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Short communication

The effects of κ-casein polymorphism on the texture and functional properties of mozzarella cheese

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ABSTRACT

The present study compared the texture and functional properties of mozzarella cheese made with milk containing different of genetic polymorphisms κ-casein (AA, AB, AE or BE). The genotype of κ-casein in the milk from individual Holstein cow was determined by pyrosequencing method. Full-fat Mozzarella cheese was made from pooled milk from 3 cows with the same κ-casein genotype and analysed 7 d after manufacture. The cheese made from type AB contained the highest level of fat and Ca/protein, and the lowest moisture content. The cheese made from type AB milk was harder and chewier than cheese made from type AE and BE milk. The cheese made from type AB and AA milk had higher stretchability but lower meltability and flowability than type AE and BE. In summary, the cheese made from type AB milk had different texture and functionality quality than that made from type AE or BE.

1. Introduction

The influence of the polymorphisms of caseins (α_{S1}, α_{S2}, β and κ) and whey proteins (α-lactalbumin and β-lactoglobulin) on milk curd formation and cheese-making properties is well established (Amigo, Recio, & Ramos, 2000; Caroli, Chessa, & Erhardt, 2009; Hallen, Allmere, Naslund, Andren, & Lunden, 2007); those of κ-casein may be the most important. Eleven κ-casein variants (A, B, C, E, F1, F2, G1, G2, H, I, J) are found in the *Bos* genus, with the A and B variants being the most common, and E also being rather common (Caroli et al., 2009). Compared with the reference κ-casein variant A, the B variants contained 2 AA substitutions (Asp169 → Ala and Thr174 → Ile), whereas the E variant contains 1 AA substitution (Ser176 → Gly).

In cheesemaking, κ-casein polymorphism is a critical factor in establishing total casein and κ-casein concentrations, casein micelle size and coagulating properties (Caroli et al., 2009). The κ-casein B (CSN3) variant reacts more quickly with rennet and has a significantly shorter rennet coagulation time compared with milk with other variants. The less-common CSN3 alleles (G and E) have had negative effects on coagulation properties (Caroli, Bolla, Budelli, Barbieri, & Leone, 2000; Erhardt et al., 1997). Cheese composition and yield are also influenced by κ-casein polymorphism. Research on Cheddar cheese shows that the κ-casein BB type results in significantly higher fat recoveries in cheese than AA. The content of κ-casein B is also closely correlated with cheese yield (Bonfatti et al., 2011; Walsh et al., 1998).

The effect of κ-casein allelic variants on the cheese manufacture process was showed in previous research (Comin et al., 2008; Mayer, Ortner, Tschager, & Ginzinger, 1997), but few studies have investigated their effect on cheese quality. The objectives of this study were to evaluate the effects of κ-casein allelic variations (with similar milk compositions) on the composition, texture and functional properties of mozzarella cheese.

2. Materials and methods

2.1. Experimental design

Blood from cows was typed for genetic variants of the κ-casein, β-casein and β-lactoglobulin using the pyrosequencing method (Hallen et al., 2007). Cows were placed into 4 groups based on κ-casein: AA, AB, AE and BE; all cows had β-casein A1A1 and β-lactoglobulin BB genotypes. Milk was pooled from the 3 cows with the same κ-casein genotype and used to make cheese, 3 vats were made on different days. A total 12 batches of cheese were made.

Milk samples were collected from 12 Holstein cows at the same dairy farm in Hangzhou (Zhengxing pasture, Hangzhou, China). Fat and protein were analysed by the infrared technique using the Milko Scan FT (Foss Electric, Denmark). The casein and κ-casein content in milks were determined by reverse phase high performance liquid chromatography (RP-HPLC) as described by Bonfatti, Grigoletto, Cecchinato, Gallo, and Carnier (2008). The calcium

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(Ca) and phosphorous (P) concentrations were measured using standard IDF atomic absorption methods (IDF, 1990, 1992).

