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RESEARCH PAPER

# Influence of buffalo chedder and mozzarella cheese amalgamation on proteolysis and organic acids of processed pizza cheese

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**Key Message:** This research study evaluates the effects of different ratios of both types of cheeses and aging of chedder on the proteolytic, physic chemical and organic acid contents of pizza cheese.

**Abstract:** There is a demand from pizza restaurants to get a proper amalgamation of cheeses with desired characteristics and longer shelf life for pizza cheese. Therefore, the present research was planned to blend the fresh buffalo milk mozzarella and ripened buffalo milk chedder cheeses at various levels into processed pizza cheese. The current study aimed to evaluate the impact of natural cheeses, amalgam of mozzarella and chedder in different ratio and aging of chedder on biochemical characteristics of processed pizza cheese (PPC) through chromatographic (HPLC) and electrophoresis techniques. The rate of proteolysis was rapid in the PPC made using a higher level of four months ripened chedder cheese. Electrophoresis (Urea PAGE) and HPLC indicated reduced intact casein with the increasing quantity and ripening months of chedder cheese in pizza cheese. Mean abundances indicated a significant change in organic acid contents of PPC. In conclusion, significant variation was observed for proteolysis and organic acids production by the difference in percentages and age of cheeses. 

**Keywords:** Casein chains, Composition, Semi-ripened cheese, Texture

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## Introduction

The processed cheese is a type of cheese obtained by amalgamation of natural cheeses, water and emulsifying salts. During processing, heat and agitation is used to produce a uniform product that can be used in multiple forms (Shirashoji et al., 2006). Chedder cheese had a great potential for its use in the preparation of processed cheeses all over the world. Ripening is an important biochemical event of cheese that represents a significant indicator of quality (Kondyli et al., 2016). The mozzarella is a variety of pasta filata cheese used extensively as a topping on baked dishes due to key physical attributes in both unmelted and melted states (Salek et al., 2017). As per recommendation of (Food and Drug Administration [FDA], 2006), the quantity of natural cheeses (Mozzarella and chedder) varied from 51% to greater that 80% in the composition of processed cheese. However, quality characteristics of natural cheeses including its composition directly influence the end quality of processed cheese (Kyung-Hoon et al., 2015). Therefore, a suitable choice of natural cheese is essential to get a processed cheese with the preferred chemical and functional characteristics.

The physicochemical characteristics of a natural cheese that influence the functional characteristics of processed cheese include Ca content, pH and amount or age of intact casein present in the natural cheese (Zehren & Nusbaum, 2000). The rate of biochemical reactions during ripening and the kind of cheeses used in the cheese processing also affects the quality of end products produced. Objective of current exploration was to study the characteristics of processed pizza cheese (PPC) manufactured by the amalgamation of chedder and mozzarella cheese using various ratio of chedder and mozzarella along with varying the age of chedder (2 month ripened, 4 month ripened). This study investigated the influence of blending of different levels and age of natural cheeses on the proteolysis and organic acid contents on processed pizza cheese (PPC).

## Materials and Methods

Buffalo milk was obtained from the Dairy Farm, Faisalabad-Pakistan. Mixed thermophilic culture (*Streptococcus thermophillus* and *Lactobacillus bulgaricus*) and mesophilic culture (*Lactococcus Lactis* subsp. *Lactis* and *Lactococcus Lactis* subsp. *Cremoris*) were used for mozzarella and chedder cheeses, respectively. Chymosin (50000 µ/G strength) were

purchased from Pharm Chemical Co., Ltd. China for both cheeses.

**Preparation of pizza cheeses**

To prepare pizza cheese, chedder and mozzarella cheese was manufactured according to the method prescribed by Ong et al. (2006); Zisu & Shah (2007), respectively with slight modifications. Processed pizza cheese was prepared following the method of Shirashoji et al. (2006). Seven PPC samples were prepared. $PPC_0$ (Pizza cheese with 100 percent mozzarella), $PPC_1$: Pizza cheese amalgam of mozzarella (75%) and chedder (25% + 2 months ripened), $PPC_2$ : Pizza cheese amalgam of mozzarella (50%) and chedder (50% + 2 months ripened) , $PPC_3$ : Pizza cheese amalgam of mozzarella (25%) and chedder (75% + 2 months ripened), $PPC_4$: Pizza cheese amalgam of mozzarella (75%) and chedder (25% + 4 months ripened), $PPC_5$: Pizza cheese amalgam of mozzarella (50%) and chedder (50% + 4 months ripened), $PPC_6$: (Pizza cheese amalgam of mozzarella (25%) and chedder (75% + 4 months ripened).

**Chemical composition of processed pizza cheese**

Moisture content, protein, fat and total calcium of all the treatments were calculated according to the reference method as mentioned in (Association of Official Analytical Chemists [AOAC], (2000); (International Dairy Federation [IDF] Standard 26A, 1993); Marshall (1993) and Metzger et al. (2000), respectively.

