

Influence of mozzarella and cheddar cheese mixing on biochemical characteristics of pizza cheese blends

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Restaurants and pizza makers in Pakistan demand a cheese that has ability to melt, stretch with a characteristics flavor and less free oil formation while applied on pizza dough. The desired characteristics can be obtained with proper amalgamation of fresh and ripened cheeses. Therefore, the present research was planned to prepare Pizza cheese blends (PCB) from fresh Mozzarella and ripened (2 and 4 months) Cheddar cheese. Seven Pizza cheese blends were prepared with fresh Mozzarella and ripened (2 and 4 months) Cheddar cheese. The quality of Pizza cheese blends were evaluated by measuring chemical composition, proteolysis, intact casein and organic acids contents. The rate of proteolysis (pH 4.6-soluble and TCA-soluble nitrogen) was rapid in PCB made with higher level of four months ripened Cheddar cheese. Electrophoresis (Urea PAGE) and High Performance Liquid Chromatography (HPLC) analysis indicated reduced intact casein in PCB that has higher level of aged (4 months) Cheddar cheese. Mean abundances indicated significant change in organic acid contents of PCB. In conclusion, significant variation was observed for proteolysis, intact casein and organic acids production with the difference in percentages and ages of cheeses. The prevalence of a comparatively large amount of variability in technological properties of Pizza cheese was confirmed. This blending of cheeses provides new insight to cheese industries which directs new strategies to improve the characteristics of Pizza cheese.

Keywords: Casein Chains; Composition; Organic acids; proteolysis; semi-ripened cheese; texture.

INTRODUCTION

Most of the cheese manufacturers are working to know how to combine different cheeses to get a customized product for pizza maker. Some customers are looking for a non-browning, easy to chip Mozzarella cheese for pizza while other demands a strong flavored and high-browning cheese for snacks (Costabel *et al.*, 2007). Mainly cheese working behavior on pizza topping depends on chemical composition and other characteristics like intact casein and organic acid content of natural cheese (Kyung-Hoon *et al.*, 2015).

In order to meet the customer requirements for pizza cheese, use of cheddar is increasing in blends with Mozzarella cheese on pizza toppings. These cheeses are encompassed mostly to improve flavor and adjust the functionality of the cheese on topping (Mei *et al.*, 2015). To increase the profitability and competition in the market, efficiency and quality are the key elements for processed cheese makers. The main challenge is to adjust the amount of natural cheese for the manufacturing of processed cheese (Kim *et al.*, 2015).

The intact casein is an important component of the natural cheese that influenced the quality characteristics of finished product. It usually reduces during aging of cheese when enzymatic hydrolysis takes place. (Ma *et al.*, 2019). Mozzarella and Cheddar cheese had variable intact casein content due to difference in storage history and composition. Although intact casein is a vital component that influence cheese quality but its estimation is not easy in different types of cheeses (Zheng *et al.*, 2016).

The production of flavor during ripening makes the cheese more acceptable as compare to fresh cheeses (Banks, 2000). Organic acids produced during ripening give unique flavor to the cheeses. These are the products that are produced from glycolysis (Fox *et al.*, 2000), lipolysis fermentation and some acids that are added in to cheese during processing (Izco *et al.*, 2002). Determination of these components is very important for the quality characteristics of many cheeses (Buffa *et al.*, 2004).

Proteolysis is a crucial process for the flavor and texture development in cheese during aging. Milk indigenous

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enzymes (plasmin), micro-organisms and rennet (chymosin) are responsible to carry out proteolysis in cheeses. Shorter chain peptides and amino acids are formed when these enzymes and microorganisms hydrolyze casein (Hashemi *et al.*, 2014). Some cheeses like Mozzarella are ripened for shorter period of time while other cheeses such as Cheddar are ripened for longer period of time (Iven *et al.*, 2017). Due to variation in ripening period, flavor of a single category of Cheddar cheese and consumer preferences are totally changed. It is documented that consumers prefer texture of Cheddar cheese of mild ripening.

Pizza cheese blend gives an appropriate coverage like stretch, melt, browning as well as also create a unique flavor. What are the chemical characteristics that contribute Pizza cheese blend to its unique characteristics is questionable? The quality characteristics of Mozzarella cheese has been comprehensively studied but comparatively slight has been described about chemical characteristics, proteolysis, intact casein and organic acid contents of Pizza cheese blend. The current study was undertaken to investigate the chemical composition, proteolysis, intact casein and organic acid contents of Pizza cheese blends using Mozzarella and Cheddar cheese at different combinations and ripening stages.

