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Physicochemical Properties of Tomato Paste Fortified Functional Cheddar Cheese

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ABSTRACT

The aim of this research is to fortify cheddar cheese's nutritional value by adding tomato paste. This study transformed ultra-heat treatment (UHT) milk into cheese through cheddaring. Tomato paste was added at 5 g/L, 10 g/L, and 15 g/L during the first curd formation, together with calcium chloride ($CaCl_2$). The type of rennet (animal and microbial) was varied at 0.25 ml/L of milk. Ripening was done in one month at 4°C. According to this study, animal rennet formed curd better than microbial rennet. The addition of tomato paste slightly decreases the curd formation, with approximately 0.37 % reduction per 5 gram of tomato paste. Increasing tomato paste to 15 g/L would increase lycopene to 0.993 – 0.996 mg/100 g. The cheese produced was categorized as extra-hard and low-fat based on the percentage of Moisture Non-Fat Basis (MNFS) and Fat on Dry Matter (FDM). The addition of tomato paste reduced the pH value, resulting in increased firmness and hardness and decreased chewiness and springiness.

Keywords: cheddar, functional cheese, animal rennet, microbial rennet, tomato paste

INTRODUCTION

Innovation in food products has been occurring rapidly. Many kinds of research are conducted to find a method to improve customers' health without sacrificing flavors and environment and offer unique customer experiences. Functional food is defined as food that provides nutrients and beneficially reduces the risk of diseases (Butnariu and Sarac, 2019).

Cheese is a food group from fermented milk produced in various flavors and textures. It is a popular product widely consumed around the globe, with the market increasing by 2.3% annually (Dairy Industries International, 2020). Cheesemaking is a complex process since it is biochemically dynamic and inherently unstable. It is comprised of milk conversion into curd and ripening. The cheese process and formulation produce different cheese products (Fox and McSweeney, 2017). Cheddar cheese is a type of cheese produced through the stirred-curd process (cheddaring) to improve the quality of the cheese due to faster acid production (Ong et al., 2017).

The initial of cheese making is to preserve the nutrients in milk. Recently, innovative functional cheese has been created for various purposes, such as extending shelf-life, dairy replacement, and improved flavors and nutritional ingredients (Farahat et al., 2021). Vegetable or fruit fortified into cheese is a popular method to ameliorate the cheese texture, tastes, and nutrients. Tomato has the potential to be incorporated into cheese due to its highly nutritious components, such as carotenoids, vitamin C, and flavonoids.

Lycopene is a major carotenoid that functions as a powerful antioxidant with a composition of approximately 8.8 – 4.2 mg/g in tomatoes. It is a natural preservative that provides human health benefits (Joshi et al., 2020). Tomato processed cheese spread was generated by fortifying tomato juice and tomato extract into processed Ras and cheddar cheese (Hassan et al., 2019; Mehanna et al., 2017). Based on the studies, the amount of tomato juice improved the lycopene content and free radical scavenging activity (%RSA). The sensory evaluation also verified the high acceptability of tomato processed cheese based on the firmness, spreading, stickiness, and crumbliness. Another study created tomato processed cheese by the addition of tomato powder (Solhi et al., 2020). The processed cheese with tomato powder had higher phenolic and lycopene content, and higher level of proteolysis. In the contrary, lipolysis index could be kept in low level. Tomato juice had also been incorporated into the making of mozzarella cheese (Abd El-Aziz and Refaey, 2017). Mozzarella cheese produced by tomato juice addition performed better in free radical scavenging activity (%RSA) and rheological tests. (Jeong et al., 2017) showed an improvement in lycopene content in Queso Blanco cheese by supplementing powdered microcapsule tomato extracts. In this method, lycopene could be protected during cheese making. The powdered microcapsules were produced using emulsion spray drying. Cheese' color and texture improvement have been reported due to the addition of tomato powdered microcapsules.

