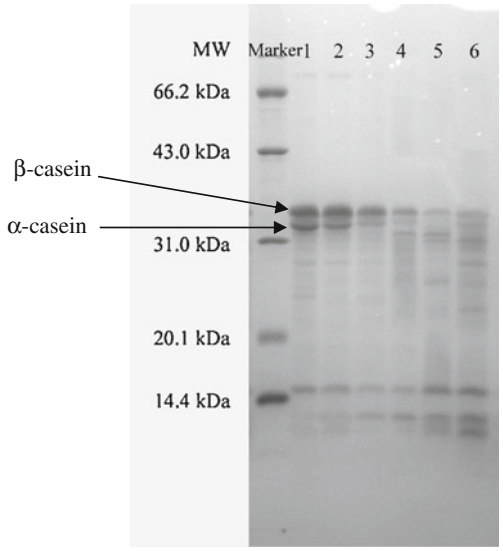

Fig. 1 SDS-PAGE profiles of pH 4.6-insoluble nitrogen fraction of cheese at different ripening periods. Standard protein markers with molecular weight—bovine serum albumin (66.2 kDa), rabbit actins (43.0 kDa), bovine carbonic anhydrase (31.0 kDa), trypsin inhibitor (20.1 kDa) and egg albumin lysozyme (14.4 kDa); Lane 1–6 depicts pH 4.6-insoluble nitrogen fraction separated from cheese ripened for 1, 7, 14, 35, 60 and 90 days respectively

there appeared mainly two bands with high molecular weight (about 32.0~33.0 kDa, β-casein and α-casein) in the fresh cheese (lane 1). As ripening progressed, the color of bands became weaker, while new bands having small molecular weight appeared, which became deeper (lane 1 vs. lane 2–6), indicating gradual degradation of caseins. When the cheese was ripened beyond 60 days, more peptides with molecular weight lower than 14.4 kDa were generated. Figure 2a showed that the migration time of standard casein was in the range of 15–30 min, exhibiting six peaks. As ripening progressed, new peaks appeared at different zones (Fig. 2b–d). For example, one peak with a migration time of about 10 min appeared, together with few peaks with migration times longer than 32 min. The peaks of casein nearly disappeared, when the cheese samples were ripened for 90 days, implying that protein degradation occurred during cheese ripening. Such decrease in peak height of α- and β-casein during cheese ripening was observed by Irigoyen et al. (2000).

Ong et al. (2007) observed several protein bands with molecular weight in the range 7.4–30.2 kDa during ripening of Cheddar cheese using SDS-PAGE technique. Vannini et al. (2008) found that on degradation of casein, more peptides with molecular weight less than 6.3 kDa were formed during ripening of Pecorino cheese.

Textural profile of cheese during ripening The textural parameters of cheese evaluated are depicted in Table 2. In general, all the textural parameters evaluated viz., hardness,