MATERIALS AND METHODS

Procurement of raw material: Mozzarella and Cheddar cheese was prepared using buffalo milk that was standardized at 3% fat level. The milk was procured from SB Dairy Farm Jhapal, Faisalabad-Pakistan. Thermophillic (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) and mesophillic culture (*Lactococcus Lactis subsp. Lactis* and *Lactococcus Lactis subsp. Cremoris*) of Chr. Hansen (Rohan Industrial Estate, Little Island, Co. Cork, Ireland) were used for Mozzarella and Cheddar cheeses respectively. Chymosin (50000u/G) was used as coagulant for the preparation of both cheeses (Pharm Chemical Co., Ltd. China).

Preparation of Pizza cheese blends (PCB): Cheddar and Mozzarella cheese were prepared adopting the method of Ong *et al.* (2006) and Zisu and Shah (2007) respectively. Ripened Cheddar (2 and 4 months) and fresh Mozzarella were used in the manufacturing of Pizza cheese blends (Shirashoji *et al.* 2006). PCB0 with 100% Mozzarella cheese was used as control. PCB1, PCB2 and PCB3 are blends of 25-75, 50-50 and 75-25% Mozzarella and ripened (2 months) Cheddar cheese while PCB4, PCB5 and PCB6 are amalgams of 25-75, 50-50 and 75-25% Mozzarella and ripened (4 months) Cheddar cheese respectively. Appropriate proportion of cheeses, water (5%), and emulsifying salts (2% Tri-sodium citrate) were cooked in a steam jacketed cooker (Blentech Corp., Rohnert Park, CA, USA) for 10 min at 80 °C. The cheese was molded, cooled, packed and stored at 5°C for further analysis.

Chemical composition of Pizza cheese blends: Chemical analysis likes moisture (AOAC, 2000), protein (FIL-IDF, 1993), fat (Marshall, 1993) and total calcium (Metzger *et al.*, 2000) of Cheddar, Mozzarella and PCB were determined according to standardized methods.

Proteolysis in Pizza cheese blends TCA soluble nitrogen: Proteolysis in cheeses was measured by estimating the TCA soluble nitrogen using Kjeldhal method (David *et al.*, 2004).

pH 4.6 soluble nitrogen: The presence of peptides in cheeses were quantified by determining the nitrogen content that is soluble at 4.6 pH using Kjeldhal apparatus (Kuchroo and Fox, 1982).

Electrophoresis separation of nitrogenous fractions: Pizza cheese blends were analyzed by urea-PAGE to identify nitrogenous fractions (Sheehan *et al.*, 2004). Samples were run in stacking gel at 112V followed by 224V for separating gel.

Proteolysis by RP-HPLC: The amount of intact casein present in Pizza cheese blends was monitored by using RP-HPLC (Verdini *et al.*, 2004). A Shim-pack C-18 CLC-ODS, 250cm × 4.6mm, 5µm column, LC-10 AT pump and UV detector SPD-10AV (Shimadzu, Japan) were used for HPLC separations at room temperature. All measurements were done at 214 nm wavelength.

Organic acids in Pizza cheese blends: High performance liquid chromatography (HPLC) was used to determine organic acids (Lactic, Acetic, Citric and Pyruvic) in Pizza cheese blends adopting the method of Akalin *et al.* (2002).

Statistical analysis: The data was analyzed using Minitab Statistical Package. The difference between means was identified using multiple degree of freedom contrast system and complete randomized design at significance level of 0.05. After significant difference between means Tukey test was used to find out comparison (Steel *et al.*, 1997).

RESULTS AND DISCUSSIONS

Chemical composition of Pizza cheese blends: The mean values for chemical composition of Pizza cheese blends are given in Table 1. It is indicated from the results that moisture content reduced in Pizza cheese blends; on the other hand protein, fat and total calcium increased in Pizza cheese blends. This difference in chemical composition of Pizza cheese blends is due to difference in moisture contents and ripening period of both cheeses. The reduction in moisture is due to loss in water from the package during storage (Perveen *et al.*, 2011). The less protein content in Pizza cheese blends with higher level of Mozzarella cheese is owing to more proteolytic activity of thermophillic cultures (*Streptococcus thermophilus* and *Lactobacillus Bulgaricus*) used for Mozzarella cheese manufacturing and higher moisture

Table 1. Chemical composition of Pizza cheese blends (PCB).