**Proteolysis in Processed Pizza Cheese**

**TCA soluble nitrogen**

Kjeldahl digestion and distillation method was used for measurement of soluble nitrogen (David et al., 2004).

**pH 4.6 soluble nitrogen**

Kjeldahl digestion and distillation were done for measurement of peptides by determining nitrogen content (Kuchroo & Fox, 1982).

**Electrophoresis**

**Separation of nitrogenous fractions**

All the treatments were tested by urea-PAGE (Sheehan et al., 2004). Samples passed through the stacking (112 V) and separating gel (224V).

**Proteolysis by RP-HPLC**

The proteolysis in processed pizza cheese insoluble fraction was determined by following the procedure as mentioned by Verdini et al. (2004). A shim-pack CLC-ODS (C-18), 250cm × 4.6 mm, 5 µm column (Phenomenex, Macclesfield, Cheshire, UK), UV detector (SPD-10AV, Shimadzu, Japan) and pump (LC-10AT, Shimadzu, Japan) at room temperature was used for chromatographic separations. Detection was carried out at 214 nm.

**Organic acids in processed pizza cheese**

High performance liquid chromatography (HPLC) was used to determine organic acids (Lactic, Acetic, Citric and Pyruvic) contents in pizza cheeses following the method of (Akalin et al., 2002).

**Statistical analysis**

The data were subjected to statistical analysis using Minitab. Difference between means at a level of significance 0.05 were calculated using complete randomized design (CRD) and multiple degree of freedom contrast system. All mean comparisons were carried out by using Turkey's test (Steel et al., 1997).

## Results and Discussion

**Basic chemical composition of cheese**

Chemical composition of mozzarella and 2 months and 4 month ripened cheese and processed pizza cheese are given in Table 1 and 2. Quality of cheese and consumer acceptability directly determined through its physicochemical composition which have a direct relation with cheese functionality (Costabel et al., 2007). The mean values indicated that mozzarella cheese (batch I and II) contained 46.50-46.95% moisture content, while in case of chedder cheese it was 35.36% and 35.26% in 2 and 4 month ripened, respectively. The protein contents of mozzarella cheese were 25.36% and 24.99%, while fat contents were 23.25% and 23.65% for batch I and II, respectively. These values are in accordance with previous outcomes of Rowney et al. (2003) who noted 25.8% protein and 24.3% fat in mozzarella cheese. The protein contents for 2 and 4 months ripened chedder cheeses were 28.23% and 28.20% while fat contents were 29.33% and 29.03% respectively. Total calcium for Mozzarella was in range of 650-655 mg/100 g and for chedder was 749-754 mg/100g. The soluble calcium was higher (310-370 mg/100 g) for mozzarella than Chedder (220-240 mg/100g soluble) due to lower pH of mozzarella cheese. These findings are in accordance with the results of Guinee et al. (2002).

**Table 1** Physicochemical composition of natural cheeses

| Parameters | Mozzarella cheese | | Chedder cheese | |
|---|---|---|---|---|
| | Batch I | Batch II | 2 months | 4 months |
| Moisture % | 46.95$^{a}$ ± 1.00 | 46.50$^{a}$ ± 0.95 | 35.36$^{a}$ ± 4.7 | 35.26$^{a}$ ± 0.20 |
| Protein % | 25.36$^{a}$ ± 1.18 | 24.99$^{a}$ ± 1.21 | 28.23$^{a}$ ± 0.20 | 28.20$^{a}$ ± 0.10 |
| Fat % | 23.25$^{a}$ ± 1.80 | 23.65$^{a}$ ± 1.73 | 29.33$^{a}$ ± 1.15 | 29.03$^{a}$ ± 1.19 |
| Total calcium (mg/1000g) | 650.32$^{a}$ ± 16.21 | 655.67$^{a}$ ± 15.79 | 754.61$^{a}$ ± 15.92 | 749.56 $^{a}$ ± 15.83 |
| Water soluble calcium (mg/1000 g) | 310.45$^{a}$ ± 15.32 | 330.87$^{a}$ ± 17.42 | 220.65$^{a}$ ± 7.22 | 240.56$^{a}$ ± 6.21 |

Means ± standard deviation. Values are the mean of triplicates, while similar letterings represent a non-significant (P≤0.05) difference.