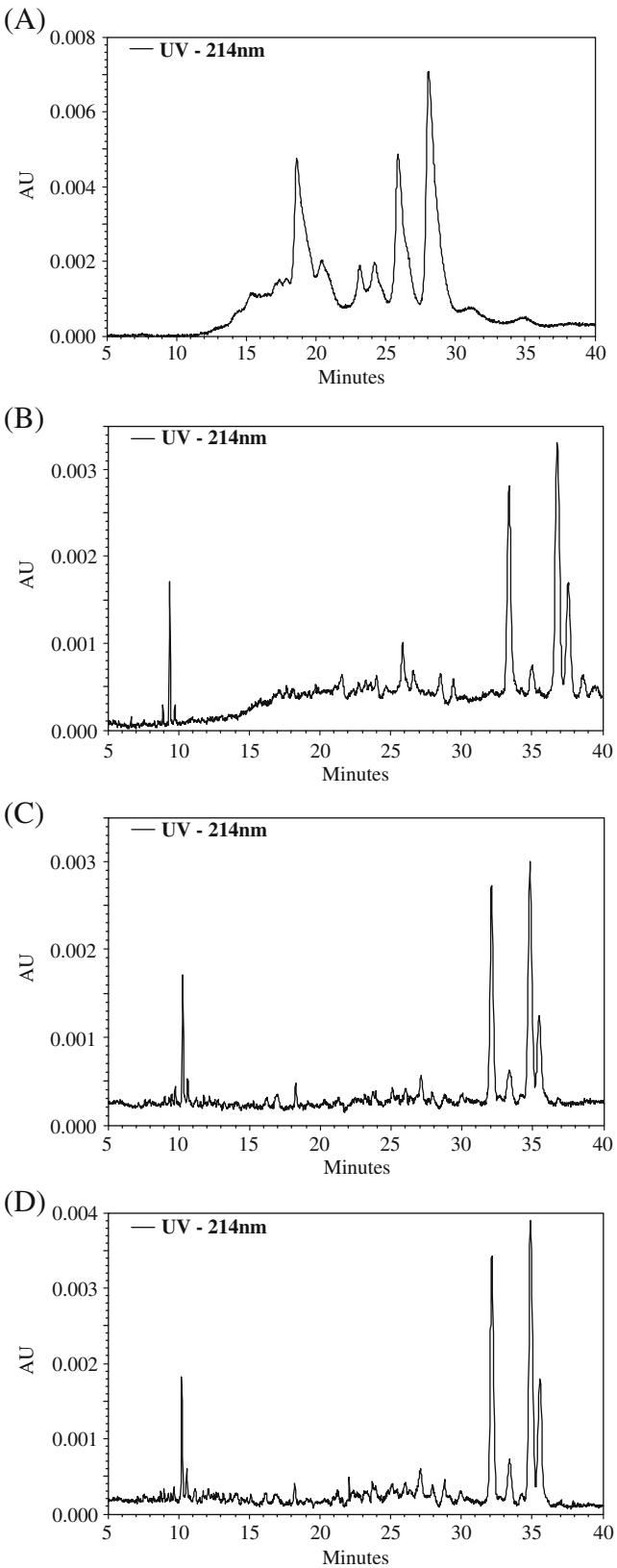

Fig. 2 CZE profile of pH 4.6-insoluble nitrogen fraction of cheese at different ripening period. **a**- standard casein, **b**, **c** and **d**—pH 4.6-insoluble nitrogen fraction separated from cheese ripened for 35, 60 and 90 days respectively

Table 2 Texture profile of cheese during different stages of ripening[a]

Time (d)	Hardness (g)	Chewiness (g)	Adhesiveness (g sec)	Cohesiveness	Springiness	Resilience
1	1348.18±499.62[a]	510.06±349.50[a]	−23.82±13.62[a]	0.80±0.03[a]	0.86±0.05[a]	0.47±0.07[a]
7	1304.85±190.75[a]	531.66±38.45[b]	−69.84±15.27[ab]	0.73±0.09[ab]	0.87±0.09[a]	0.47±0.01[a]
14	950.44±38.07[bc]	396.59±68.27[c]	−96.08±7.69[ab]	0.65±0.05[b]	0.90±0.01[b]	0.39±0.09[b]
21	908.89±67.86[bc]	334.16±29.10[c]	−123.11±57.37[b]	0.62±0.06[b]	0.82±0.04[ac]	0.30±0.07[b]
28	766.68±131.44[c]	348.95±94.48[c]	−247.47±139.12[c]	0.66±0.05[b]	0.61±0.06[d]	0.24±0.06[b]
35	439.98±68.14[d]	219.52±58.49[d]	−569.32±128.33[d]	0.61±0.07[bc]	0.78±0.25[c]	0.20±0.03[c]
60	413.23±36.06[d]	163.86±58.12[e]	−392.03±127.13[cd]	0.58±0.04[c]	0.62±0.04[d]	0.21±0.03[c]
90	436.42±159.61[d]	234.23±114.56[d]	−187.48±99.33[bc]	0.59±0.06[c]	0.58±0.06[d]	0.25±0.03[c]

[a] Values expressed as mean ± standard deviation; number of trials—three; Superscripts bearing different alphabets indicates values differ significantly ($p<0.05$) from each other

chewiness, cohesiveness, adhesiveness, springiness and resilience, showed a decrease in value with progress of cheese ripening. In general, all the textural parameters evaluated viz., hardness, chewiness, cohesiveness, adhesiveness, springiness and resilience, showed a decrease in value with progress of cheese ripening. The hardness of cheese decreased continuously significantly ($P<0.05$) from 1348.18 to 413.23 g at 60th day, after which it tended to increase slightly to 436.42 g at 90th day. The chewiness and cohesiveness of cheese also behaved in a manner similar to that for hardness. The springiness of cheese tended to decrease throughout ripening. The adhesiveness