Components	Pizza cheese blends (PCB)													
	PCB0	PCB1		PCB2		PCB3		PCB4		PCB5		PCB6		
	100%	75%	25%	50%	50%	25%	75%	75%	25%	50%	50%	25%	75%	
	M ^a	M ^a	C1 ^b	M ^a	C1 ^b	M ^a	C1 ^b	M ^a	C2 ^b	M ^a	C2 ^b	M ^a	C2 ^b	
Moisture %	*51.23±0.87A	*50.80±0.62B		*50.00±0.90C		*47.54±0.57D		*49.72±1.00C		*47.81±0.57D		*46.58±0.33E		
Protein %	*25.37±0.54B	*26.07±0.28B		*26.70±0.49AB		*27.51±0.208A		*26.04±0.52B		*26.40±0.57AB		*27.58±0.43A		
Fat %	*23.00±1.00B	*24.32±0.66B		*26.67±0.33A		*27.64±0.66A		*24.33±0.33B		*27.32±0.88A		*28.35±0.88A		
Total calcium mg/100g	*652±26D	*676±22CD		*702±24ABC		*728±28AB		*689±26BCD		*722±29ABC		*748±30A		

Values presenting in the table are mean ± SE of three replicates; *Means sharing different letters in a row are statistically significant (P<0.05); a M (Mozzarella cheese); b C1 (Two months ripened Cheddar cheese) and C2 (Four months ripened Cheddar cheese)

Table 2. Effect of Mozzarella and Cheddar cheese amalgamation on organic acid contents (mg/kg) of Pizza cheese blends.

Components	Pizza cheese blends (PCB)												
	PCB0	PCB1		PCB2		PCB3		PCB4		PCB5		PCB6	
	100%	75%	25%	50%	50%	25%	75%	75%	25%	50%	50%	25%	75%
	M ^a	M ^a	C1 ^b	M ^a	C1 ^b	M ^a	C1 ^b	M ^a	C2 ^b	M ^a	C2 ^b	M ^a	C2 ^b
Lactic acid	*8036±68.35D	*9025±118.37C		*9079±57.49C		*9383±319.42BC		*10010±286.99B		*10697±299.79A		*11184±161.64A	
Acetic acid	*745.7±14.50D	*790.67±17.61D		*1044±28.29C		*1197±36.56B		*1154±18.34B		*1273±18.45A		*1291±30.43A	
Citric acid	*2040±22.06C	*2127±20.03B		*2248±21.70A		*2299±28.88A		*1929±18.27DE		*1913±21.70E		*1995±26.89CD	
Pyruvic acid	*127.55±5.38A	*111.77±4.34B		*92.04±1.56D		*73.38±1.63E		*108.91±3.70BC		*100.30±2.59CD		*64.07±1.73E	

Values presenting in the table are mean ± SE of three replicates; *Means sharing different letters in a row are statistically significant (P<0.05); a M (Mozzarella cheese); b C1 (Two months ripened Cheddar cheese) and C2 (Four months ripened Cheddar cheese)

content (Castillo *et al.*, 2006). The decrease in fat content in Pizza cheese with increased Mozzarella cheese is to the effect of high gelation temperature (37°C) of Mozzarella which lost more fat through whey because milk fat melting is more at 37°C as compared to 31°C as in case of Cheddar cheese (Hussain *et al.*, 2012).

Organic acid contents of Pizza cheese blends: Organic acid contents of Pizza cheese blends are depicted in Table 2. Mean values indicated that lactic and acetic acid contents increased from PCB0 to PCB6 with the lowest were recorded in control sample that have 100% Mozzarella cheese while highest were observed in Pizza cheese blends with 75% Cheddar cheese (4 months ripened) and 25% Mozzarella cheese combination. The increase in lactic acid content in Pizza cheese blends with increasing level and age of Cheddar cheese is a consequence of more lactic acid production by the conversion of residual lactose with the help of *Lactococcus lactis subsp. lactis* and *Lactococcus lactis subsp. cremoris* in Cheddar cheese (McSweeney and Fox, 2004).

Increase in acetic acid in Pizza cheese blends with increasing level and age of Cheddar cheese is due to more protein breakdown and production of amino acids in Cheddar cheese that provide precursor for acetic acid formation (McSweeney and Sousa, 2000). It is also a result of fermentation of lactose into acetic acid with microorganism via fructose-6-phosphate shunt pathway (Nayak, 2015).

