

INFLUENCE OF MOZZARELLA AND SEMI-RIPENED CHEDDAR CHEESE AMALGAMATIONS ON THE CHEMICAL COMPOSITION AND TEXTURAL ATTRIBUTES OF PROCESSED PIZZA CHEESE

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Processed pizza cheeses (PPC) are the mixture of Mozzarella and other cheeses which is pasteurized with the addition of emulsifier to get the desired characteristics of cheese, used as pizza topping. Mostly being used cheese on pizza topping is fresh Mozzarella but it has some week quality attributes due to lack of biochemical reactions that takes place during ripening. Present study was designed in which PPC was prepared by blending different percentages of Mozzarella cheese (MC) and semi-ripened Cheddar cheese (SRCC). Seven PPC were prepared: Control (100% MC), PPC₁, PPC₂, PPC₃ with (75:50), (50:50), (25:75) MC and 2 months SRCC and PPC₄, PPC₅, PPC₆ with (75:50), (50:50), (25:75) MC and 4 months SRCC respectively. The quality of PPC was accessed by analyzing the chemical composition and textural attributes. The outcome of chemical composition suggested that protein-protein interactions in PPC were improved due to less electrostatic repulsion caused by pH reduction. Moisture retention also increased in PPC perhaps due to disintegration of paracaseinate complex and mechanical shear and temperature of processing. Protein, fat and ash contents of PPC were increased while texture results indicated that hardness, cohesiveness, springiness, gumminess and chewiness decreased in PPC with greater share of SRCC. Based on the physicochemical and texture results it was concluded that cheese with better quality can be obtained by blending 75% MC and 25% two months SRCC.

Keywords: Composition, texture, pizza cheese, casein chains, semi-ripened cheese.

INTRODUCTION

Chemistry and biochemistry of natural cheeses influence the functional properties of resultant Processed pizza cheese. These variations are due to difference of natural cheese source and age. Natural cheese differed due to fat content, moisture content (Lee *et al.*, 2005), pH (Lee and Klostermeyer, 2001), total calcium content, intact casein (Garimella *et al.*, 2006), lactose content, and whey protein content. The melt and stretch performance of cheese when baked on pizza is influenced by cheese composition, pH history (especially extent of acidification at coagulant addition), insoluble colloidal calcium phosphate content, and the amount of intact casein (Johnson and Lucey, 2006).

The major component of most Processed cheese manufacture in the United States is Cheddar cheese. A typical Processed cheese contains about 15 to 20% aged cheese. Young cheese is preferred for textural and performance issues, whereas aged cheese offers better flavor (Hickey *et al.*, 2007). In addition to lack of flavor, young cheeses suffer from textural problems such as excessive firmness, rubbery texture, and decreased melting properties. The properties of Cheddar cheese used in Processed cheese making have direct impact on the quality attributes of the final product. As cheese ages, residual proteolytic activity of the coagulant can quickly hydrolyze

sufficient casein to greatly increase flowability and decrease the stretch of cheese, both to the extent that bake performance may be negatively affected (Moynihan *et al.*, 2013).

The functional properties of Processed cheese are determined by the ingredients used in the formulation (i.e., type of natural cheese, age of natural cheese, amount of natural cheese, type and amount of emulsifying salt) as well as processing conditions (i.e., cooking temperature, cooking time, and mixing speed during manufacture). The variations in the chemical properties of a Processed cheese that arise during a cheese formulation significantly influence its functional properties therefore, it is important to control the formulation parameters of Processed cheese to achieve a product with consistent functional properties (Kapoor *et al.*, 2007). A lot of work has been done on Processed cheese prepared from Cheddar and other natural cheeses of different ages. There is no work in which Mozzarella and Cheddar cheese amalgam was used for manufacturing of Processed pizza cheese. Therefore, a study was designed to develop Processed pizza cheese by using fresh Mozzarella and semi ripened Cheddar cheese in different combinations.

The aim of the present study was to access the changes in chemical composition and texture of Processed pizza cheese developed by using Mozzarella and semi-ripened Cheddar cheese in combination.

