Comparative analysis of mozzarella cheeses fortified with whey protein hydrolysates, diverse in hydrolysis time and concentrations

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Abstract The objective of the present study was to improve the quality of mozzarella cheese using whey protein concentrates (WPCs) hydrolyzed for varying lengths of time (1 and 3 h). Four types of cheeses were made incorporating hydrolyzed WPCs in milk 3 and 6 % level and evaluated for nutritional, structural, and functional properties during 28 days storage at 4 °C. Whey protein hydrolysates (WPHs) incorporation increased protein, lactose, minerals, water-soluble-protein, non-protein-nitrogen. Mozzarella incorporated with WPHs hydrolyzed for 3 h had higher fat contents, favorable meltability and lower browning effect, stretchability, brittleness, springiness, and cohesiveness compared to mozzarella fortified with WPHs hydrolyzed for 1 h. The incorporation of hydrolyzed WPCs significantly influenced rheological and functional characteristics of mozzarella cheese. The cheeses made with hydrolyzed WPCs showed fewer changes in whiteness than control during storage. It was observed that both extent of hydrolysis and levels of WPHs incorporation had significant effect on the characteristics of mozzarella cheeses.

Keywords Whey protein hydrolysates · Mozzarella cheese · Low-fat cheese · WPHs-fortified cheese · Mozzarella analysis

Introduction

Cheese is considered as an ideal food due to its high nutritional value, convenience, variety, availability, and pleasant taste (Bogue et al. 1999). Compared to other types of cheese, mozzarella cheese is highly consumed worldwide, and it is classified as a “pasta filata” which involves skillfully stretching the curd in hot water during production. Mozzarella cheese is soft, white, unripened, lightly salted and consumed shortly without a long aging period. Its melting and stretching characteristics are highly appreciated in the manufacture of pizza (Banville et al. 2013; Jeewanthi et al. 2015a). Nowadays, producing high quality cheeses that meet consumers’ expectations is crucial for cheese makers to remain competitive. The soluble components of cheese like lactose, whey proteins (WPs), and minerals are separated with the whey stream and are slightly retained in the final cheese product. Whey protein concentrates (WPCs) have a high nutritional value and are an important source of bioactive peptides (Clare and Swaisgood 2000; FitzGerald and Meisel 2003). Failure to incorporate milk protein solids into the cheese curds represents a significant loss of protein. Several methods of recovering whey proteins in made cheese by adding WPCs into cheese milk have been studied (Kosikowski 1977). Microparticulation is generally suggests that any product that does not interact with milk proteins during the cheese-making process is only trapped within the casein network (Jovanović et al. 2005). However, it is still unclear whether WPCs act as active or inert particles in mozzarella. Hydrolyzing intact WPs improves the functionality like gel forming properties and water holding capacity, which are important characteristics in mozzarella manufacturing that helps to increase the cheese yield and promote coagulation, respectively. β -lactoglobulin (β -Lg), represents the major

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protein of WPCs, and is reported to have high water holding capacity (Kilara 2004). Hydrolyzed β -Lg also has the ability to form networks associated with gels like cheese (Foegeding et al. 2002). WP also has emulsifying properties allowing fat globules to be structural elements in heat induced WP gels (Jeewanthi et al. 2015b). These emulsifications are useful for develop cheese products. However, incorporating high amounts of whey protein hydrolysates (WPHs) into cheese can induce undesirable properties like unacceptable taste and texture due to high concentrations of bitter peptides and high amounts of lactose, respectively. The present study was undertaken to investigate the effect of incorporation of enzymatically modified ultrafiltrated WPCs on the characteristics of four types of mozzarella cheeses.

Materials and methods

Materials

The starter culture used for mozzarella cheese was Thermophilic Y 082 D (Clerici Sacco International Srl, Cadorage Como, Italy), a mixed culture containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Double strength (IMUC 290) calf rennet was obtained from NATUREN products (CHR. HANSENS laboratory Inc., Victoria, Australia).