2.2. Cheese manufacture

Non-standardised milk (30 L) containing either κ-casein polymorphisms AA, AB, AE or BE was pasteurised at 63 °C for 30 min, then cooled to 35 °C. Mozzarella cheese was made as described by Kuo and Gunasekaran (2009) with some modifications. The milk was inoculated with 10 g L^{-1} starter culture (Taiming biotechnology company, Shangdong, China), consisting of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in a 1:1 ratio. When the pH of the milk had decreased by 0.1 pH units, chymosin (Marzyme™, Danisco, Vinay, France), a microbial coagulant with optimum temperature at 35 °C was added at a level of 0.01 g L^{-1}. After 30 min, the curd was cut with 1-cm knives and allowed to heal for 15 min. The temperature was increased to 42 °C at a rate of 0.2 °C min^{-1}, and the curd was cooked for 30 min. After the whey was removed, the curd was trenched, cut into slabs, turned, and stacked until the pH dropped to about 5.2–5.3. Curds were then milled and dry salted (2%, w/w, of curd), prior to being mechanically heated, stretched and moulded under hot water (77 °C). Curds were formed into 1-kg loaves, immersed in cold water for 1 h, and then in brine for 90 min, and stored in vacuum-sealed barrier bags at 4–5 °C for 7 d prior to analysis.

2.3. Compositional and water activity analysis of cheese

The fat content of the cheese was determined by the Gerber method (Kleyn, Lynch, Barbano, Bloom, & Mitchell, 2001), moisture content was determined by the oven drying method (IDF, 1958), and protein content by the macro-Kjeldahl method (IDF, 1993). The pH was measured by inserting a calibrated Unicam glass Ag/AgCl-combination pH electrode attached to a pH meter (SevenEasy™ Plu, Mettler-Toledo, Switzerland) directly into the cheese at three randomly chosen locations. Water activity (a_w) was measured using a HygroPalm AW1 (Rotronic, Bassersdorf, Switzerland). All analyses were performed in triplicate. The moisture in non-fat substances (MNFS), salt in moisture (S/M) and the fat in dry matter (FDM) were calculated based on the results of the individual constituents. The calcium (Ca) content was determined as described in section 2.1.

2.4. Texture profile analysis

Textural properties were measured using a texture analyser (TA-XTPlus, Stable Micro System, England) fitted with a P/N 0.5 probe (10 mm diameter) moving at a speed of 1 mm s^{-1} for a distance of 8 mm using cycle or normal programs. Hardness, cohesiveness, springiness, gumminess and adhesiveness were calculated as described previously (Ayad, Awad, El Attar, de Jong, & El-Soda, 2004; Bourne, 1978). The analyses were conducted in triplicate.

2.5. Cheese functionality after heating

Cheese functionality, including meltability, stretchability and flowability, was tested after heating the cheeses. Flowability was measured by a modified Schreiber method, defined as the percentage increase in the diameter of a disc of cheese (Mounsey & O'Riordan, 1999). The meltability of the cheeses was determined using the Arnott test (Park, Rosenau, & Peleg, 1984), which measured the change in sample height after heating at 100 °C for 15 min. The stretchability of the molten cheese (280 °C for 4 min) was measured using an uniaxial extension apparatus operating at a velocity of 0.066 m s^{-1}, as described by Guinee and O'Callaghan (1997) and Sheehan and Guinee (2004). All tests were performed in triplicate.

2.6. Cryo-scanning electron microscopy

Cheese samples of approximately 1 mm × 5 mm × 10 mm, were cut using a razor blade from the interior of the cheese block. Samples were frozen in the machine using liquid nitrogen. Cryo-scanning electron microscopy (cryo-SEM) was conducted using a PP3000T Cryo-Preparation System (Hitachi S-3000N Scanning Microscope, Ibaraki, Japan) as previously described (Montesinos-Herrero, Cottell, O'Riordan, & O'Sullivan, 2006). Three samples were analysed from each batch of Mozzarella cheese.

2.7. Statistical analysis

Three replicate cheese-making trials were undertaken. Two-way analysis of variance (two-way ANOVA) was applied to the experimental data ($n = 3$) using the general linear model using an SPSS statistical software package (17.0), with replicate blocks and genetic variants of κ-casein as experimental factors. All differences were considered as statistically significant at $P < 0.05$. In the case of significant differences, Tukey pairwise comparisons were performed.

3. Results

3.1. Milk and cheese composition

The composition of milk with different κ-casein genotypes is shown in Table 1. The total protein and fat content, κ-casein/total protein, and Ca levels of milk were similar; however, differences ($P < 0.05$) in casein/total protein and P contents were observed. No significant difference of milk composition was found among the three batches of raw milk.