MATERIALS AND METHODS

Procurement of raw materials: Raw buffalo milk for the preparation of Mozzarella and Cheddar cheeses were procured from the SB Dairy Farm Jhapal, Faisalabad. 100 L of milk was used for preparation of each batch of Mozzarella and Cheddar cheese. Mixed mesophillic culture *Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* and thermophillic culture *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Chr. Hansen Ireland Ltd., Rohan Industrial Estate, Little Island, Co. Cork, Ireland) were used for Cheddar and Mozzarella cheese respectively. Chymosin of 50000u/G strength of Pharm Chemical Co., Ltd. China was used as coagulant in both cheeses. Emulsifying salts disodium phosphate and trisodium citrate of Fooding Group Limited was procured from local supplier for manufacturing of Processed pizza cheeses. Three replicates of seven Processed pizza cheeses were prepared for the study.

Preparation of Processed pizza cheeses (PPC): The milk was standardized at 3% fat level for both Mozzarella and Cheddar cheese. Cheddar cheese was prepared by following the method described by Ong *et al.* (2007) while Mozzarella cheese was manufactured by following the method of Zisu and Shah, (2007) with slight modification as milling was done at 4.9 pH in order to standardize the processing steps for buffalo Mozzarella that is well suited to prepare PPC. Processed pizza cheeses were prepared by following the method of Shirashoji *et al.* (2006) with some variation of emulsifying salts concentration as 2% emulsifying salt was used in the study. PPC were prepared in a Blentech twin screw cooker (Blentech Corp., Rohnert Park, CA) equipped with indirect steam injection. Seven PPC were prepared: Control (100% MC), PPC₁, PPC₂, PPC₃ with (75:50), (50:50), (25:75) MC and 2 months SRCC and PPC₄, PPC₅, PPC₆ with (75:50), (50:50), (25:75) MC and 4 months SRCC, respectively. PPC blocks of 2 Kg weight were then repacked, vacuum-sealed and stored again at 5°C for further analysis.

Physicochemical analysis: Physicochemical analysis like moisture, acidity (AOAC, 1990), pH (Ong *et al.*, 2007), protein (IDF, 1964), fat (Marshall, 1992) and total calcium (Metzger *et al.*, 2000) of natural and PPC were determined according to standardized methods.

Texture profile analysis: Texture profile of PPC was determined by performing the texture profile analysis (TPA) on TA-XT Plus Texture Analyzer (Stable Micro Systems, Godalming, Surrey, UK) using Compression plate (Zisu and Shah, 2007). Definitions and calculated TPA parameters of the TA-XT2i texture analyzer used to measure cheese samples texture are given in Table 1.

Statistical analysis: The results were analyzed statistically by using Minitab statistical package. Complete randomized design and multiple degree of freedom contrast system were used to find out the difference between control and other

treatment samples. The means were compared using Tukey's test after their significant difference (Steel *et al.*, 1997).

Table 1. Definitions and calculated TPA parameters.

Parameters	Descriptive definition
Hardness	Force F_{max} required for 30% compression (g)
Length-I	Distance required to reach F_{max} (mm)
Area-I	Work of first compression (integration of area under first compression curve) (g/mm)
Length-II	Distance from probe contact to reach 30% compression (mm)
Area-II	Work of second compression (integration of area under second compression curve) (g/mm)
Cohesiveness	Area-II/Area-I (ratio)
Springiness	Length-II/Length-I (ratio)
Gumminess	Hardness x Cohesiveness (unitless)
Chewiness	Hardness x Cohesiveness x Springiness (unitless)

RESULTS AND DISCUSSION

Physicochemical composition of natural cheeses: The mean of the physicochemical composition of Mozzarella and Cheddar cheese (2 and 4 months ripened) are given in Table 2.

Table 2. Mean values of physicochemical composition of natural cheeses.

Parameters	Mozzarella cheese	Cheddar cheese	
		2 months ripened	4 months ripened
Moisture	46.95±1.00	35.36a±0.47	35.26b±0.20
pH	4.88±0.01	5.21a±0.05	5.0b±0.04
Acidity	0.9±0.2	0.88b±0.005	0.82a±0.01
Protein	25.36±1.18	28.23±0.20	28.20±0.10
Fat	20.01±1.73	22.33±1.15	22±1.00
Total Ca g/100g	0.7±0.005	0.82±0.03	0.84±0.02

Physicochemical composition of Processed pizza cheeses (PPC): Mean squares for physicochemical composition of PPC are depicted in Table 3. The results demonstrated that blending significantly ($P<0.05$) changes physicochemical parameters (moisture, pH, acidity, protein, fat and total calcium) of PPC. Moisture, pH and acidity of PPC significantly ($P<0.05$) affected by the ripening months of Cheddar cheese while protein, fat and total calcium content is not affected by the ripening months of Cheddar cheese. Level of cheeses (Mozzarella and Cheddar) also have significant ($P<0.05$) effect on the physicochemical composition of PPC however, interaction of ripening months of Cheddar cheese × level of cheeses has non-significant ($P>0.05$) on physicochemical parameters of PPC.