It is apparent from the results (Table 2) that citric acid contents increase as the Cheddar cheese increases. Interestingly, it has been noted that as the level of ripened Cheddar cheese (2 months) increases in PCB, the citric acid contents also increases while increasing level of four months

ripened Cheddar cheese decreases the citric acid in processed pizza cheeses. Increasing level of citric acid is a result of formation of citrate in Cheddar cheese from the breakdown of carbohydrates, fat and protein in early days of ripening, later on as the citrate is utilized in the Krebs where it is used as substrate and product as well then its concentration decreases (McMurry *et al.*, 2009). It is due to the reason of its utilization by secondary microflora as a carbon source during ripening and also due to its conversion in flavor compounds by lactic acid bacteria (Kwak and Chung, 2002).

Pyruvic acid content considerably varied with blending of Mozzarella and Cheddar cheeses but ripening did not influence its content. Higher level of pyruvic acid in fresh cheeses is due to its rapid formation through the glycolytic pathway and lesser amount in ripened Cheddar cheese was due to its utilization as it is used as substrate in ethanol, formic acid, acetoin, diacetyl and 2,3-butylene glycol formation.

Proteolysis in Pizza cheese blends pH 4.6 and TCA soluble nitrogen (SN): The mean values shown that PCB0 exhibited lowest (1.76%) while PCB6 possessed higher pH 4.6 SN value (8.30%) followed by the PCB5, PCB4, PCB3, PCB2 and PCB1 containing 7.48, 5.34, 4.90, 4.20 and 3.66% (Table 3). The amount of pH 4.6 and TCA-SN in PCB was increased with increasing level of Cheddar cheese, but increase was higher with four month old Cheddar cheese than with two month old Cheddar cheese. The increase in pH 4.6 SN and TCA- SN with increasing level of Cheddar cheese is attributed due to presence of high amount of hydrolytic protein of Cheddar cheese. The milk indigenous enzymes, starter culture, residual rennet and nonstarter lactic acid

Table 3. Effect of Mozzarella and Cheddar cheese amalgamation on proteolysis of Pizza cheese blends (PCB).

Table 1. Effect of Mozzarella and Cheddar cheese amalgamation on proteolysis of Pizza cheese blends (PCB).															
Components		Pizza cheese blends (PCB)													
		PCB0		PCB1		PCB2		PCB3		PCB4		PCB5		PCB6	
		100% M ^a	75% M ^a	25% C1 ^b	50% M ^a	50% C1 ^b	25% M ^a	75% C1 ^b	75% M ^a	25% C2 ^b	50% M ^a	50% C2 ^b	25% M ^a	75% C2 ^b	
pH 4.6 soluble nitrogen	*1.76±0.11G	*3.66±0.06F		*4.20±0.15E		*4.90±0.03D		*5.34±0.22C		*7.48±0.19B		*8.30±0.12A			
TCA soluble nitrogen	*2.05±0.03G	*3.27±0.26F		*4.20±0.15E		*5.03±0.09D		*5.57±0.28C		*6.15±0.10B		*7.03±0.03A			

Values presenting in the table are mean of three replicates; *Means sharing different letters in a row are statistically significant ($P < 0.05$); a M (Mozzarella cheese); b C1 (Two months ripened Cheddar cheese) and C2 (Four months ripened Cheddar cheese)

bacteria convert casein fractions and larger peptides into smaller peptides and amino acids (Sulejmani and Ali, 2016).

Urea polyacrylamide gel electrophoresis (Urea-PAGE): Urea-PAGE chromatograms of the different Pizza cheese blends are shown in Figure 1. Casein segments are being categorized according to Viotto and Grosso (1999). Electrophoretogram showed mainly the difference in casein components (α s1- and β -casein) of different pizza blends. The electrophoretogram indicated that the hydrolytic product (α s1-I-CN) of α s1-casein increased as the level of ripened (2 months) Cheddar cheese increased in Pizza cheese blends from PCB1-PCB3. However, in case of blends that have ripened (4 months) Cheddar cheese; the α s1-I-CN fragment becomes less visible from PCB4-PCB6. In PCB5 and PCB6 it was further hydrolyzed as indicated by the band below the α s1-I-CN.