**Chemical composition of processed pizza cheese**

Control cheese samples significantly (p<0.05) differ from other treatments regarding moisture, protein, fat and total calcium contents of pizza cheeses. Pizza cheeses prepared by incorporation of 2 months ripened chedder cheese and a greater proportion of mozzarella cheese ($PPC_1$) has higher mean value (50.80 ± 0.62%) for moisture content than those prepared by amalgamation of 4 months ripened chedder cheese ($PPC_6$) with mozzarella cheese (46.58 ± 0.33%). Different functional properties (Melting and stretching) of different cheese varieties is contributed by its protein contents (Guinee et al., 2007). Pizza cheeses made with the addition of 75% mozzarella and 25% chedder cheese ($PPC_1$ and $PPC_4$) showed lowest mean value (26.07 ± 0.28% and 26.04 ± 0.52%) of protein and lowest mean value of fat (24.32 ± 0.66 and 24.33 ± 0.33), while the highest values 27.58 ± 0.43% and 27.51 ± 0.208% of protein and 27.64 ± 0.66% and 28.35 ± 0.88% fat contents was observed in pizza cheeses prepared with the incorporation of 75% Chedder in 25% mozzarella ($PPC_6$ and $PPC_3$), respectively. It might be due to greater amount of moisture in mozzarella than that of chedder cheese. Protein reduction of pizza cheese with increased level of mozzarella cheese might be high gelation temperature (37 ºC) of mozzarella cheese as compared to chedder cheese (31 ºC). Higher temperature in mozzarella cheese that results in more proteolysis and protein loss through whey. These results are in close proximity with the findings of Castillo et al. (2006a) they reported that excess proteolysis in curd leads to weakening of protein network. However ripening time (2 months and 4 months) of chedder cheese does not have a significant effect (P>0.05) on fat, protein and calcium contents. The control sample (100% mozzarella) exhibited the lowest (652 ± 26 mg/100 g) while $PPC_6$ (Pizza cheese amalgam of mozzarella (25%) and chedder (75% + 4 months ripened) showed highest (748 ± 30 mg/100 g) total calcium content. The reason of calcium reduction with increased level of mozzarella in pizza cheese is probably a function of pH reduction (4.88) in mozzarella during cheddering that solubilizes colloidal calcium from the casein network. By reducing pH, bonds between sub micelles weakens due to the loss of hydrophobic binding sites which in turn solubilized calcium (Kimura et al., 1992).

**Table 2** Chemical composition of processed pizza cheeses

| Components | $PPC_0$ | Processed Pizza Cheeses | | | | | |
|---|---|---|---|---|---|---|---|
| | | $PPC_1$ | $PPC_2$ | $PPC_3$ | $PPC_4$ | $PPC_5$ | $PPC_6$ |
| Moisture % | 51.23$^{a}$±0.87 | 50.80$^{b}$±0.62 | 50.00$^{c}$±0.90 | 47.54$^{d}$±0.57 | 49.72$^{c}$±1.00 | 47.8$^{d}$±0.57 | 46.58$^{e}$±0.33 |
| Protein % | 25.37$^{b}$±0.54 | 26.07 $^{b}$±0.28 | 26.$^{70ab}$±0.49 | 27.51$^{a}$±0.21 | 26.04$^{b}$±0.52 | 26.40$^{ab}$±0.57 | 27.58$^{a}$ ±0.43 |
| Fat % | 23.00$^{b}$±1.00 | 24.32$^{b}$± 0.66 | 26.67$^{a}$±0.33 | 27.64$^{a}$ ±0.66 | 24.33$^{b}$ ±0.33 | 27.32$^{a}$±0.88 | 28.35$^{a}$ ±0.88 |
| Total calcium | 652$^{d}$ ±26 | 676$^{cd}$±22 | 702$^{abc}$±24 | 728$^{ab}$±28 | 689$^{bcd}$ ±26 | 722$^{abc}$±29 | 748$^{a}$ ±30 |

Values presenting in the table are the mean of three replicates; $PPC_0$ (Pizza cheese with 100 percent mozzarella), $PPC_1$: Pizza cheese amalgam of mozzarella (75%) and chedder (25% + 2 months ripened), $PPC_2$ : Pizza cheese amalgam of mozzarella (50%) and chedder (50% + 2 months ripened) , $PPC_3$: Pizza cheese amalgam of mozzarella (25%) and chedder (75% + 2 months ripened), $PPC_4$ : Pizza cheese amalgam of mozzarella (75%) and chedder (25% + 4 months ripened), $PPC_5$ : Pizza cheese amalgam of mozzarella (50%) and chedder (50% + 4 months ripened) , $PPC_6$ : Pizza cheese amalgam of mozzarella (25%) and chedder (75% + 4 months ripened). Total calcium was measured in mg/100 g.

**Organic acid contents of processed pizza cheese**

**Lactic acid**

It is indicated from the results that lactic acid contents increased from $PPC_0$ to $PPC_6$ (Table 3). Lactic acid produced in cheeses is a consequence of metabolic activity of microorganisms. At 0-day of both cheeses, lactic acid was slightly higher in mozzarella cheese as compared to chedder cheese perhaps associated with greater activity of microbial culture of mozzarella cheese (Ong & Shah, 2009). This elevated amount of lactic acid in mozzarella cheese might also be associated with lower pH value of mozzarella cheese (Buffa et al., 2004). The increase in lactic acid content of Processed Pizza cheese with increasing level and age of Chedder cheese could be a consequence of more lactic acid produced by the conversion of residual lactose with the help of *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* in Chedder cheese (McSweeney & Fox, 2004).