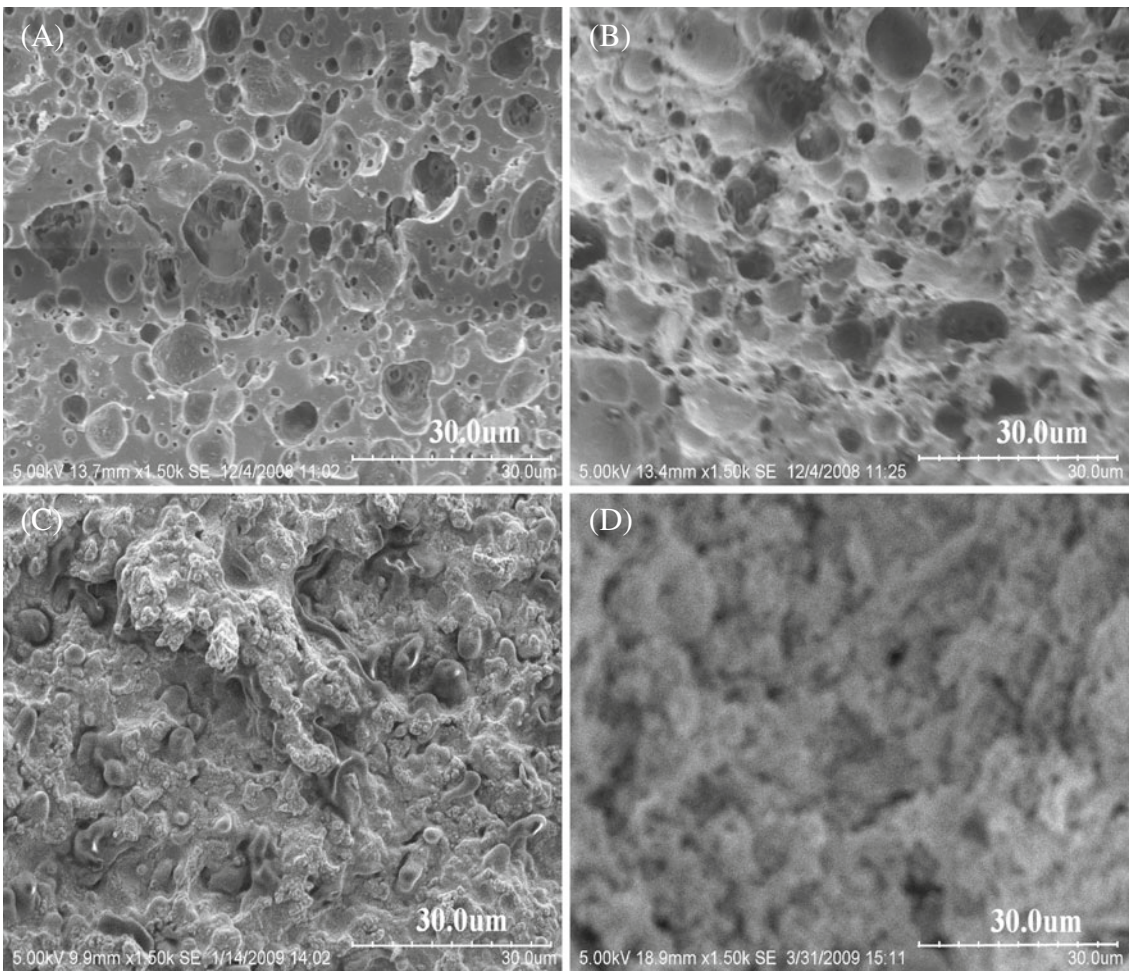

Fig. 3 Scanning Electron Microscopic structure of cheese as affected by ripening. Magnification ×1,500. SEM micrographs of cheese ripened for 1 (**a**), 35 (**b**), 60 (**c**) and 90 (**d**) days

of the cheese increased significantly ($P<0.05$) during initial 35 days of ripening but decreased subsequently. The resilience of the cheese decreased gradually till 35th day and then did not show much change.

Cohesiveness, defined as the strength of the internal bonds making up the body of the product (Szczesniak 1963), was weakened as a result of reduction in the protein interaction. Such proteolysis induced by the *Mucor spp.* led to improvement in the texture of ripened cheese as is evident from the results of textural profile analysis.