The composition of the cheeses made from milk with different κ-casein genotypes is summarised in Table 2. Compared with the other cheeses, the cheese made from type AB milk had the highest fat content and Ca/protein ratio, and the lowest protein and moisture content. Accordingly, the MNFS in the cheese made from type AB milk was significantly lower. The FDM in the cheese made from type AB milk was higher than that made from type AE and BE. These results are consistent with those of other studies (Guinee, Auty, & Fenelon, 2000; Sheehan & Guinee, 2004). Although a higher Ca/protein was found in the cheese made from type AB milk, the Ca content was similar among the four types of milk.

Table 1
The composition of milk with different κ-casein polymorphisms.[a]

Composition	Milk type			
	AA	AB	AE	BE
Protein (%, w/w)	3.25 ± 0.12	3.20 ± 0.11	3.16 ± 0.14	3.19 ± 0.18
Fat (%, w/w)	3.61 ± 0.26	3.53 ± 0.15	3.58 ± 0.33	3.66 ± 0.14
Casein/total protein (%)	84.58 ± 1.57[b]	86.23 ± 1.95[a]	82.35 ± 1.79[c]	83.11 ± 1.47[c]
κ-Casein/total protein (%)	7.89 ± 0.39	8.11 ± 0.28	7.86 ± 0.13	7.97 ± 0.27
Ca (mg 100 g^{-1} milk)	98.5 ± 3.56	101.6 ± 4.42	99.3 ± 3.42	98.9 ± 2.76
P (mg 100 g^{-1} milk)	76.4 ± 2.11[b]	79.4 ± 2.37[a]	79.2 ± 3.23[a]	74.8 ± 2.78[c]

[a] Milk samples were collected from cattle with different κ-casein polymorphisms but the same genotype of β-casein (A1A1) and β-lactoglobulin (BB). Values are means ± standard deviation; values within the same row not sharing a common letter differ significantly, $P < 0.05$.

Table 2
The composition of cheese made from milk with different κ-casein polymorphisms.[a]

Composition[b]	Cheese type			
	AA	AB	AE	BE
Moisture	47.05 ± 0.55[c]	46.93 ± 0.46[c]	48.81 ± 0.42[a]	48.02 ± 0.38[b]
Fat	22.95 ± 0.99[c]	24.69 ± 0.62[a]	23.58 ± 0.71[ab]	23.22 ± 0.58a[a]
Protein	21.68 ± 0.43[a]	20.94 ± 0.60[b]	20.73 ± 0.58[b]	21.53 ± 0.46[a]
MNFS	62.31 ± 1.29[b]	60.99 ± 0.96[c]	65.18 ± 0.81[a]	62.53 ± 0.87[b]
FDM	46.52 ± 1.78[a]	46.98 ± 1.21[a]	43.31 ± 1.13[b]	44.64 ± 1.28[ab]
Ca/protein (mg g^{-1})	27.48 ± 1.28[ab]	28.98 ± 0.72[a]	25.52 ± 0.97[b]	26.13 ± 0.69[b]
S/M	3.45 ± 0.24	3.47 ± 0.35	3.29 ± 0.29	3.36 ± 0.42
pH	5.33 ± 0.01[c]	5.34 ± 0.02[c]	5.44 ± 0.03[a]	5.39 ± 0.02[b]
a_w	0.952 ± 0.002[ab]	0.943 ± 0.002[c]	0.965 ± 0.003[a]	0.959 ± 0.004[b]

[a] Cheese made from milk with different κ-casein polymorphisms but the same genotype of β-casein (A1A1) and β-lactoglobulin (BB). Values are means ± standard deviation; values within the same row not sharing a common letter differ significantly, $P < 0.05$.
[b] Abbreviations are: MNFS, moisture in non-fat substances; FDM, fat in dry matter; S/M, salt in moisture.

3.2. pH, a_w and microstructure of cheese

The pH and a_w of the experimental cheeses are summarised in Table 2. The cheese made from type AE or BE milk had significantly higher pH values than that made from type AA or AB type cheeses. The cheese made from type AB milk had significantly lower a_w than that made from type AE or BE. The cryo-SEM images of the different types of cheeses showed a porous protein matrix with embedded lipid droplets (Fig. 1). The fat globules in the cheese made from type AB milk appear to be larger in size, mostly spherical in shape, and uniformly distributed in a continuous protein matrix. The porosity of the protein matrix in cheese made from type AE or BE milk was larger than that made from type AA and AB. This could contribute to the former cheeses having higher moisture content.