**Acetic acid**

Mean values for acetic acid content of processed pizza cheese indicated that mozzarella cheese ($PPC_0$) possessed comparatively lower acetic acid content than that of amalgamated pizza cheeses (Table 3). Although, the acetic acid content was greater in fresh mozzarella as compared to fresh chedder cheese due to greater activity of thermophilic culture, while ripening increased the concentration of acetic acid in chedder cheese comparative to mozzarella cheese. It is possibly because of more protein breakdown and production of amino acids in chedder cheese that provide precursor for acetic acid formation (McSweeney & Sousa, 2000). Acetic acid contents increased in PPC with increasing level and age of ripened cheese as a result of fermentation of lactose into acetic acid with microorganism via fructose-6-phosphate shunt pathway (Nayak., 2015). During ripening of chedder, greater breakdown of amino acids leads to the increase in acetic acid contents in contrast to mozzarella (Manolaki et al., 2006).

**Citric acid**

It is apparent from the results that overall mean values of citric acid increases as the chedder cheese increases (2048-2147 mg/kg) (Table 3). The variation arises due to difference of culture, processing and ripening period used for manufacturing of mozzarella and chedder cheese (Manolaki et al., 2006; Ong & Shah, 2009). However, it is interesting to note that as the level of two months ripened chedder cheese increases in PPC, the citric acid contents also increases while increasing the level of four months ripened chedder cheese decreases the citric acid in PPC. In the former samples increasing level of citric acid is a result formation of citrate in chedder 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). The ripening months of chedder cheese significantly decreases the citric acid contents. 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 & Chung, 2002).

**Pyruvic acid**

Mean values regarding pyruvic acid content of PPC are shown in Table 3 which indicated that amalgamation significantly affected the pyruvic acid content of PPC. Higher concentration of pyruvic acid in fresh cheeses is a result of its rapid formation through the glycolytic pathway and lesser amount in ripened chedder cheese was due to its utilization as it is used as substrate in various metabolic reactions such as formation of ethanol, formic acid, acetoin, diacetyl and 2,3-butylene glycol. No significant variation was found in pizza cheeses made with two and four months ripened chedder cheese.

**Table 3** Effect of mozzarella and chedder cheese amalgamation on organic acid contents (mg/kg) of processed pizza cheese

| Treatments | Lactic acid | Acetic acid | Citric acid | Pyruvic acid |
|---|---|---|---|---|
| $PPC_0$ | $8036.00^{d} \pm 68.35$ | $745.67^{d} \pm 14.50$ | $2040.33^{c} \pm 22.06$ | $127.55^{a} \pm 5.38$ |
| $PPC_1$ | $9025.00^{c} \pm 118.37$ | $790.67^{d} \pm 17.61$ | $2127.00^{b} \pm 20.03$ | $111.77^{b} \pm 4.34$ |
| $PPC_2$ | $9079.67^{c} \pm 57.49$ | $1044.33^{c} \pm 28.29$ | $2248.00^{a} \pm 21.70$ | $92.04^{d} \pm 1.56$ |
| $PPC_3$ | $9383.33^{bc} \pm 319.42$ | $1197.33^{b} \pm 36.56$ | $2299.33^{a} \pm 28.88$ | $73.38^{e} \pm 1.63$ |
| $PPC_4$ | $10010.00^{b} \pm 286.99$ | $1154.33^{b} \pm 18.34$ | $1929.33^{de} \pm 18.27$ | $108.91^{bc} \pm 3.70$ |
| $PPC_5$ | $10697.33^{a} \pm 299.79$ | $1273.00^{a} \pm 18.45$ | $1913.00^{e} \pm 21.70$ | $100.30^{cd} \pm 2.59$ |
| $PPC_6$ | $11184.00^{a} \pm 161.64$ | $1291.33^{a} \pm 30.43$ | $1995.00^{cd} \pm 26.89$ | $64.07^{e} \pm 1.73$ |

Mean ± Standard error. Means sharing similar letters in a column are statistically non-significant ($P \leq 0.05$).

**Proteolysis in processed pizza cheese**

**pH 4.6 and TCA soluble nitrogen (SN)**

The mean values revealed that $PPC_0$ exhibited lowest while $PPC_6$ possessed higher pH 4.6 and TCA-SN. The difference might be due to variation in mozzarella and chedder cheese protein hydrolysis during manufacturing and ripening. The thermophilic cultures used in mozzarella cheese are more proteolytic in nature therefore cause greater hydrolysis of protein and reduced pH 4.6 and TCA soluble nitrogen in mozzarella (Najafi et al., 2008). As the level of chedder cheese increased in PPC, the amount of pH 4.6 and TCA-SN also increased, but the increase is higher with four month old chedder cheese than that with two month old chedder cheese (Table 4). The increase in pH 4.6 SN and TCA- SN with increasing level of chedder cheese is attributed to the greater hydrolytic protein of chedder cheese. The hydrolytic protein further increased during ripening of chedder cheese therefore the pH 4.6 and TCA-SN also increased in PPC. The nitrogen contents usually increased as ripening time increases, consequently the continues breakdown of casein fractions and larger peptides into smaller peptides and amino acids by the action of NSLAB, starter culture, enzymes and residual rennet that increase the pH 4.6 and TCA soluble nitrogen in cheese (Sulejmani & Ali, 2016).