Proteolysis contributes to texture modification in ripened cheese as a result of breakdown of the protein network (Fox 1989). Van Hekken et al. (2004) found that the hardness of Monterey Jack goat's milk cheese decreased significantly during initial stage of ripening; the springiness was also increased over the first 4 weeks of ripening and thereafter did not change appreciably in either hardness or springiness over the next 5 months of ripening. Pinho et al. (2004) also observed decrease in hardness, gumminess and chewiness of Terrincho cheese during ripening.

Microstructure of cheese SEM micrographs of the ripening cheese are presented in Fig. 3a to d. The microstructure of 1 day old cheese had a smooth protein matrix, interspersed with several pore spaces. The size and shape of pore spaces were irregular and asymmetric, with diameters in the range of 5–30 μm. The microstructures of the cheeses ripened for 35, 60 and 90 days were different, validating the proteolytic role played by *Mucor* during cheese ripening.

On continued fermentation for 35 or 60 days, the cheese had a large number of smaller pore spaces, which brought about uniformity and compactness in the microstructure (Fig. 3b, c). The network structure was destroyed and the aggregates of casein micelles degraded to numerous small aggregates. At 90th day of ripening, it was difficult to discern the pore spaces in the cheese. More loose and symmetrical state in protein matrices was found, however the 3-dimensional network structure was maintained.

Luna and Bressan (1986) obtained similar finding for Fromage Fermier cheese. Fallico et al. (2006) reported that the microstructure of Piacentinu Ennese cheese after 2, 4 and 6 months of ripening changed drastically; in the end of ripening, more uniform holes were observed. The microstructure of cheese corroborated the texture modification in cheese during ripening.

Conclusion

A strain of *Mucor spp.* separated from a fermented soybean food was applied to semi-hard cheese to study its applicability in surface smeared cheeses. SDS-PAGE and CZE analysis revealed gradual degradation of caseins during cheese ripening, forming several peptides with molecular weight lower than 31.0 kDa. The ratios of WSN, pH 4.6-SN and TCA-SN to total nitrogen (TN) showed three to 4-fold increases during 90 days of cheese ripening as a result of proteolysis. Texture profile analysis revealed decrease in hardness, chewiness, cohesiveness, adhesiveness, springiness and resilience of cheese during ripening with attendant improvement in its texture. SEM micrographs authenticated the texture modification in ripened cheese, leading to uniform and compact network structure. The *Mucor spp.* studied may have potential application in manufacture of surface smear mould ripened cheeses. Further studies on associated lipolysis and flavor formation in cheese needs to be attempted.

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References

Anderson DL, Mistry VV (1994) Reduced fat cheddar cheese from condense milk. 2: microstructure. J Dairy Sci 77:7–15

Bárcenas P, Pérez-Elortondo FJ, Albisu M (2007) An international ring trial for the sensory evaluation of raw ewes' milk cheese texture. Int Dairy J 17:1139–1147

Cakmakci S, Dagdemir E, Hayaloglu AA, Gurses M, Gundogdu E (2008) Influence of ripening container on the lactic acid bacteria population in Tulum cheese. World J Microbiol Biotechnol 24:293–299

Carminati D, Gatti M, Bonvini B, Neviani E, Mucchetti G (2004) High-pressure processing of Gorgonzola cheese: influence on *Listeria monocytogenes* inactivation and on sensory characteristics. J Food Prot 67:1671–1675

Dall'Asta C, De Dea LJ, Galaverna G, Dossena A, Neviani E, Marchelli R (2008) The occurrence of ochratoxin A in blue cheese. Food Chem 106:729–734

Durrieu C, Degraeve P, Chappaz S, Martial-Gros A (2006) Immunomodulating effects of water-soluble extracts of traditional French Alps cheeses on a human T-lymphocyte cell line. Int Dairy J 16:1505–1514

Ercolini D, Hill PJ, Dodd CER (2003) Bacterial community structure and location in Stilton cheese. Appl Environ Microbiol 69:3540–3548

Fallico V, Tuminello L, Pediliggieri C, Horne J, Carpino S, Licitra G (2006) Proteolysis and microstructure of Piacentinu Ennese cheese made using different farm technologies. J Dairy Sci 89:37–48