3.3. The effect of κ-casein polymorphisms on cheese texture

The texture parameters (hardness, gumminess, adhesiveness, cohesiveness, springiness, chewiness, resilience) of cheese are shown in Table 3. The hardness, gumminess and chewiness of the cheese samples made from milk with the AB type were significantly higher than the other cheeses. However, for cohesiveness, the cheese made from type AE and BE milk were significantly higher than that of cheese made from type AA or AB. A positive relationship between hardness and dry matter content was shown in this study, as reported by Ayad et al. (2004). A positive relationship was also found between hardness and gumminess in this study. However, a negative relationship was apparent between both the hardness and adhesiveness, and the hardness and springiness of the cheese samples. Cheese made from milk with the B allele of κ-casein was associated with higher hardness and chewiness, while the E allele of κ-casein was correlated with cohesiveness and resilience of the cheese.

3.4. The effect of κ-casein polymorphism on cheese functional properties

The functional characteristics of the different types of cheeses are shown in Table 4. The meltability of cheese made from type AB or AA milk was significantly lower than that made from type AE and BE milk. Similar to meltability, the mean flowability of cheeses from milk types AB or AA was significantly lower than that of AE and BE cheeses. In contrast, the stretchability of the cheese made from type AB milk was significantly higher than the other cheeses (Table 4). This disagrees with the positive correlation between flowability

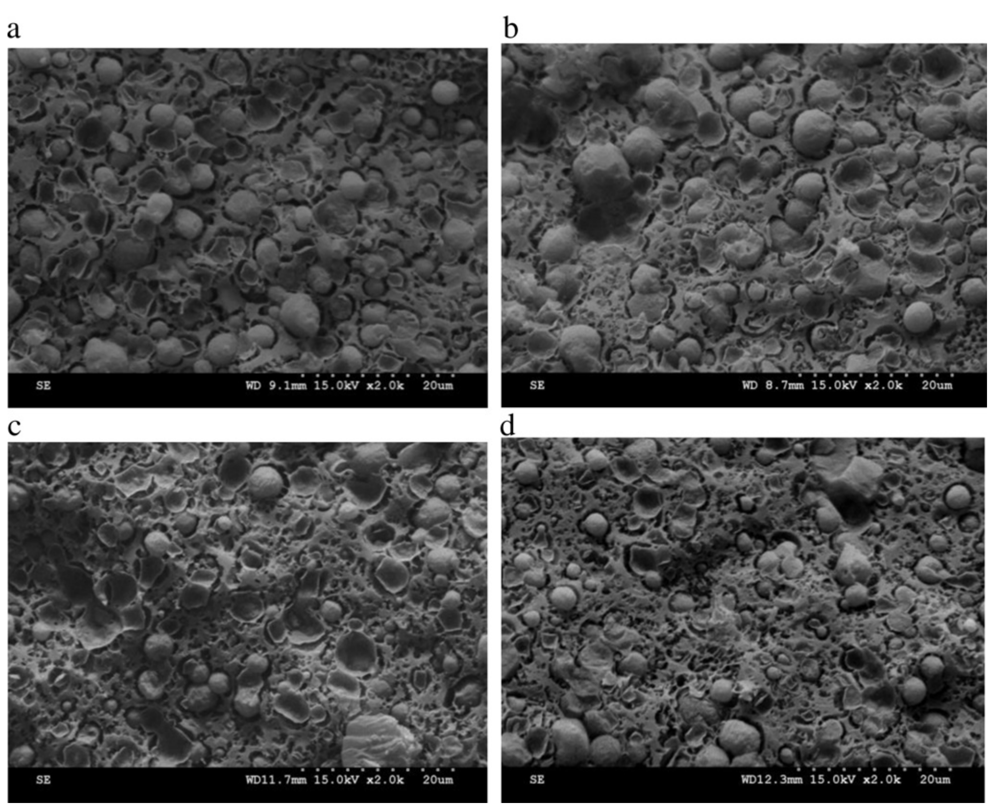

Fig. 1. Cryo-scanning electron micrographs of cheeses made from milk with different κ-casein polymorphisms: panel a, AA; panel b, AB; panel c, AE; panel d, BE.