Preparation of WPC hydrolysates

WPC consists of 50 % proteins (WPC-50) in dry material were prepared using ultrafiltration and spray drying (Jeewanthi et al. 2014) and then was hydrolyzed using trypsin enzyme derived from porcine stomach mucosa (Wako Pure Chemical Industries. Ltd, Osaka, Japan; EC 3.4.23.1). Hydrolysis was conducted for 1 and 3 h, by shaking at 180 rpm, 37 °C in a shaking incubator (VS-8480S, Vision Scientific Ltd, Daejeon, Korea). The solutions were then placed in a 95 °C water bath for 10 min to inactivate the enzyme and were cooled to room temperature. Then the solutions were centrifuged at 3000 rpm (Combi-514R, Hanil Sciencee Industrial, Incheon, Korea) for 30 min at 4 °C, and the supernatant was dried completely using a freeze dryer (FDU-1200, EYELA, Rikakikai Co. Ltd., Tokyo, Japan) for approximately 24 h. The resulting dried WPHs powder was sealed and stored at 4 °C until further use in cheese production.

Milk preparation and analysis

Cow milk was obtained from a local Korean dairy farm. Four different mozzarella cheese types named, A, B, C, and

D were prepared by distinct WPH fortifications as follows. Control: cow milk only. A: added 1 h hydrolyzed WPH 3 % (protein: 1.5 %) into cow milk. B: added 3 h hydrolyzed WPH 3 % (protein: 1.5 %) into cow milk. C: added 1 h hydrolyzed WPH 6 % (protein: 3 %). D: added 3 h hydrolyzed WPH 6 % (protein: 3 %) into cow milk. After adding WPHs, milk was pasteurized at 72 °C for 15 s using a cheese pasteurizer. Then, 80 mL sample was taken from the milk base and analyzed using a Milk analyzer (Milko scan minor, Foss, Hillerod, Denmark).

Cheese manufacturing

Cheeses were prepared according to the method described by Jeewanthi et al. 2015a. Briefly, prepared milk was heated to 30 °C and inoculated with cheese starter culture (2 %). After adding rennet (3 %), milk was ripened for 45 min. The resulted curd was cut with a cheese wire knife into 1 cm cubes. The curds were cooked to 38 °C by slowly increasing temperature at the rate of 1 °C rise per 5 min with agitation. Whey was drained and curds were cheddared at 38 °C and flipped every 15 min until the pH dropped to 5.4–5.5. The curds were milled and formed into a ball which was put into 75–85 °C hot water and kept stretching until curds were exposed to enough heat. Then the curd ball was put into a 2 % w/w salted solution for 2 h. Finally, all samples were vacuum packaged and stored at 4 °C.

Compositional analysis of cheese

Values of pH were measured with a pH meter (SevenEasy, Mettler-Toledo, Seoul, Korea). Total solid (TS) and ash contents were measured according to the AOAC method (2000). Cheese slurry was prepared by blending 20 g of grated cheese with 12 mL of distilled water and used for analysis. Water soluble proteins (WSPs) and non protein nitrogen (NPN) of cheese slurries were analyzed by the micro-Kjeldahl method (AOAC 2000) and according to a method by Lowry et al. (1951), respectively. Lactose levels of cheese slurries were determined by the method of Munson–Walker (AOAC 2000). The Kjeldahl method was used to measure protein content, and Mojonnier method mentioned in AOAC (2000) was used to measure fat content. All chemical measurements were done in triplicate at 0, 7, 14, 21, and 28 days of ripening and mean values were presented.

Physical and rheological properties of cheese

Color of the mozzarella cheeses was evaluated by measuring the L (100 = white; 0 = black), a (+, red; –, green), and b (+, yellow; –, blue) values using a Hunter

tristimulus colorimeter (D25-9; Reston, Virginia, USA). The L^* values indicates lightness, the a^* and b^* values are chromaticity coordinates (a^* , from green to red; b^* , from blue to yellow) (Jeewanthi et al. 2015a). The reported values are the means of three replicates. Textural and rheological analyses were performed for springiness, brittleness, gumminess, and cohesiveness of mozzarella. These parameters were measured at 25 °C using a Fudoh Rheometer (Model NRM-2001, Fudoh Kogyo Co., Tokyo, Japan). Cheese specimens were taken in 25 mm × 25 mm × 20 mm (length, width, and height) sections. Specimens were removed at different angles relative to the axis of the cheese to avoid effects due to curd orientation. The maximum load used was 2000 g, table speed was 60 mm/min, adaptor No. 5, with an intrusion distance of 10 mm.