Fernández-Salguero J (2004) Internal mould-ripened cheeses: characteristics, composition and proteolysis of the main European blue vein varieties. Ital J Food Sci 16:437–445

Fox PF (1989) Proteolysis during cheese manufacture and ripening. J Dairy Sci 72:1379–1400

Gaborit P, Menard A, Morgan F (2001) Impact of ripening strains on the typical flavour of goat cheese. Int Dairy J 11:315–325

Gripon JC (1993) Mould-ripened cheeses. In: Fox PF (ed) Cheese: chemistry, physics and microbiology, vol 2, 2nd edn. Chapman and Hall, London, pp 111–136

IDF (1982) Determination of the total solids contents: Gravimetric (Cheese and processed cheese). IDF Standard 4 A, Brussels, Belgium

IDF (2001) Milk—Determination of nitrogen content—Part 1: Kjeldahl method. IDF Standard 20-1. Brussels, Belgium

Irigoyen A, Izco JM, Ibáñez FC, Torre P (2000) Evaluation of the effect of rennet type on casein proteolysis in an ovine milk cheese by means of capillary electrophoresis. J Chromatogr 881:59–67

Kuchroo CN, Fox PF (1982) Soluble nitrogen in Cheddar cheese: comparison of extraction procedures. Milchwissenschaft 37:331–335

Laemmli UK (1970) Cleavage of structural protein during the assembly of the head of bacteriophage T4. Nature 227:680–685

Luna JA, Bressan JA (1986) Mass transfer during brining of cuartirolo argentino cheese. J Food Sci 51:829–831

O'Sullivan N, Jsousa M, O'Grady-Walsh D, Uniacke T, Kelly A, McSweeney PLH (2005) Ripening of Camembert-type cheese made from caprine milk using calf rennet or kid rennet as coagulant. Int J Dairy Technol 58:13–20

Oberg CJ, McManus WR, McMahon DJ (1993) Microstructure of Mozzarella cheese during manufacture. Discussion. Food Struct 12:251–258

Ong L, Henriksson A, Shah NP (2007) Proteolytic pattern and organic acid profiles of probiotic Cheddar cheese as influenced by probiotic strains of *Lactobacillus acidophilus*, *Lb. paracasei, Lb. casei* or *Bifidobacterium sp*. Int Dairy J 17:67–78

Pinho O, Mendes E, Alves MM, Ferreira IMPLVO (2004) Chemical, physical and sensorial characteristics of "Terrincho" ewe cheese: changes during ripening and intravarietal comparison. J Dairy Sci 87:249–257

Polychroniadou A, Michaelidou A, Paschaloudis N (1999) Effect of time, temperature and extraction method on the trichloroacetic acid soluble nitrogen of cheese. Int Dairy J 9:559–568

Sienkiewicz-Szapka E, Jarmo Owska B, Krawczuk S, Kostyra E, Kostyra H, Iwan M (2009) Contents of agonistic and antagonistic opioid peptides in different cheese varieties. Int Dairy J 19:258–263

Sihufe GA, Zorrilla SE, Mercanti DJ, Perotti MC, Zalazar CA, Rubiolo AC (2007) The influence of ripening temperature and sampling site on the lipolysis in Reggianito Argentino cheese. Food Res Int 40:1220–1226

Sousa MJ, Ardö Y, McSweeney PLH (2001) Advances in the study of proteolysis during cheese ripening. Int Dairy J 11:327–345

Sparringa RA, Owens JD (1999) Protein utilization during soybean tempe fermentation. J Agr Food Chem 47:4375–4378

Szczesniak AS (1963) Objective measurements of food texture. J Food Sci 28:410–420

Van Hekken DL, Tunick MH, Park YW (2004) Rheological and proteolytic properties of monterey jack goat's milk cheese during aging. J Agr Food Chem 52:5372–5377

Vannini L, Patrignani F, Ucci L, Ndagijimana M, Vallicelli M, Lanciotti R, Guerzoni ME (2008) Effect of a pre-treatment of milk with high pressure homogenization on yield as well as on microbiological, lipolytic and proteolytic patterns of "Pecorino" cheese. Int J Food Microbiol 128:329–335

Zhao XH, Zheng XT (2009) A primary study on texture modification and proteolysis of mao-tofu during its fermentation. Afr J Biotechnol 8:2294–2300