Table 3
The texture of cheese made from milk with different κ-casein polymorphisms.[a]

Parameter[b]	Cheese type			
	AA	AB	AE	BE
Hardness (g)	329.6 ± 14.8[b]	599.6 ± 34.5[a]	191.1 ± 12.8[d]	244.7 ± 11.9[c]
Gumminess (g s^{-1})	154.8 ± 16.5[b]	290.2 ± 8.9[a]	132.8 ± 10.2[c]	156.8 ± 11.5[b]
Adhesiveness (g s^{-1})	−28.44 ± 3.45[c]	−21.61 ± 2.47[c]	−83.57 ± 3.66[a]	−48.98 ± 2.84[b]
Cohesiveness (ratio)	0.45 ± 0.04[d]	0.51 ± 0.02[c]	0.72 ± 0.05[a]	0.65 ± 0.04[b]
Springiness (mm)	0.88 ± 0.05[c]	0.83 ± 0.02[c]	0.99 ± 0.04[a]	0.93 ± 0.04[b]
Chewiness (g mm^{-1})	126.92 ± 8.25[b]	259.02 ± 10.35[a]	128.34 ± 5.47[b]	128.87 ± 6.85[b]
Resilience (ratio)	0.19 ± 0.01[c]	0.28 ± 0.02[b]	0.29 ± 0.01[b]	0.32 ± 0.02[a]

[a] Values are means ± standard deviation; values within the same row not sharing a common letter differ significantly, $P < 0.05$.
[b] Hardness, gumminess, adhesiveness, cohesiveness, springiness, chewiness, and resilience were calculated as described by Bourne (1978).

and stretchability reported by others (Guinee, Feeney, Auty, & Fox, 2002; Sheehan & Guinee, 2004).

4. Discussion

Cheese composition, texture and functional properties can be influenced by many factors, however, in this experiment, Ca and its interactions with protein may be an important factor.

Compared with other κ-casein variants, a high Ca content was found in the κ-casein AB milk, and also a high Ca/protein ratio in the cheese made from type AB milk. Higher levels of Ca in the cheese made from type AB milk means that less soluble Ca is removed from the curds during whey drainage. The protein matrix is cross-linked by Ca ions to form a bridge between proteins; a higher Ca levels can lead to a more cross-linked protein matrix (Pastorino, Ricks, Hansen, & McMahon, 2003). On the other hand, the strong protein–protein interactions also cause a higher exclusion of moisture from the cheese matrix in the syneresis step of cheese-making (McMahon, Paulson, & Oberg, 2005). The higher Ca content in the cheese made from type AB led to a lower-moisture, more rigid structure, and resulted in increased hardness and decreased melt of the cheese. This is consistent with reports by Guinee et al. (2002) and McMahon et al. (2005) that the moisture content increased as the Ca content in the cheese decreased. The inverse relationship is also consistent with the findings of model studies involving dilute suspensions of casein or para-casein (Sood, Gaind, & Dewan, 1979).

The differences in protein structure caused by different levels of Ca also can explain the differences in texture and functional properties. A higher content of Ca causes the protein fibres in the cheese matrix to contract and expel more whey (McMahon et al., 2005; Pastorino et al., 2003), which may increase the cheese hardness and decrease cohesiveness. McMahon et al. (2005) and Sheehan and Guinee (2004) reported that cheese with higher Ca content was firmer than cheese with lower Ca content. In contrast with hardness, adhesiveness of the proteins in the cheese with low Ca content was increased compared with cheese with high Ca. Similar with hardness, this can also be explained by the protein matrix in cheese. For the Cheese made from type AB milk with the higher Ca level, the proteins are highly aggregated. Thus, when the cheese made from type AB milk was cut, the proteins maintain their self-association and low adhesiveness, however, for the cheese made from type AE and BE milk with lower Ca levels, the protein are more unfolded and available to interact with other surfaces (McMahon et al., 2005).

Table 4
The effect of κ-casein polymorphism on cheese functional properties.[a]

Parameter	Cheese type			
	AA	AB	AE	BE
Flowability (%)	38.13 ± 1.24[c]	33.43 ± 0.85[c]	53.21 ± 2.51[b]	67.54 ± 2.34[a]
Meltability (%)	63.75 ± 1.25[b]	58.08 ± 3.42[b]	78.76 ± 2.59[a]	74.41 ± 1.89[a]
Stretchability (cm)	25.67 ± 2.18[b]	48.33 ± 2.54[a]	13.67 ± 1.15[c]	16.83 ± 1.53[c]

[a] Values are means ± standard deviation; values within the same row not sharing a common letter differ significantly, $P < 0.05$.