Functional characteristics analysis

Meltability was determined by the Schreiber test (Lee et al. 2006). Area Increase was measured from cheese samples taken from a no. 10 cork borer with a 5 mm height, after heating at 232 °C for 2 min and were cooled to room temperature. Shredded cheese was evenly distributed on the pizza base and then placed in an electric fan oven at 280 °C for 4 min to evaluate stretchability according to methods described by Chatherine et al. (1998). Then the fork test was performed, in which cheese is baked on a pizza and then evaluated for how far it will stretch (USDA 1980). Browning results are presented as the difference in color before and after baking. This test was performed for all batches of mozzarella using the Hunter tristimulus colorimeter based on the procedure described by Mukherjee and Hutkins (1994).

Statistical analysis

Mean values of three replicates and SD were calculated using SAS (2004). One-way analysis of variance (ANOVA) and Duncan's multiple range tests ($p \leq 0.05$) were used to evaluate the significance of differences between groups.

Results and discussion

Composition of cheese milk

Milk type used in the preparation of cheese has a profound influence on compositional, textural, and functional characteristics of the final cheese. Different peptide compositions and ionization of the added WPHs influenced cheese composition and their functional qualities. Chemical

composition of the cheese milk types in this study are shown in Table 1. Adding WPHs into milk resulted in increased protein, lactose, and TS content of the milk. TS was increased in cheese milk within the range of 1.76–2.8 % after the incorporation of WPHs and adding more extensively (4 h) hydrolyzed proteins resulted in increased TS contents. This is expected because of the increased solubility of more hydrolyzed WPHs. The protein to fat ratio of milk for mozzarella cheese production varied with the addition of various WPHs protein levels.

Composition of mozzarella cheese

The drop of pH values in WPHs incorporated mozzarella cheese might be due to the increased amino acids and peptide contents in cheese milk (Fig. 1a). More hydrolyzed, more concentrated samples had lower pH values. TS contents of WPHs-fortified cheeses were less than TS contents of control cheeses that might be due to water retention capacity of WPHs (Fig. 1b). This suggests that incorporating WPHs makes cheese softer. Denatured WP aggregates are known to bind water effectively and may discourage whey drainage, thus water retention becomes higher in cheese (Salama 2015). Our results agreed that by showing more hydrolyzed WPHs had higher water retention capacity in cheese. Gradually increase of the addition of more WPHs before heating could be lead to the increase immobilization of free water in the casein matrix with increased compactness. Furthermore, slight decrease in the pH of cheese milk reduces calcium ion concentration and increase moisture retention in the curd (Zisu and Shah 2005). Minerals play a vital role in creating the para-casein matrix that influences functional properties of mozzarella. In our study, concentration of WPHs affected mineral composition but hydrolysis time did not (Fig. 1c). However, in higher concentrated samples, mineral content reduced obviously within the first week of storage. Protein is the major component of the cheese body helping to maintains desirable firmness, stretchability, and meltability. Protein content of cheese samples examined in this study are presented in Fig. 1d. Protein content was markedly increased in the prepared cheese with the WPHs fortification. The concentration of WPHs influenced the protein content of the final cheese, but not the hydrolysis time of WPHs. During storage, WSP contents increased, whereas protein contents decreased (Fig. 1e). More extensively hydrolyzed and more concentrated samples showed greater WSP values. These samples may have more short chained proteins trapped/incorporated within the casein structure. Yun (1999) reported that aging has a large impact on the soluble nitrogen content of cheese. WPHs-fortified samples increased its NPN values comparing to that of control cheese, more extensively