Despite many studies evaluating tomato functional cheese products, most of these studies used processed cheese. The tomato cheddar cheese has not yet been considered. Cheddar cheese is a hard cheese that gets through the cheddaring process and is usually applied in many savory food products, such as pizza, burgers, and soups. Fortified tomato into cheddar cheese was expected to promote texture, nutrients, and practicality innovation. Tomato paste was selected due to its higher lycopene content than tomato juice and powder (Górecka et al., 2020). The type of rennet used also varied between animal and microbial rennet to see the difference in cheese's composition and mechanical properties. This research aimed to produce fortified tomato paste cheddar cheese by varying tomato paste concentration (0, 5, 10, and 15 g/L of milk) and rennet type (animal and microbial rennet).

MATERIALS AND METHODS

Materials

Commercial ultra-high-temperature (UHT) milk (Ultrajaya, Indonesia) was employed as a milk base. The ripe and red tomatoes were obtained from a local store and selected for paste preparation. Mesophilic Aromatic Type B (Biena, Canada) was determined as cheese culture. Liquid animal and microbial rennet (Dupont Danisco, USA) and food-grade calcium chloride (CC Food Tetra, Finland) were used for curd coagulation. All chemicals (NaCl, K_2SO_4, and $CuSO_4$) and solvents (NaOH, HCl, H_2SO_4, H_3BO_3, Hexane, and Ethanol) were analytical grades obtained from Merck, Germany. Lycopene analytical standard (purity 90%, Sigma Aldrich, Germany) was applied to determine lycopene content.

The preparation of tomato paste

The clean tomato was blanched using steam for 5 minutes, and its seeds and skins were separated. The peeled tomato was crushed in a food processor until it formed tomato pulp, then cooked at 90 °C for 2 hours until the °Brix value was around 24–28. The °Brix was measured using a refractometer (ATAGO, Japan). The tomato paste was stored in a heatproof container and pasteurized for 15 minutes using boiling water.

The preparation of cheese

The cheese preparation followed the method by (Arlene *et al.*, 2015) with adjustment. Commercial UHT milk consisted of fat and protein content of around 8 g/L each. Milk was pasteurized at 62 °C for 30 minutes and then cooled to 30 °C. Milk was inoculated with a mesophilic culture of around 0.1 g/L of milk, then let sit for 30 minutes. Tomato paste (0, 5, 10, and 15 g/L) and $CaCl_2$ (0.25%-v/v) were added into the milk, followed by rennet (animal and microbial) addition around 0.025%-v/v to coagulate for 30 minutes. The coagulum was cut to around 3 x 3 x 3 cm^3 and left for 5 minutes. The curd was then slowly heated until it reached 38 °C then the whey was separated by cheesecloth, which was previously sterilized in boiling water. The curd yield was calculated by calculating the weight of curd obtained from the initial milk weight. The curd was stacked and formed block shapes, then cut and turn it over every 10 minutes. This cheddaring process was conducted four times. Approximately 2.5%-w/w NaCl was added to the curd. The curd was then placed in a container (10 x 15 cm^2) and pressed with 2 kg weight for 12 hours. The cheese was stored in a refrigerator at 4 °C for 30 days.

The chemical analysis

The moisture, fat, and protein content of cheese products were analyzed according to the Indonesia National Standard for processed cheese (SNI 2980:2018) (National Standardization Agency of Indonesia, 2018). The moisture, fat, and protein content used gravimetry, soxhlet, and Kjeldahl methods (Kjeltec 8100). The moisture non-fat basis (MNFS) and fat in dry matter (FDM) were calculated according to Equations 1 and 2, respectively. MNFS and FDM are parameters to categorize the type of cheese produced.

$$\text{MNFS (\%)} = \frac{m_{moisture}}{m_{cheese} - m_{fat}} \times 100 \qquad (1)$$

$$\text{FDM (\%)} = \frac{m_{fat}}{m_{cheese} - m_{moisture}} \times 100 \qquad (2)$$