**Table 4** Effect of mozzarella and chedder cheese amalgamation on proteolysis in processed pizza cheese

| Treatments | pH 4.6-soluble nitrogen | TCA-soluble nitrogen |
|---|---|---|
| $PPC_0$ | $1.76^{g} \pm 0.11$ | $2.05^{g} \pm 0.03$ |
| $PPC_1$ | $3.66^{f} \pm 0.06$ | $3.27^{f} \pm 0.26$ |
| $PPC_2$ | $4.20^{e} \pm 0.15$ | $4.20^{e} \pm 0.15$ |
| $PPC_3$ | $4.90^{d} \pm 0.03$ | $5.03^{d} \pm 0.09$ |
| $PPC_4$ | $5.34^{c} \pm 0.22$ | $5.57^{c} \pm 0.28$ |
| $PPC_5$ | $7.48^{b} \pm 0.19$ | $6.15^{b} \pm 0.10$ |
| $PPC_6$ | $8.30^{a} \pm 0.12$ | $7.03^{a} \pm 0.03$ |

Means sharing similar letter in a column are statistically non-significant (P≤0.05).

**Urea polyacrylamide gel electrophoresis (Urea-PAGE)**

Urea–PAGE chromatograms of the different Pizza cheeses are shown in Fig. 1. Casein fractions are being labelled according to (Viotto and Grosso, 1999). Electrophoretogram showed mainly the difference in casein fractions (αs1- and β-casein) of different pizza cheeses. The electrophoretogram indicated that the hydrolytic product ($\alpha s_1$-I-CN) of αs1-casein increased as the level of two months ripened chedder cheese increased in Pizza cheeses from $PPC_1$-$PPC_3$ but in case of Pizza cheeses that have four months ripened chedder cheese by increasing the level of chedder, the $\alpha s_1$-I-CN fragment becomes less visible from $PPC_4$-$PPC_6$. In $PPC_5$ and $PPC_6$ it was further hydrolyzed as indicated by the band below the $\alpha s_1$-I-CN. McSweeney et al.(1993); Fox and McSweeney (1996) also observed remarkable increase of αS1-I-casein at the beginning and decreased later with ripening time.This behavior can be explained taking into account that αS1-I-casein is the fragment (f24–199) of αs1-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 cheeses (Akin & Kirmaci, 2015). The degradation is more noticeable in the PPC with four months ripened chedder cheese as compared to two months ripened chedder cheese. It is because of the reason that protein continuously degrades during maturation and casein fractions break into their hydrolytic products (Phelan et al., 1973; Fenelon & Guinee, 2000). A band between αs1-casein and β-casein that is also prominent in control ($PPC_0$) and its intensity decrease with the increasing level and age of chedder cheese but it remains slightly visible in all PPC due to presence of fresh Mozzarella cheese (McSweeney et al., 1994).