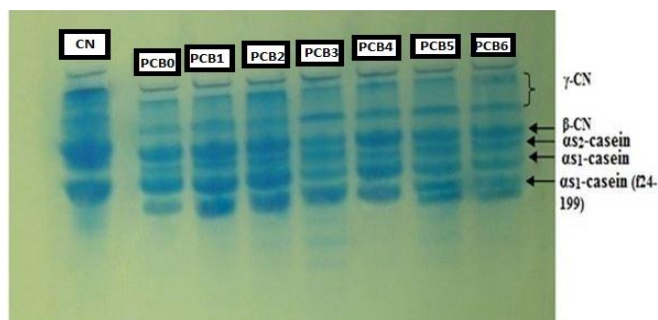


Figure 1. Electrophoretogram showing proteolysis in Pizza cheese blends, CN (Casein standard), PCB0 (Pizza cheese with 100% Mozzarella cheese), PCB1 (Pizza cheese blend with 75% Mozzarella cheese and 25% two months ripened Cheddar cheese), PCB2 (Pizza cheese blend with 50% Mozzarella cheese and 50% two months ripened Cheddar cheese), PCB3 (Pizza cheese blend with 25% Mozzarella cheese and 75% two months ripened Cheddar cheese), PCB4 (Pizza cheese blend with 75% Mozzarella cheese and 25% four months ripened Cheddar cheese), PCB5 (Pizza cheese blend with 50% Mozzarella cheese and 50% four months ripened Cheddar cheese), PCB6 (Pizza cheese blend with 25% Mozzarella cheese and 75% four months ripened Cheddar cheese).

This behavior can be explained taking into account that α s1-I-casein is the fragment (f24–199) of α s1-casein formed by initial cleavage and is susceptible to further proteolytic attack by different proteinases and peptidases resulting in smaller fragments which disappear virtually in the later Pizza cheese blends (Akin and Kirmaci, 2015). The degradation is more noticeable in the PCB with four months ripened Cheddar cheese as compared to two months ripened Cheddar cheese. It is because of the reason that protein continuously degraded during maturation and casein fractions breaks into their hydrolytic products (Phelan *et al.*, 1973; Fenelon and Guinee, 2000).

Detection of casein hydrolysis in Pizza cheese blends by HPLC: The amount of casein breakdown in PCB with different levels of cheeses (Mozzarella and Cheddar) and ripening months of Cheddar cheese was monitored. Casein proteolysis was studied by using water insoluble fraction of Pizza cheese blends. Casein standard from bovine milk was used to measure the degree of hydrolysis in Pizza cheese blends (Fig. 2A). In Pizza cheese blends three peaks of α s1 and α s2 caseins and β -casein were recognized by matching their retention time with that of casein standard.

α s1-casein, α s2-casein and β -casein: The peak areas for α s1-casein, α s2-casein and β -casein of PCB indicated that maximum peak areas were observed in PCB0 while PCB6 exhibited lowest peak areas (Fig. 2 B and Fig. 2H). The reduced peak area was due to greater amount of Cheddar cheese present in PCB and further reduction were noticed when four months ripened Cheddar cheese was used in PCB. It is due to presence of higher level of hydrolytic protein in Pizza cheese blends. The increased level of hydrolytic protein was attributed due to continuous breakdown of casein fractions in Cheddar cheese by the action of coagulant, peptidases and proteinases of the starter bacteria that decreased the levels of intact casein (Miloradovic *et al.*, 2017). The content of intact caseins (α s-1, α s-2 and β -casein) in PCB also decreased with ripening months of Cheddar cheese is due to formation of peptides and amino acids from casein that reduces the intact casein quantity. A band between α s1-casein and β -casein that is prominent in control (PCB0) and its intensity decrease with increasing level and age of Cheddar cheese due to presence of fresh Mozzarella cheese. This reduction is complemented with the growth of α s1-I

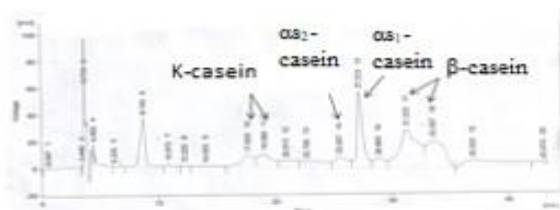


Fig. 2 A (Standard)

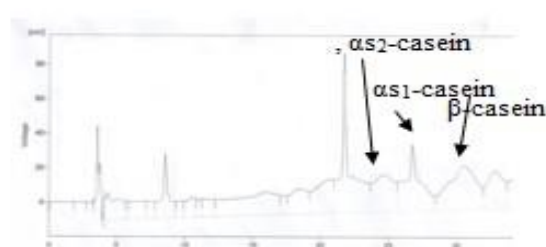


Fig. 2 B Control (Mozzarella cheese)