The lycopene content was measured using the spectrophotometry method according to Ramadhany *et al.* (2021) method with adjustment. Approximately 0.1 g sample was extracted with 10 ml of solvent mixture (2:1:1 v/v/v of hexane, acetone, and ethanol) in the 50 ml Erlenmeyer flask. The solution was mixed for 10 minutes using a reciprocal shaker at 280 rpm. The mixture was filtered using a Buchner funnel to remove solid particles. The filtrate was combined with 1.5 mL of distilled water, followed by mixing for 5 minutes, and non-polar solution layers were formed. Around 4 mL of the top layer was pipetted. Absorbances were measured using a UV-Vis spectrophotometer (Genesys 20) at 503 nm.

The milk clotting activity (MCA)

The milk clotting activity was measured to determine the number of enzymes required to coagulate 1 mL of milk in 2400 s at 35 °C. Around 0.5 mL of liquid rennet was added to 1.5 mL of milk. The time until milk coagulated was measured. The measurement was conducted in triplicate. The MCA quantification was calculated following reports by (Silva *et al.*, 2014) and (Liburdi *et al.*, 2019) as shown in Equation 3.

$$\text{MCA (U)} = \frac{2400 \times S}{T \times E} \qquad (3)$$

where S = milk volume (mL), T = coagulation time (s), and E = coagulant volume (mL).

The color analysis

The color of the cheese sample was determined by the Color Lab application and expressed in CIELAB parameters (L*, a*, b*). Variable L*, a*, and b* specify the perceptual lightness, red-green chromatism, and blue-yellow chromatism, respectively.

The mechanical properties analysis

The mechanical properties were measured using Texture Analyzer (Brookfield CT 3). The texture analyzer was set to texture profile analysis (TPA) with initial condition 5.0 g trigger, 10 mm deformation, and 0.5 mm/s initial speed. The sample (3 x 3 x 3 cm^3) was measured for firmness, hardness, adhesiveness, cohesiveness, chewiness, gumminess, and springiness. Firmness is related to the force required to penetrate the sample with fingers, while hardness is the force required to perforate the sample with a knife. Adhesiveness indicates the work done to excel attractive forces between the surface of materials. Cohesiveness shows how a good product withstands deformation. Chewiness is related to the energy required to masticate the product, and gumminess is the energy required to disintegrate the product, ready for swallowing. Springiness specifies how a good product springs back after being deformed.

The statistical analysis

The ANOVA two-way test with replication defines variables that influence the product. The test was conducted in Microsoft Excel©. Partial Least Square Regression (PLSR) by Unscrambler™ was selected to evaluate further the correlation between rennet type and tomato paste to mechanical properties. Both statistical analyses were done at a confident interval of 95%.

RESULTS AND DISCUSSION

The pH, °Brix, and lycopene value of tomato paste

The pH, °Brix, and lycopene values of tomato paste can be seen in Table 1. The pH acquired in this study was around 4.45 ± 0.07. The acidity of tomato paste is related to its ripeness and acid compositions. Most commercial tomato pastes pH were in the range of 3.39 – 4.92. However, the tomato paste pH should be kept under 4.6 to intercept the activity of pathogenic microorganisms (Aykas *et al.*, 2020; FAO and WHO, 2013). Therefore, the result attained in this study was still low enough from the theoretical. The overall °Brix value of tomato paste was 25.24 ± 0.55. Similar values were also reported (Aykas *et al.*, 2020; Devseren *et al.*, 2021). According to (FAO and WHO, 2013), the tomato paste °Brix value should be at least 24 °Brix. Nevertheless, most commercial tomato paste usually ranges between 26 – 30 °Brix (Aykas *et al.*, 2020). Thus, the obtained value in this study was still close to the previous study.

The bioavailability of lycopene in tomato paste should be higher than the tomato juice due to the rupture of plant cell walls during the heating processes (Soares *et al.*, 2017). According to Table 1, the tomato paste consisted of 6.97 ± 0.34 mg lycopene/100 g. According to some studies (Joshi *et al.*, 2020; Soares *et al.*, 2017), lycopene in tomato paste was approximately 5.4 – 15 mg/100 g depending on the variety, ripeness, and processing condition.