Mean values for moisture content of PPC are given in Table 4. The results revealed that control pizza cheese (100%

Table 3. Mean squares of physicochemical composition of Processed pizza cheese.

Sources of variation	df	Moisture	pH	Acidity	Protein	Fat	Total calcium
Blending	6	13.4696*	0.266**	0.001727**	1.95289*	4.9035*	0.006442**
Control VS others	1	0.3779 ^{NS}	0.0224 ^{NS}	0.004000**	4.66331*	0.7685 ^{NS}	0.009257**
Months (M)	1	2.6450*	0.0512*	0.002938*	0.03380 ^{NS}	0.0018 ^{NS}	0.001800 ^{NS}
Levels (L)	2	38.8588*	0.0392*	0.001672**	3.45515*	14.3223*	0.013650*
M×L	2	0.387 ^{NS}	0.0038 ^{NS}	0.000038 ^{NS}	0.05495 ^{NS}	0.0031 ^{NS}	0.000150 ^{NS}
Error	14	0.5411	0.0077	0.000238	0.62604	0.4861	0.000782

NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01)

Table 4. Mean values (n = 3) of the physicochemical composition of Processed pizza cheese samples.

	Processed pizza cheese						
	PPC ₀	PPC ₁	PPC ₂	PPC ₃	PPC ₄	PPC ₅	PPC ₆
Moisture	51.90±0.58a	49.75±0.37b	46.15±0.06b	44.95±0.07bc	49.05±0.30b	45.50±0.21bc	44.00±0.18c
pH	4.89±0.06b	4.88±0.06 b	4.92±0.05b	4.99±0.03b	4.94±0.04b	5.02±0.03ab	5.15±0.04a
Acidity	0.92±0.01a	0.89±0.02bcd	0.87±0.09de	0.85±0.08e	0.91±0.09ab	0.90±0.01abc	0.88±0.08cde
Protein	25.37±0.68b	26.07±0.48b	26.70±0.46ab	27.51±0.42a	26.04±0.28b	26.40±0.29ab	27.58±0.26a
Fat	18.23±1.00c	19.21±0.66bc	19.78±0.33b	21.29±0.66a	19.20±0.33c	19.72±0.88b	21.32±0.88a
Total Ca	0.71±0.09d	0.73±0.01d	0.78±0.09bc	0.83±0.02a	0.72±0.02d	0.75±0.01cd	0.81±0.01ab

Means sharing similar letters in a column are statistically non-significant (P>0.05)

Mozzarella cheese) has higher moisture content than all other blends. This could be due to impact of moisture differences of natural Mozzarella and Cheddar cheeses as indicated in Table 2. The higher level of moisture in Processed pizza cheeses with high percentage of Mozzarella cheese might be due to *Streptococcus thermophilis* used as starter culture which has the ability to produce EPS that entrap moisture and reduces the whey expulsion in Mozzarella cheese (Petersen *et al.*, 2000). Higher moisture content in Processed pizza cheese with greater Mozzarella cheese in the present investigation is perhaps a consequence of lower level of calcium in Mozzarella cheese that results in increased casein hydration (Keller *et al.*, 1974). The reduction of moisture in Processed pizza cheeses with ripening and increasing level of Cheddar cheese could be a result of change of physical state of water in cheese, as during ripening the water gets chemically bound which reduce the moisture content in ripened Cheddar cheese. Mean values for pH of PPC are expressed in Table 4. The highest pH (5.15) was found in PPC₆ while PPC₁ exhibited lowest pH (4.88). The results also illustrated that increasing level of Cheddar cheese increases the pH of PPC. Moreover, PPC that was prepared with the addition of four months ripened Cheddar cheese has slightly higher pH as compared to PPC that was prepared with the amalgamation of two months ripened Cheddar cheese. In PPC minor differences in pH values might be attributed to the fact that pH of fresh Mozzarella and Cheddar (2 and 4 months ripened) used in the pizza cheese was different (Table 2). In similar study conducted by Kaminarides *et al.* (2007), the pH of Processed cheese blends prepared by mixing of Halloumi and Kopanisti cheeses and strained yoghurt were detected dissimilar due to pH difference of initial products used in the combinations.