Table 1 Nutrition composition of cheese milk

Sample	Addition level of WPHs		Fat content (%)	Protein content (%)	Lactose content (%)	Total solids content (%)
	Concentration (%)	Hydrolysis time (h)				
Control			4.02 (0.04) ^{ab}	3.11 (0.02) ^a	5.22 (0.02) ^a	12.70 (0.01) ^a
A	3	1	2.85 (0.01) ^a	4.38 (0.07) ^b	6.36 (0.12) ^a	14.56 (0.07) ^b
B	3	3	2.90 (0.02) ^a	4.43 (0.06) ^a	6.32 (0.09) ^b	15.05 (0.07) ^a
C	6	1	2.75 (0.09) ^c	5.11 (0.09) ^b	8.03 (0.04) ^a	15.21 (0.03) ^a
D	6	3	2.82 (0.11) ^b	5.25 (0.02) ^a	8.43 (0.23) ^{bc}	15.60 (0.02) ^a

A–D represent different milk mixtures that were used to make mozzarella cheeses

Average mean values (SD) and ^{a–c} Means within a column with different superscripts are significantly different by Duncan's multiple range test ($P \leq 0.05$)

hydrolyzed WPHs added cheese samples showed more values in the range of 0.6–0.89 (Fig. 1f). Fat contents of cheese decreased with storage time in all cheese samples (Fig. 1g). More extensively hydrolyzed 6 % WPHs added cheeses has increased the fat content of the cheeses comparing to control. Their fat content ranged 18.28–21.81 % during storage for 28 days while control cheese was less than this range with a fat content of 14.92–16.25 %. The protein dominated microstructure of low fat cheese causes hard and rubbery texture (Madadlou et al. 2005). Several researchers suggested that the addition of denatured WP to cheese milk would produce low-fat mozzarella (Ismail et al. 2011). However, our results suggested that adding more WPHs (6 %) increased the fat binding effect resulting in a fattier mozzarella than control. Increased fat binding capacity is associated with increased protein hydrophobicity (Voutsinas and Nakai 1983). WPHs-fortified cheeses had lactose contents ranging from 2.58–4.28 % and 2.52–3.32 % for 3 and 6 % WPHs, respectively, during the storage period (Fig. 1h). WPHs-fortified cheeses had reduced the lactose content during aging compared to control cheese.

Physical and rheological properties of mozzarella cheese

Color characteristics

Whiteness is an important quality characteristic of mozzarella cheese. Whiteness (L) was increased in WPHs-fortified mozzarella and more than 81 % whiteness was maintained during 28 days of storage (Table 2). WPHs-fortified cheeses 6 % (C and D) showed higher values for green color comparatively. Cheeses fortified with more extensively hydrolyzed WPHs were whiter in color than the other cheese preparations were. Whey protein concentrations (4 %) were reported to have whitening properties when added to chicken meat (Prabhu 2007). In yogurt fortified with tryptic WPHs (2 h hydrolysis) higher

L and b values were observed compared to the control (Lim et al. 2011).

Rheological properties

Measurements of cheese rheology reveal the relationships among stress, strain, and time scale to understand the effects of processing on food products' structure and, texture. Generally moderate toughness and adequate stringiness are preferable characteristics of mozzarella. The rheological values of WPHs-fortified cheese just after production (0 days storage) are shown in Fig. 2. Gumminess results from hardness and cohesiveness of cheeses. Gumminess was increased in 6 % WPHs-fortified cheese samples. This result suggests that the gumminess increases with increasing concentrations of WPHs added to cheese milk. More extensively hydrolyzed WPHs added samples B and D types with same concentration showed decreased values for cheese rheology parameters, such as springiness, cohesiveness, brittleness, and gumminess. These findings suggest that the strength of the internal bonds of mozzarella that promote cohesiveness was reduced upon the incorporation of more extensively hydrolyzed WPHs. This might be due to the ability of water retention capacity of high concentration of short peptides in WPHs-fortified cheeses as previously mentioned.