The milk clotting activity (MCA) and curd yield

The enzyme plays a significant role in cheese coagulation and as a bioprotective agent. The milk clotting activity (MCA) is a variable to determine the capability of the enzyme to hydrolyze protein, specifically κ-casein. However, protein breakdown or proteolysis should not occur too much since it will degrade protein further into smaller size, causing whey loss and reducing curd formation (Ivens *et al.*, 2017).

According to Table 2, animal rennet only required 737 ± 136 enzyme units to coagulate 1 mL of milk at 35 °C, while microbial rennet required 1224 ± 364 U/mL. It showed that animal rennet is a more effective milk clotting enzyme. Animal rennet consists of predominantly chymosin, followed by pepsin. Chymosin is effective in splitting κ-casein at Phe_{105} – Met_{106} and releasing macropeptide, while pepsin is less specific than chymosin (Jaros and Rohm, 2017). Microbial rennet refers to *Mucor miehei* lipase. Microbial enzymes are less specific than chymosin in proteolysis or protein breakdown, resulting in lower milk clotting activity. Microbial rennet is also more thermal stable than chymosin, requiring more enzymes to curd milk (Jaros and Rohm, 2017). In this study, each rennet was kept constant at 0.25 mL/L. Therefore, animal rennet with lower MCA resulted in better curd yield, as observed in Table 2. Previous studies also showed similar result (Manuelian *et al.*, 2020).

The pH is an essential parameter in the curding phase of cheese production. Its value can change due to culture activity or the addition of acid components. Low pH speeds up proteolysis, reduces electrostatic repulsion between casein micelles, and changes calcium distribution between the micelle and serum phases (Ong *et al.*, 2012a). Splitting κ-casein at low pH lessens the surface potential and steric repulsion between the casein micelle, permitting quicker protein aggregation (Holt *et al.*, 2013). Decreasing pH value also enhances Ca^{2+} activity that supports the salt bridges between the casein micelle and provides faster aggregation (Lazzaro *et al.*, 2017). However, the pH value should be controlled since lowering its value further can exacerbate the proteolytic process and cause lower yield (Ivens *et al.*, 2017).

The influence of tomato paste on curd yield was also assessed (Table 2). The control cheese had the highest curd yield, and adding tomato paste decreased the curd yield slightly. The addition of tomato pastes resulted in a slight pH reduction based on the pH profile during the curding process (as shown in Table 3). The initial milk pH was around 7.01 ± 0.03. However, after the addition of rennet (control), the value dropped between 6.60 and 6.43. The addition of tomato pastes even further reduced the pH to around 6.35. The standard renneting pH for cheddar cheese is around 6.5 (Ong *et al.*, 2012a). Thus, it was presumed that the pH decrement due to tomato pastes encouraged other proteolytic activity that caused curd loss due to protein degradation into smaller molecules. This result also follows the study by (Nugroho *et al.*, 2018) and (Wiedyantara *et al.*, 2017), where the addition of fruit extracts decreased the pH of the milk and curd yield.