The increase in pH with increased level of Cheddar cheese was owing to higher pH for both two and four months ripened Cheddar cheese. The higher pH of Cheddar cheese might be due to higher milling pH (5.3) while in case of Mozzarella cheese milling was carried out at lower pH (4.88). In previous studies milling pH of Mozzarella and Cheddar cheese were found in the range of 5.2 to 5.4 with different processing conditions (Yun *et al.*, 1993; Shakeel-Ur-Rehman *et al.*, 2003; Hassan *et al.*, 2004). In the present study milling pH of the Mozzarella cheese was 4.9 because at this pH the buffalo milk curd exhibited more stretch properties. This increase in pH of PPC due to increase of more ripened Cheddar cheese perhaps associated with the slow increase of hydration of para-casein and formation of various protein residues in Cheddar cheese during ripening. These breakdown products e.g. carboxyl groups of glutamic and aspartic acids have ability to attach with H⁺ during storage and thus reducing the hydrogen ion concentration of the Cheddar cheese that subsequently increase pH of PPC (Guo *et al.*, 1997; Sheehan *et al.*, 2004).

Table 4 shows the mean values for acidity of PPC. It was obvious from the results that the acidity of control pizza cheese was highest from all other amalgams. It possessed greater acidity might be due to Mozzarella cheese in which pH decreased to 4.88 during cheddaring and more lactose is converted into lactic acid. In present experiment as the level of SRCC increased the acidity decreased. It might be due to higher (5.3) milling pH of Cheddar cheese than Mozzarella cheese (4.88). Moreover, acidity of PPC with four months SRCC were greater than PPC with two months SRCC. It could be due to greater activity of culture that converts more sugars and residual lactose in to lactic acid and other acids.

Amarita *et al.*, (2001) showed similar results that the acidity increased as the sugars converted into acids. Furthermore, during ripening the acidity was further increased due to biochemical changes like proteolysis, lipolysis and conversion of residual lactose to lactate and citrate (McSweeney, 2004).

The mean values for protein of PPC are demonstrated in Table 4. It was indicated that control pizza cheese has lowest protein content as compared to other cheese blends. Similarly the PPC with higher levels of Mozzarella cheese had less protein contents and PPC with higher levels of SRCC showed greater protein content. The protein varied in cheese blends might be due to Mozzarella and Cheddar cheese protein difference. This variation might be due to difference in cheese making ingredients and conditions in both types of cheeses. In Mozzarella cheese thermophillic cultures (*Streptococcus Thermophilus* and *Lactobacillus Bulgaricus*) were used with more proteolytic activity and caused a decrease in protein in Mozzarella cheese due to loss of protein in whey. In case of Cheddar cheese protein recovery was more due to less proteolytic activity of mesophillic cultures (*Lactococcus Lactis* *sb. Lactis*; *Lactococcus Lactis* *sb. Cremoris*) (Najafi *et al.*, 2008).

The reduction of protein content in Mozzarella cheese might also be due to difference of gelation temperature in both cheeses. In case of Cheddar cheese gelation was carried out at 27°C while in Mozzarella cheese curd was set at 37°C. In both type of cheeses chymosin was used which is very sensitive to temperature and its proteolytic activity increased due to higher temperature therefore the protein content of Mozzarella decreased due to more loss of protein in whey. The results of the present study correlated with the finding of Castillo *et al.* (2006) who reported that excess proteolysis in curd supported the weakening of protein network. This network rearranged with more shrinkage but syneresis increased the loss of protein and fat (Hussain *et al.*, 2012).