Functional properties of mozzarella cheese

The functionality of heated mozzarella cheese depends on several factors like meltability, stretchability, and browning. We evaluated each of these parameters and the results are discussed below.

Meltability

Meltability is the ability of cheese particles to flow together and form a continuous melted mass (Kindstedt 1993). The meltability of every cheese tested increased during storage

Fig. 1 Compositional changes **a** pH; **b** total solid (%); **c** ash (%); **d** protein (%); **e** water soluble protein; **f** non-protein-nitrogen; **g** fat (%); and **h** lactose (%) of WPHs fortified-mozzarella cheeses during 4 weeks refrigerated storage at 4 °C. *white circle* control (cow milk); *black circle* A, cow milk added WPHs (1 h, 3 % of WPH-50); *white up-pointing triangle* B, cow milk added WPHs (3 h, 3 % of WPH-50); *black up-pointing triangle* C, cow milk added WPHs (1 h, 6 % of WPH-50); *white square* D, cow milk added WPHs (3 h, 6 % of WPH-50)

due to the proteolysis (Fig. 3a). Cheeses with 6 % WPHs had higher meltability that of superior to cheeses added 3 % WPHs. Control cheese showed the highest meltability. The cheese with lower fat content and thus greater volume fractions of the casein matrix forms thicker para-casein

fibers with fewer inclusions of fat serum-channels between them (McMahon et al. 1999; Merrill et al. 1996), resulting in firmer less meltable cheese. Cheeses with 3 % WPHs had moderate meltability which was acceptable for mozzarella cheese.

Table 2 Changes in color values of whey protein hydrolysates fortified-mozzarella cheeses during 4 weeks refrigerated storage at 4 °C

Storage time (week)	CEI scale	Cheese types				
		Control	A	B	C	D
0	L* ^c	87.01 (0.21) ^{ab}	87.31 (0.09) ^a	91.04 (0.08) ^a	88.74 (0.01) ^a	92.36 (0.31) ^a
	a*	−4.36 (0.01) ^a	−4.44 (0.03) ^a	−4.24 (0.08) ^a	−4.99 (0.11) ^a	−4.76 (0.01) ^a
	b*	12.43 (0.07) ^c	15.2 (0.69) ^b	11.94 (0.08) ^d	13.33 (0.01) ^b	12.69 (1.78) ^b
1	L*	86.81 (0.18) ^a	86.73 (0.39) ^b	90.52 (0.07) ^a	88.72 (0.01) ^a	91.85 (0.39) ^a
	a*	−4.28 (0.06) ^a	−4.42 (0.03) ^a	−4.1 (0.06) ^a	−4.89 (0.04) ^a	−4.74 (0.03) ^a
	b*	12.55 (0.06) ^c	16.07 (0.66) ^a	12.65 (0.55) ^c	13.34 (0.03) ^b	12.88 (0.25) ^a
2	L*	85.22 (0.12) ^a	85.48 (0.46) ^c	89.42 (0.46) ^b	88.48 (0.02) ^a	91.07(0.2) ^b
	a*	−4.16 (0.12) ^b	−4.41 (0.01) ^a	−3.96 (0.08) ^a	−3.56 (0.14) ^b	−3.65 (0.08) ^b
	b*	13.21 (0.07) ^{cb}	16.19 (0.52) ^a	12.77 (0.25) ^b	13.36 (0.15) ^b	13.28 (0.28) ^a
3	L*	83.14 (0.15) ^a	83.33 (0.08) ^a	89.39 (0.35) ^b	87.89 (0.31) ^b	90.65 (0.35) ^b
	a*	−4.02 (0.04) ^c	−4.39 (0.01) ^a	−3.48 (0.06) ^b	−3.53 (0.13) ^b	−3.61 (0.1) ^b
	b*	13.86 (0.03) ^b	16.72 (0.11) ^a	13.82 (0.31) ^{ab}	14.06 (2.95) ^a	13.96 (0.26) ^a
4	L*	82.68 (0.92) ^b	81.27 (0.52) ^d	88.84 (0.22) ^b	82.83 (0.11) ^c	89.13 (0.04) ^c
	a*	−3.88 (0.04) ^d	−3.52 (0.03) ^b	−2.99 (0.21) ^c	−3.3 (0.11) ^c	−3.1 (0.14) ^c
	b*	14.93 (0.1) ^a	16.81 (0.17) ^a	14.75 (0.43) ^a	15.88 (1.45) ^a	14.11 (0.31) ^a