The cheese composition

The cheese composition is summarized in Tables 4 and 5. The cheese is comprised of approximately 28.67 – 32.33 (30.52 ± 1.17) % moisture, 15 – 15.75 (15.42 ± 0.24) % fat, 19.10 – 20.24 (19.63 ± 0.30) % protein, and 0 – 1.069 (0.50 ± 0.39) mg lycopene/100 g. The average moisture content of the control cheese was about 32.17 ± 0.24 %, and its amount decreased by the tomato paste insertion. As shown in Table 3, the cheese product pH was lower by fortifying the tomato paste during the process and promoting syneresis. Similar results also occur in other studies (Farahat *et al.*, 2021; Jeong *et al.*, 2017). Under normal conditions, the protein appears as a (casein) micelle, a colloidal aggregate. Colloidal calcium phosphate (CCP) and counter ions make up this aggregate. This CCP maintains the integrity of the micelles and can hold a significant amount of water (3 g/g casein) (Huppertz *et al.*, 2017). However, these micelles lost their surface charge and steric repulsion during the proteolytic process. It causes CCP solubilization and an increase in protein interaction. Consequently, it decreases the water holding capacity and stimulates syneresis (Meletharayil *et al.*, 2015). From Table 4, it can also be observed that microbial rennet resulted in higher moisture content than animal rennet. Due to the effectiveness of animal rennet's milk clotting activity (MCA), it advocated more syneresis than microbial rennet. In order to see the influence of rennet type and tomato paste on the moisture content, ANOVA two-way test was performed (Table 5). A p-value lower than 0.05 indicates the influential parameters. It is noticed that both rennet type and tomato paste had a p-value smaller than 0.05, proving a prominent influence of the moisture content. However, the interaction between rennet type and tomato was insignificant. These results confirmed the previous explanation.

Protein content in cheese was influenced by rennet type and tomato paste, as displayed in Table 4. Its value declined with the application of microbial rennet and tomato paste. As previously explained, animal rennet's chymosin is more selective in splitting κ-casein compared to microbial rennet. Thus, the microbial implementation resulted in lower protein content. The addition of tomato paste was related to pH reduction and protein loss in the whey during renneting. It was presented in Table 3 that the pH further decreased during the cheddaring and ripening process, in which pH dropped to around 6.03 – 5.47 with the addition of tomato paste. It was stipulated that more protein loss in the whey compared to control cheese. Similar events also occurred in other studies (Mehanna *et al.*, 2017; Ong *et al.*, 2012a). From ANOVA two-way test results (Table 5), rennet type, tomato paste, and interaction of both variables were corroborated to affect the cheese protein content ($p < 0.05$).

As indicated in Table 4, the rennet type and tomato paste did not significantly affect the cheese fat content. Since the process was constant for all samples, the mechanical process did not affect the cheese fat content. ANOVA two-way confirmed the evaluation, in which the p-value of rennet type, tomato paste, and interaction of both variables were larger than 0.05. It was stipulated that these variables did not affect fat content. However, comparing the fat content in this study to others (Ibáñez *et al.*, 2016; Ong *et al.*, 2012a; Zheng *et al.*, 2016), the cheese fat amount in this study was relatively small, with an average of 15.42 ± 0.24 %. Fat globules are generally trapped in the cheese pores and behave as a non-reactive filler. The size of fat globules is affected by forming a protein network during renneting (Ong *et al.*, 2012a). At low pH, the rate of protein network formation is faster and reduces the movement of fat globules. It creates restrictions for fat coalescence and derives smaller fat globules. Small globules increase the opportunity for fat loss, where the fat loss in cheese making is usually related to a mechanical process, such as agitation, cutting mechanisms, and whey removals (Logan *et al.*, 2015). This study's curd formation pH was lower than the typical renneting pH for cheddar cheese (pH = 6.5), so there was less entrapped fat in the curd during the whey removals.

According to SNI of Processed Cheddar Cheese (SNI 01-2980-1992), the cheddar composition should have composition of water (< 45%), protein (> 19.5 %), and fat (> 25%). The moisture and protein content of the obtained cheeses were according to the SNI standard. However, increasing the tomato paste to 10 g/L reduced the protein content less than the SNI standard. The fat content of fortified cheddar cheeses was lower than the SNI standard. Improvement is required to fulfill the SNI standard.

The lycopene composition

The lycopene and color for each cheese sample are displayed in Table 4. Control cheese consisted of no lycopene, but adding

5 g/L of tomato paste increased the lycopene content to 0.324 – 0.327 g/100 g. Further, increasing the tomato paste to 15 g/L improved the lycopene content to 0.993 – 0.996 mg/100 g. The obtained results were better than the study by (Solhi *et al.*, 2020), in which adding 20 g of tomato powder per kg of processed cheese increased the lycopene to 0.224 mg/100 g. Lycopene value in tomato paste is 3 – 16 times higher than in the dried forms (Górecka *et al.*, 2020). According to ANOVA two-way test (Table 5), the lycopene content was solely influenced by tomato paste ($p < 0.05$).