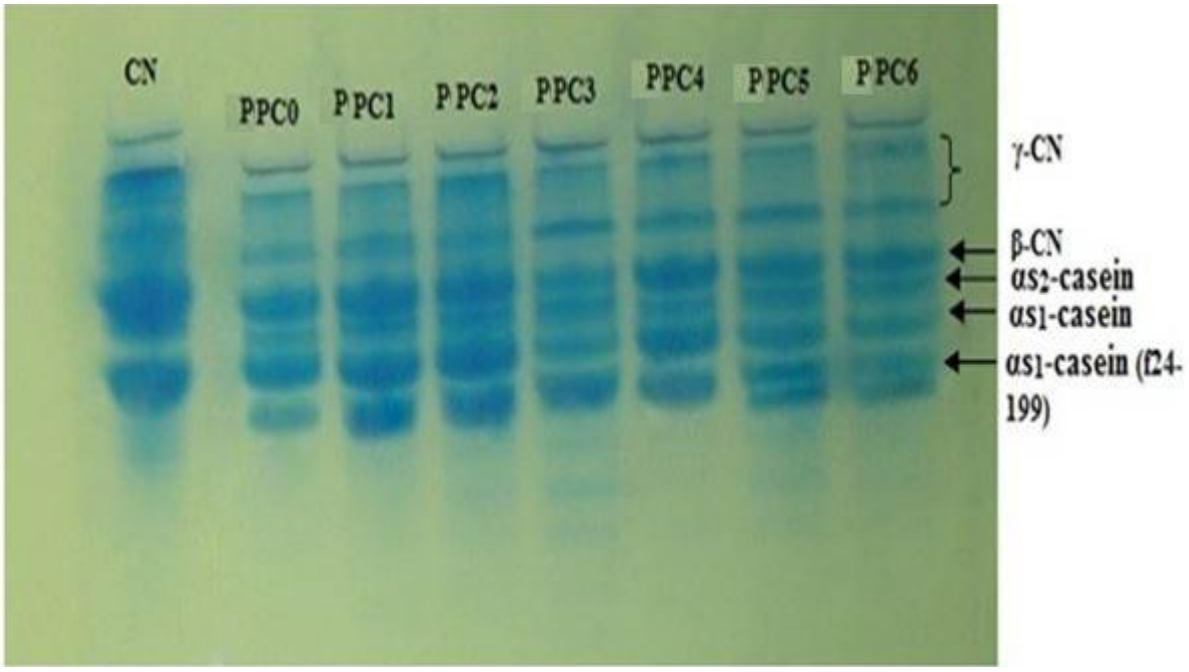


**Fig. 1** Electrophoretogram showing proteolysis in processed pizza cheese.
CN (Casein standard), $PPC_0$ (Pizza cheese with 100 percent mozzarella), $PPC_1$: Pizza cheese amalgam of mozzarella (75%) and chedder (25% + 2 months ripened), $PPC_2$: Pizza cheese amalgam of mozzarella (50%) and chedder (50% + 2 months ripened) , $PPC_3$ : Pizza cheese amalgam of mozzarella (25%) and chedder (75% + 2 months ripened), $PPC_4$ : Pizza cheese amalgam of mozzarella (75%) and chedder (25% + 4 months ripened), $PPC_5$: Pizza cheese amalgam of mozzarella (50%) and chedder (50% + 4 months ripened) , $PPC_6$ : (Pizza cheese amalgam of mozzarella (25%) and chedder (75% + 4 months ripened).

**Reverse phase high performance liquid chromatography (RP-HPLC)**

The amount of casein breakdown in PPC at different levels of natural cheeses (Chedder and mozzarella) and ripened chedder was monitored. Proteolysis of casein in pizza cheeses were studied from water insoluble fraction using standard (Casein) to assess the degree of hydrolysis in pizza cheeses (Fig. 2). In processed pizza cheeses three peaks of αs-1and αs-2 caseins and β-casein were identified by comparing their retention time of casein standard.

**$\alpha s_1$-casein, $\alpha s_2$-casein and β-casein**

The peak areas for $\alpha s_1$-casein, $\alpha s_2$-casein and β-casein of PPC are depicted in Fig. 3 and Fig. 4(a), 4(b), 4(c), 4(d), 4(e) and 4(f). These peaks indicated that maximum peak areas were observed in $PPC_0$ (Fig. 3), while $PPC_6$ exhibited lowest peak areas for $\alpha s_1$-casein (Fig. 4f ). These were reduced as the level of chedder cheese increased in PPC and further reduction were noticed when four months ripened chedder cheese was used in PPC. With increasing levels of ripened chedder, intact casein decreased. It happened due to the continuous breakdown of all casein fractions (action of coagulant or peptidases and proteinases of the starter bacteria) which leads to an increase in proportion of hydrolytic protein in pizza cheeses (Miloradovic et al., 2017). The content of intact caseins (αs-1, αs-2 and β-casein) in PPC also decreased with ripening months of chedder cheese might be due to formation of water soluble peptides and amino acids from casein that reduces the intact casein fractions. In case of αS1-casein, the reduction is accompanied by an increase in αS1-I fraction which originate as a result of αS1-casein hydrolysis (Ceruti et al., 2012).