A ~ D represent mozzarella cheeses (*Control*, cow milk; A, cow milk added WPH (1 h, 3 % of WPC-50); B, cow milk added WPH (3 h, 3 % of WPC-50); C, cow milk added WPH (1 h, 6 % of WPC-50); D, cow milk added WPH (3 h, 6 % of WPC-50)

Average Mean Values (SD) and ^{a–g} Means within a column with different superscripts are significantly different by Duncan's multiple range test ($P \leq 0.05$)

L* value = degree of lightness from black (−) to white (+), a* value = degree of green (−) and red (+), b* value = degree of blue (−) and yellow (+)

Fig. 2 Rheological properties of WPHs fortified-mozzarella cheeses before storage. First Y axis represents *filled square* springiness (m); *light grey square* cohesiveness; *thick grey square* gumminess (N). Second Y axis represents *filled circle* brittleness (N). *Control*, cow milk; A, cow milk added WPHs (1 h, 3 % of WPH-50); B, cow milk added WPHs (3 h, 3 % of WPH-50); C, cow milk added WPHs (1 h, 6 % of WPH-50); D, cow milk added WPHs (3 h, 6 % of WPH-50)

Stretchability

Young cheese typically dehydrates during pizza baking and results scorching and hardening. Cheese generally requires 1–3 weeks of aging in the refrigerator for optimum stretchability (Jeewanthi et al. 2015a; Kindstedt et al. 2004). Stretching ability of WPHs-fortified mozzarella is shown in Fig. 3b. Cheeses fortified with more concentrated, more hydrolyzed

WPHs had the least stretchability. D cheese (3 h hydrolysis, 6 % WPHs) had 14–15.5 cm stretchability after heating. When the pH of cheese reduces, casein molecules begin to disconnect and reorganize, resulting in a loss of stretchability. During stretching, high curd temperatures strongly favor hydrophobic protein-to-protein interactions which cause the para-casein matrix to aggregate and contract (Pastorino et al. 2002).

Browning effect

High browning color like “burnt” is not preferred by the consumer after heating mozzarella at high temperatures. L* (lightness to darkness) and a* (red to green) were the most relevant indicators of browning (Beatriz et al. 1994). Cheese A had the lowest browning effect ($\Delta L = -0.82$, $\Delta a = -10.44$) whereas B had the highest ($\Delta L = -1.35$, $\Delta a = -13.19$) (data not shown). Cheeses fortified with 6 % WPHs showed a moderate browning effect.

Conclusion

This study indicates that WPHs fortification has a beneficial effect on mozzarella cheese quality. Adding WPHs into cheese milk influences cheese properties, and the

Fig. 3 The effect of storage on functionality of WPHs fortified-mozzarella during a 4 weeks storage period at 4 °C. **a** Meltability; **b** stretchability. *white circle* control (cow milk); *black circle* A, cow milk added WPHs (1 h, 3 % of WPH-50); *white up-pointing triangle*

B, cow milk added WPHs (3 h, 3 % of WPH-50); *black up-pointing triangle* C, cow milk added WPHs (1 h, 6 % of WPH-50); *white square* D, cow milk added WPHs (3 h, 6 % of WPH-50)

quality is affected by hydrolysis time and the concentration of WPHs. WPHs addition results in reduced pH, increased protein content, and lower fat content of mozzarella cheeses, with improved nutritive quality and functionality. Whiteness improves in WPHs added cheeses than non added cheeses. However, the addition of WPHs may decrease the shelf life of mozzarella cheese due to the resulting increased proteolytic activity compared to control cheese. Therefore, WPHs fortification of cheese is suitable for freshly consumed cheese or cheese that is stored for a short time.

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