The melt and flowability of cheese is strongly related to the Ca content and protein matrix. As stated earlier, the protein interactions within the cheese increased as the Ca content increases, and thus more energy is required to break this interaction and to allow the proteins to flow. In the cheese made from type AE or BE milk, higher protein hydration and lower protein interactions are found at lower Ca levels, and thus less energy was needed to break the bonds between the proteins (McMahon, Fife, & Oberg, 1999). However, in contrast to the trends noted for flowability and meltability, the stretchability of the cheese made from type AB milk was significantly higher than that of the other cheeses. This can also be attributed to the protein interaction and hydration caused by Ca. When the cheese made from type AB milk was stretched, more power was needed to break the protein interactions, so that cheese could be stretched further under the same applied force. In contrast, other researchers have reported that the stretchability was correlated with flowability (Guinee et al., 2002; Sheehan & Guinee, 2004), and meltability (Wadhwani, McManus, & McMahon, 2011). These differences may be attributed to the difference in making procedure and storage time.

The genetic variation of κ-casein may be another important factor that can influence the composition, texture and functional properties of cheese. Prior studies have shown that the allelic variants of κ-casein were associated with variation in the total casein and κ-casein concentrations in milk, variation in casein micelle size, and differences in coagulating properties (Hallen et al., 2007; Vandenberg, Escher, Dekoning, & Bovenhuis, 1992; Walsh et al., 1998). In this study, higher casein/total protein ratios and κ-casein/total protein (although not significant) were found in κ-casein type AB milk. This may lead to a firmer milk gel, because of higher protein density and more cross-linked casein matrix in curd.

Because the κ-casein genetic variation also relates to protein structure and function, the protein quality influences milk coagulation traits, more than protein quantity (Hallen et al., 2007; Pagnacco & Caroli, 1987). Different amino acid sequence, glycosylation sites and phosphorylation sites of κ-casein are found among the different genetic variants of κ-casein (Jensen, Holland, Poulsen, & Larsen, 2012), and may be a contributory factor for the significant difference of coagulation traits between the milk with κ-casein type B and other types (G and E). Conformation changes in κ-casein that alters the binding site which would then influence κ-casein degradation and gel formation. Phosphorylation sites of κ-casein are associated with the ability of casein to bind with calcium phosphate (Mamone et al., 2003). These differences may also be related to protein cross-linking and protein hydration. Further studies are needed to understand these differences in protein quality, especially in phosphorylation among the κ-casein variants (A, B and E) to explain their effect on cheese texture and functional properties.

The pH of all cheeses at day 7 was higher than that of the curds at salting, as reported by Guinee et al. (2002) and Guo, Gilmore, and Kindstedt (1997). The increase in pH may be partly due to microbial activity. During storage, NH_3 was produced as a result of proteolysis, which may increase the pH. The increase in pH may also be associated with loss of lactic acid, soluble Ca and P in the stretch water, and also to the resolubilisation of micellar calcium phosphate on cooling the cheese after plasticisation (Guinee et al., 2002). Compared with the cheese made from type AE and BE milk, the cheese made from type AA and type AB milk had significantly lower pH values. This variance may be partly due to a higher ratio of Ca to protein caused by higher moisture content in the cheeses made from types AE and BE milk, which improves their buffering capacity (Czulak, Conochie, Sutherland, & van Leeuwen, 1969). Other factors, such as Ca and P levels and a_w may also influence the difference in pH. The a_w value reflects the mobility of protons in the water, and may be related to the tightness of the protein matrix, pH and water content (El-Bakry, Duggan, O'Riordan, & O'Sullivan, 2010). The higher a_w and lower Ca content in cheese made from type AE and BE milk may have been beneficial to the growth of starter bacteria, which then produce more NH_3 to increase the pH.

5. Conclusions

Polymorphism of κ-casein markedly influenced the composition, texture and functional properties of Mozzarella cheese. The κ-casein cheese made from type AB had significantly higher hardness, chewiness, and stretchability than that made from milk with κ-casein type AE and BE. This difference can be attributed to the cheese composition, parameters such as Ca/protein, fat and moisture, and their interaction within the protein matrix. Understanding how the κ-casein variants influence the functional and textural properties of cheese will give insight to improving the quality of cheese.

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