Table 4 demonstrates the mean values for fat content of PPC. It is indicated from the results that control pizza cheese has less fat content as compared to other PPC. It is also obvious from the results that in PPC as the concentration of SRCC increased the fat content increased respective to the levels of Cheddar used in the formulation. The greater value of fat content with higher level of Cheddar cheese might be due to greater fat content of Cheddar cheese (Table 2). Although the

milk was standardized at same fat level but high level in Mozzarella cheese could be due to greater moisture retention in Mozzarella cheese. While in case of Cheddar cheese pressing decreased moisture contents therefore the fat content on dry matter basis increased. By num and Olson (1982) observed that in Cheddar cheese firmness leads to reduction of fat and casein losses therefore the fat recovery in Cheddar cheese is greater than Mozzarella cheese. Furthermore, even though the levels of SRCC were same in two and four months blends but a little reduction of fat content was observed but this difference was non-significant. Wang *et al.*, (2012) observed similar results that there was non-significant variation in the contents of fat for both natural Cheddar cheeses and Processed cheeses during ripening.

Total calcium content of PPC are depicted in Table 4 illustrated that total calcium content in control pizza cheese was less than all other PPC. The less calcium content in control cheese perhaps associated with decreased pH (4.88) in Mozzarella cheese during cheese manufacturing which solubilize calcium. Total calcium content increased as the level of Cheddar cheese increased. It might be due to less solubilization of colloidal calcium phosphate in Cheddar cheese that results higher level of calcium. Kimura *et al.* (1992) worked on the effect of calcium on cheese quality and concluded that colloidal calcium phosphate is dissociated from the casein during pH reduction which reduced the hydrophobic binding sites of submicelles therefore weaken the bond between submicelles and solubilize calcium.

Cheese texture: The mean squares concerning texture profile analysis (TPA) of PPC are depicted in Table 5 which revealed that blending, ripening months of Cheddar cheese and levels of cheeses (Mozzarella and Cheddar) has significant ($P > 0.05$) effect on hardness, cohesiveness, springiness, gumminess and chewiness of PPC while interaction of ripening months of Cheddar cheese \times levels of cheeses has non-significant effect on the TPA of PPC.

The means for textural parameters (hardness, cohesiveness, springiness, gumminess and chewiness) of PPC are depicted in Figures 1, 2, 3, 4 and 5 which pointed out that blending of Mozzarella and Cheddar cheese considerably changes the TPA of PPC. Control pizza cheese exhibited highest while PPC₆ possessed lowest values for textural parameters. It is also indicated that in PPC hardness decreased with increasing concentration and age of Cheddar cheese.

Table 5. Mean squares of texture parameters of Processed pizza cheese.

Sources of variation	df	Hardness	Cohesiveness	Springiness	Gumminess	Chewiness
Blends	6	2611860**	0.005577**	0.011444**	3652587**	3065726**
Control vs others	1	7207838**	0.0099556**	0.022400**	9827977**	8248660**
Months (M)	1	849821**	0.0072000**	0.005000**	1789522**	1344496**
Levels (L)	2	3784254**	0.0077389**	0.020217**	5122837**	4371184**
M \times L	2	22498 ^{NS}	0.0004167 ^{NS}	0.000417 ^{NS}	26176 ^{NS}	29417 ^{NS}
Error	14	15465	0.0007000	0.000514	16090	14484

NS = Non-significant ($P > 0.05$); * = Significant ($P < 0.05$); ** = Highly significant ($P < 0.01$)

The changes in the texture characteristics of PPC might be attributed due to difference in physico-chemical composition of Cheddar and Mozzarella cheese (Table 2). No doubt, the moisture content of control pizza cheese (100% Mozzarella cheese) was higher, but it was negatively correlated with the textural attributes of PPC. It could be related to the higher level of intact case in which incorporates higher values of textural attributes to PPC.

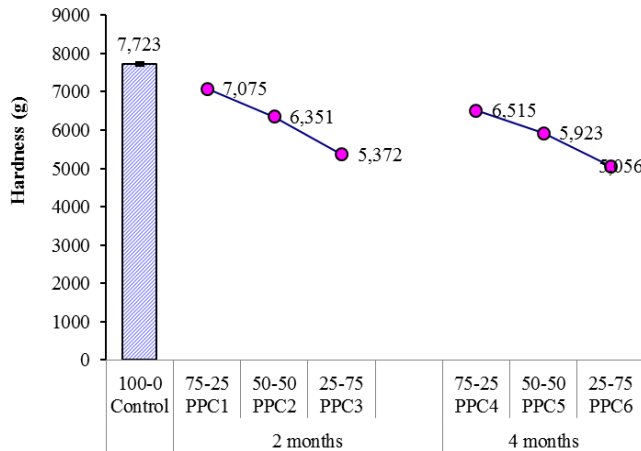


Figure 1. Hardness of Processed pizza cheeses, Control-100% Mozzarella cheese, PPC1, PPC2, PPC3 blends with 75:25, 50:50 and 25:75 Mozzarella: 2 months semi-ripened Cheddar cheese while PPC4, PPC5 and PPC6 blends with 75:25, 50:50 and 25:75 Mozzarella: 4 months semi-ripened Cheddar blends.