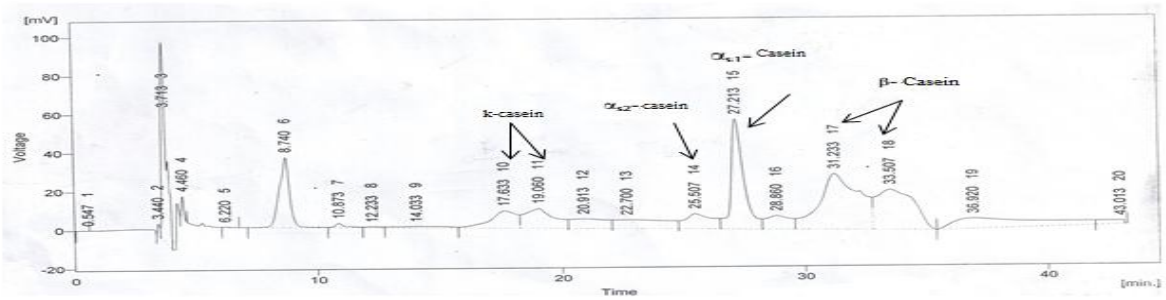


**Fig. 2** Peak areas for casein fractions of standard

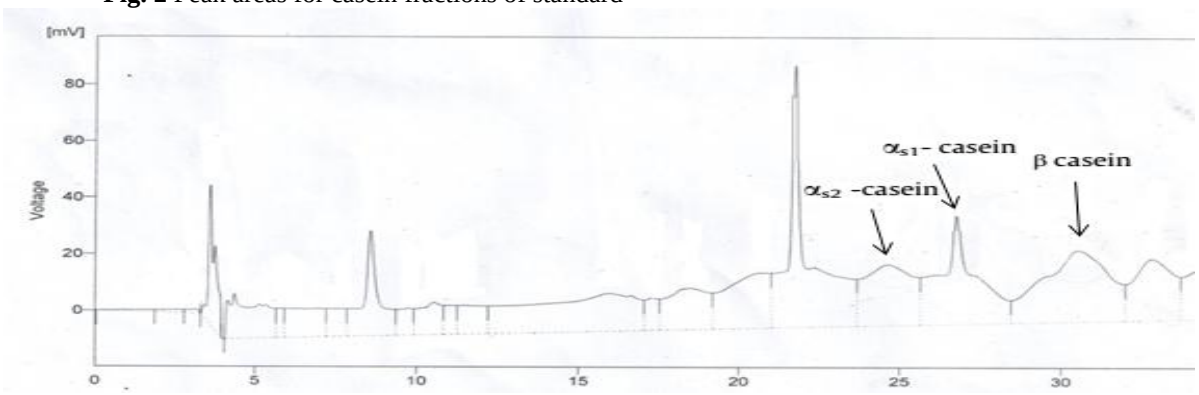


**Fig. 3** Peak areas for casein fractions of control ($PPC_0$)

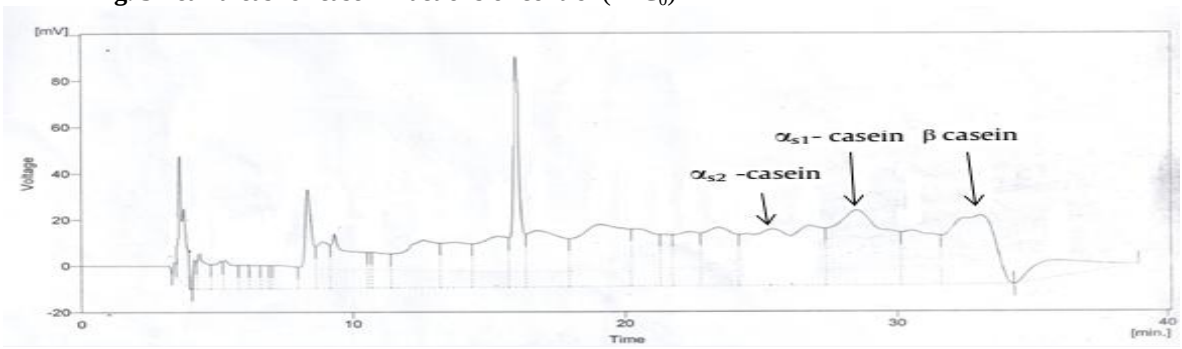


**Fig. 4(a)** Peak areas processed pizza cheese ($PPC_1$)

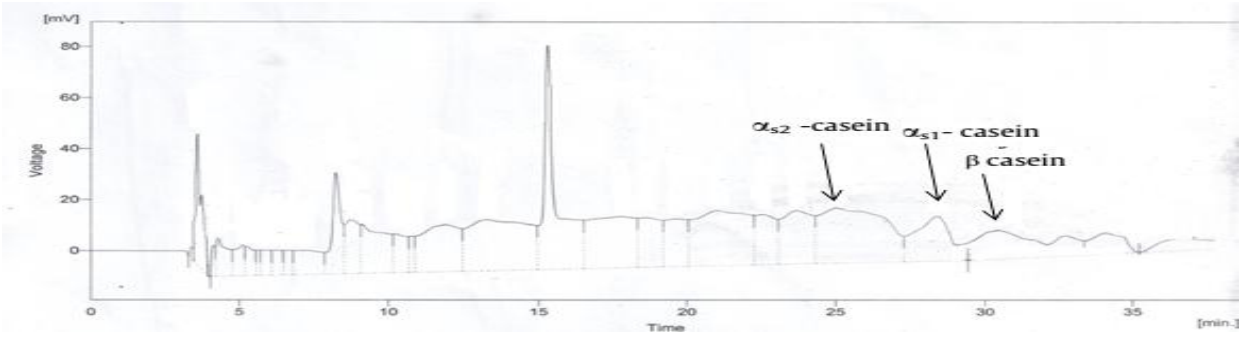


**Fig. 4(b)** Peak areas of processed pizza cheese ($PPC_2$)

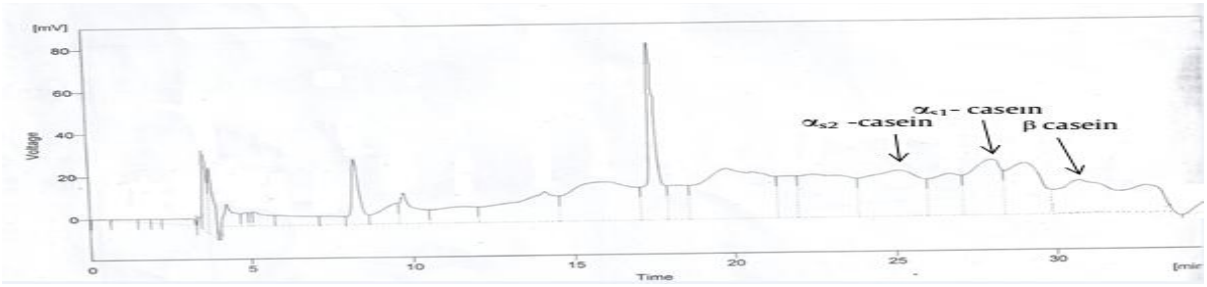


**Fig. 4(c)** Peak areas of processed pizza cheese ($PPC_3$)

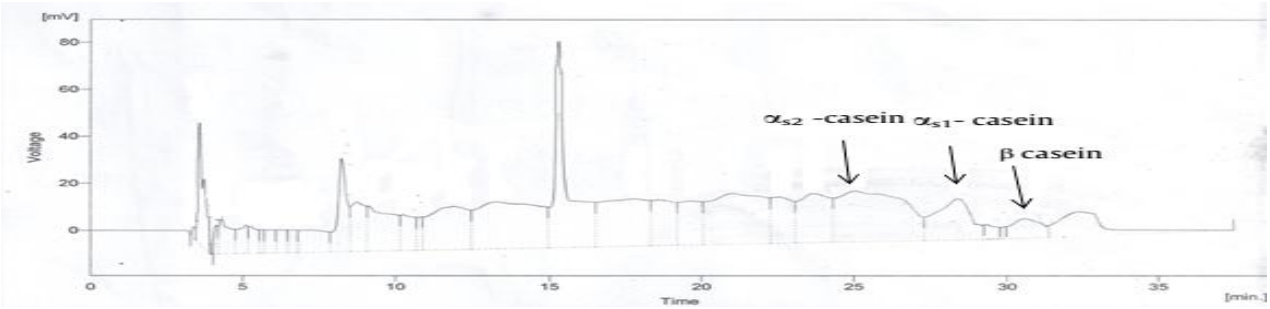


**Fig. 4(d)** Peak areas of processed pizza cheese ($PPC_4$)

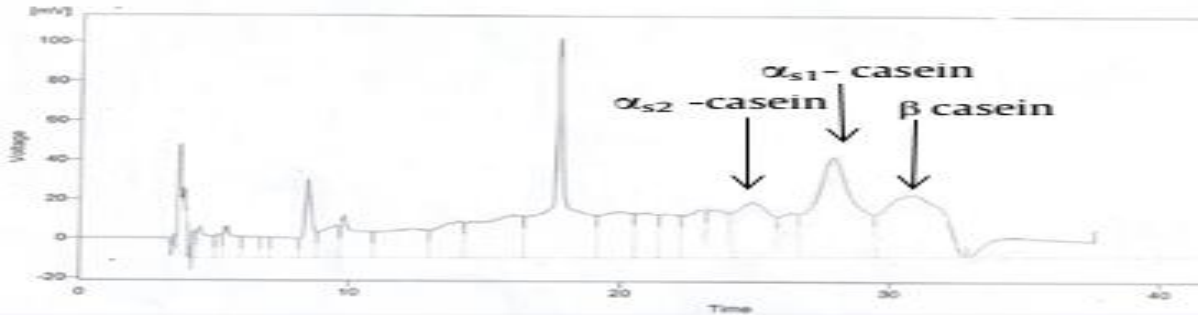


**Fig. 4(e)** Peak areas of processed pizza cheese ($PPC_5$)


**Fig. 4(f)** Peak areas of processed pizza cheese ($PPC_6$)

## Conclusion and Recommendations

The results of organic acids, the lactic acid and acetic acid contents increased from $PPC_0$ to $PPC_6$, while the citric acid content of processed pizza cheeses ($PPC_1$, $PPC_2$ and $PPC_{3)}$ was higher than ($PPC_4$, $PPC_5$ and $PPC_6$). The pH 4.6 soluble and TCA soluble N varied significantly due to amalgamation of cheeses at their different levels and with ripening months of chedder cheese. The pH 4.6 soluble and TCA soluble N were higher (8.30%, 7.03%) in $PPC_6$as compared to control pizza cheese (1.76% and 2.05%). Both nitrogen increased with the increasing level and ripening period of chedder cheese. Electrophoresis and reverse phase HPLC results indicated that the level of intact casein decreased with increasing the proportion and ripening time of chedder in the pizza cheese. Based on these findings, high quality processed pizza cheese with a good nutritional profile could be manufactured locally in Pakistan for the stakeholders.

**Author Contribution Statement:** Nabila Gulzar performed the experiment. Aysha Sameen gave an idea of the present study and provided the technical assistance. Aqsa Qayyum wrote the manuscript. Nuzhat Huma provided the materials used for the study. Saima Rafiq assist in performing the experiment. Muhammad Issa Khan analyzed the data statistically.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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