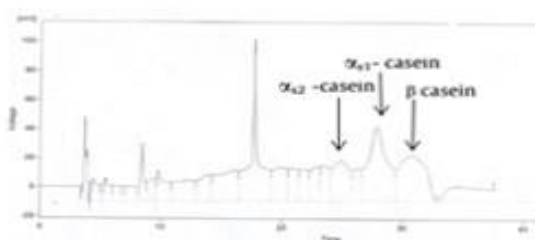


Fig. 2 C PCB1

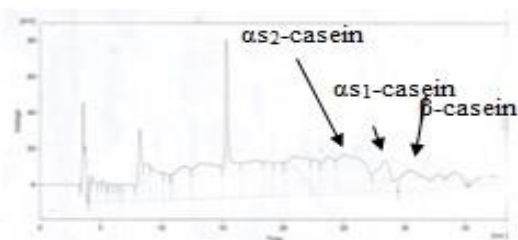


Fig. 2 D PCB2

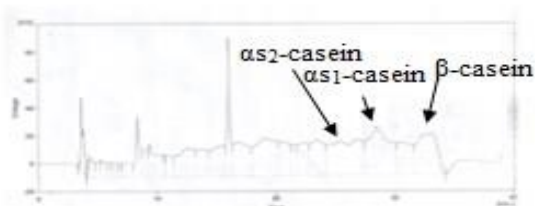


Fig. 2 E PCB3

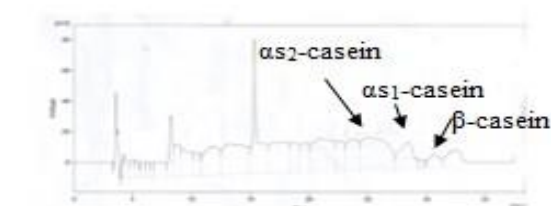


Fig. 2 F PCB4

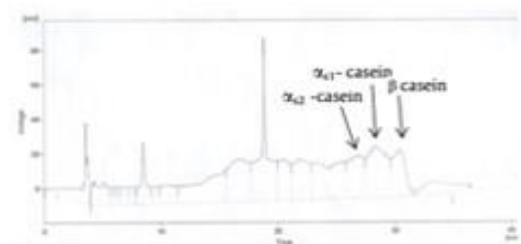


Fig. 2 G PCB5

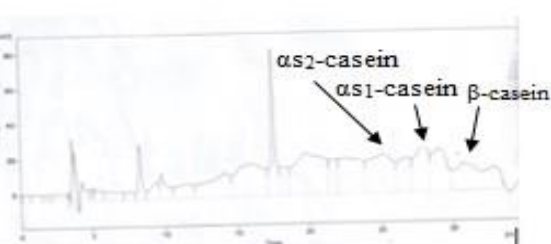


Fig. 2 H PCB6

Figure 2. Peak areas showing α s1-casein, α s2-casein and β -casein fractions of Pizza cheese blends. Standard (Casein), PCB0 (Pizza cheese with 100% Mozzarella cheese), PCB1 (Pizza cheese blend with 75% Mozzarella cheese and 25% two months ripened Cheddar cheese), PCB2 (Pizza cheese blend with 50% Mozzarella cheese and 50% two months ripened Cheddar cheese), PCB3 (Pizza cheese blend with 25% Mozzarella cheese and 75% two months ripened Cheddar cheese), PCB4 (Pizza cheese blend with 75% Mozzarella cheese and 25% four months ripened Cheddar cheese), PCB5 (Pizza cheese blend with 50% Mozzarella cheese and 50% four months ripened Cheddar cheese), PCB6 (Pizza cheese blend with 25% Mozzarella cheese and 75% four months ripened Cheddar cheese).

fraction which is created as a result of hydrolysis of α s1-casein (Ceruti *et al.*, 2012).

Conclusion: It was concluded that use of ripened Cheddar with Mozzarella cheese in Pizza cheese blends can effectively make a customized product for pizza maker. By using ripened (4 months) Cheddar cheese (up to 75%) in blends, a product

with better texture and flavor characteristics can be obtained simultaneously. It is because of ripening process of Cheddar cheese that results in increased production of organic acids, and proteolytic products which are important for texture and flavor development.

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