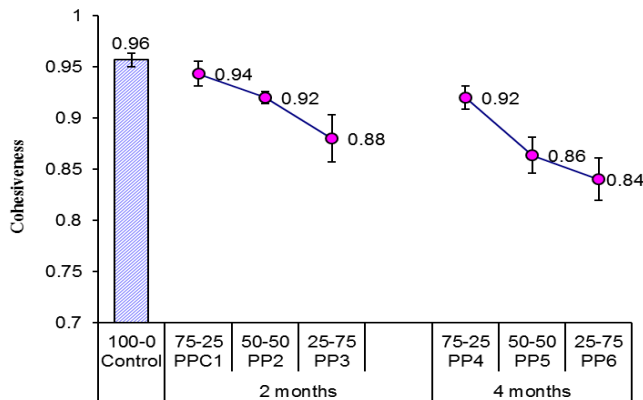


Figure 2. Cohesiveness of Processed pizza cheeses, Control-100% Mozzarella cheese, PPC1, PPC2, PPC3 blends with 75:25, 50:50 and 25:75 Mozzarella: 2 months semi-ripened Cheddar cheese while PPC4, PPC5 and PPC6 blends with 75:25, 50:50 and 25:75 Mozzarella: 4 months semi-ripened Cheddar blends.

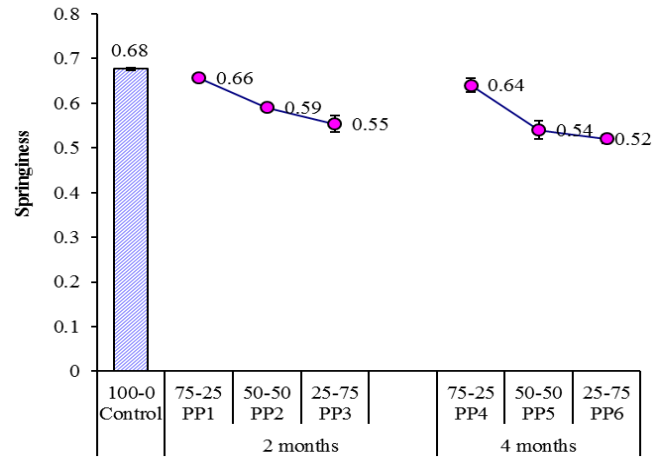


Figure 3. springiness of Processed Pizza cheese, Control-100% Mozzarella cheese, PPC1, PPC2, PPC3 blends with 75:25, 50:50 and 25:75 Mozzarella: 2 months semi-ripened Cheddar cheese while PPC4, PPC5 and PPC6 blends with 75:25, 50:50 and 25:75 Mozzarella: 4 months semi-ripened Cheddar blends.

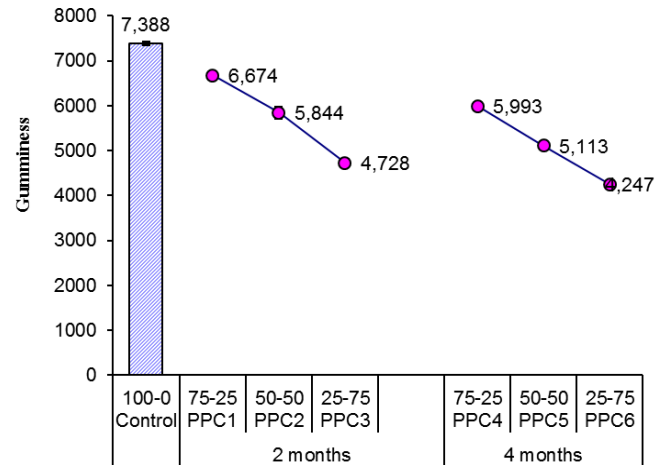


Figure 4. Gumminess of Processed pizza cheeses, Control-100% Mozzarella cheese, PPC1, PPC2, PPC3 blends with 75:25, 50:50 and 25:75 Mozzarella: 2 months semi-ripened Cheddar cheese while PPC4, PPC5 and PPC6 blends with 75:25, 50:50 and 25:75 Mozzarella: 4 months semi-ripened Cheddar blends.

The higher levels and longer ripening of Cheddar cheese are associated with greater proportion of hydrolytic protein due to formation of more amino acids and peptides during ripening. The interaction between protein networks becomes weak and PPC with softer texture are obtained (Sousa and McSweeney 2001); therefore, values for all texture attributes reduced with increased ripened cheese.

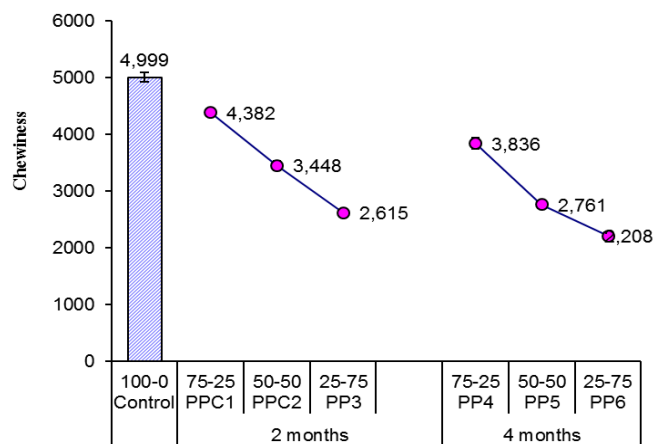


Figure 5. Chewiness of Processed pizza cheese, Control-100% Mozzarella cheese, PPC1, PPC2, PPC3 blends with 75:25, 50:50 and 25:75 Mozzarella: 2 months semi-ripened Cheddar cheese while PPC4, PPC5 and PPC6 blends with 75:25, 50:50 and 25:75 Mozzarella: 4 months semi-ripened Cheddar blends.

In PPC, changes in TPA might be due to difference of structural components arrangements. The cheeses made with the addition of more percentage of Mozzarella showed less porosity due to strong protein network thereby, have higher value of textural attributes while in Pizza cheese containing ripened Cheddar cheese, the values decreased, which might be due to more structural disintegration and expanded protein network (micrograph not shown here) (Awad *et al.*, 2005; Brickley *et al.*, 2008; Lu *et al.*, 2008; Ong *et al.*, 2012).

pH is also another important factor that may affect the texture of PPC. It has been observed that as the concentration of Cheddar cheese increased in Pizza cheese blend, the pH value also increased. Higher pH produce negative charge on the casein which leads to repulsion forces in casein matrix, that may weakens the protein structure and all the texture attributes (Bunka *et al.*, 2014).

The present investigation showed some conflicting results to that of previous studies. cohesiveness were observed in the range of 0.95-0.84 and it was reduced during ripening but in a study conducted by Hladka *et al.* (2014) cohesiveness of processed cheese manufactured from Edam cheese at different maturity levels (1, 8 and 16 weeks) were in the range of 0.47-0.53. They reported that there was no dependence of maturity level on the cohesiveness level of cheese.

Conclusion: Mozzarella is the most popular cheese used for pizza topping. Although, fresh Mozzarella cheese is preferred but in addition to lack of flavor, it suffers from textural problems such as excessive firmness, rubbery texture, and decreased melting properties. In order to reduce these limitations Mozzarella and semi-ripened Cheddar cheese

were processed in different combinations to obtain better foundation properties from Mozzarella while soft and melting characteristics from Cheddar. Mozzarella cheese was milled at pH 4.9 which has greater electrostatic repulsion thereby becomes more hard while Cheddar cheese possessed greater pH (5.4) with less repulsion forces. The results of chemical composition indicated that protein-protein interactions in Processed pizza cheese were decreased due to less electrostatic repulsion contributed by semi-ripened Cheddar cheese as indicated by microstructure (micrograph is not given here). Moisture retention also increased in blends perhaps due to disintegration of paracaseinate complex and mechanical shear and temperature of processing. Hardness, cohesiveness, springiness, gumminess and chewiness decreased in blends with greater semi-ripened Cheddar cheese due to reducing level of intact casein, more α -s1 hydrolysis and casein hydration, less number of cross-linkages induced by Ca.

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