The CIELAB color parameters are displayed in Table 4. The color of the control cheese was white, and with the addition of tomato paste, the color shifted to orange (Figure 1). The color transformation is related to the cheese's concentration of tomato paste or lycopene.

The commercial tomato paste has the lycopene content in the range between 7 – 15.6 mg/100 g (Soares *et al.*, 2019). In this study, the lycopene content in the functional cheese was much lower than commercial products of tomato paste. Lycopene's oxidation during the cheese making might be the cause of lycopene reduction. Interaction between lycopene with other cheese composition (such as moisture) will also accelerate lycopene degradation though isomerization (Ramadhany *et al.*, 2021). Therefore, despite enhancement of nutritional value of fortified cheese, the cheese making processing and storage need to be evaluated to prevent further lycopene degradation.

The cheese classification

The moisture non-fat basis (MNFS) categorized the cheese from extra hard to soft. The fat in dry matter (FDM) classified the cheese from high fat to skim cheese. From Table 6, the MNFS was around 33.93 – 38.26 %, and the FDM was between 21.43 and 23.16 %. According to (Goosen, 2014), cheese with MNFS lower than 41% and FDM between 10 – 25% is listed as extra-hard and low-fat cheese. Low-fat cheddar cheese is suggested to have MNFS and FDM around 53.79 – 57.58 % and 14.24 – 29.57 %, respectively (Amelia *et al.*, 2013; Zheng *et al.*, 2016). The MNFS obtained in this study was much lower than the suggestion due to the low-fat content. Cheese with low MNFS has been suggested to require a longer time to mature (Ong *et al.*, 2017).

The FDM of acquired functional cheeses were still lower than 25% of SNI standard (SNI 01-2980-1992). Cheese with low FDM is categorized as skim cheese according to SNI.

The cheddar cheese's mechanical properties

The mechanical properties evaluated are firmness, hardness, adhesiveness, cohesiveness, chewiness, gumminess, and springiness, as shown in Tables 7 and 8. Firmness represents the force required to press and penetrate the sample by fingers. From Table 7, it can be noted that cheese from animal rennet was firmer than microbial rennet. It was suggested that the protein matrix formed by the animal rennet gives rise to the rigid form of the cheese. Fat content also played a role in the cheese texture. Around 15 – 30% of fat formed firm and less smooth cheese (Ong *et al.*, 2017). In this study, fat content was approximately 15.42 ± 0.24 %, resulting in a firm and solid cheese. Similar results were also performed in other studies (Zheng *et al.*, 2016). Tomato paste fortification also increased the firmness of the cheese to 34.07, and 40.48 N. Casein breakdown at low pH due to tomato paste greatly enhanced the solubilization of CCP and contributed to the change of texture. When the peptide bond is broken, two new ionic groups are formed, and each of these competes for the available water in the system, resulting in a firmer, less easily deformed cheese (Brigiano *et al.*, 2022). According to ANOVA two-way test (Table 9), rennet type and tomato paste significantly influenced the firmness of cheese ($p < 0.05$). Hardness indicates the force required to penetrate the cheese with a knife. Similar to firmness, cheese hardness with animal rennet was higher than microbial rennet due to the effectivity of animal rennet in casein splitting and new protein network formation. Tomato paste also increased the cheese hardness for a similar reason as the increase of cheese firmness. Based on the ANOVA test, rennet type and tomato paste were the influential variables in determining the cheese hardness ($p < 0.05$).

Adhesiveness is the work necessary to remove the cheese's attractive force to the mouth surface. Protein and moisture content are the dominant factors in determining cheese adhesiveness. An increase of water–protein matrix interaction implies an elevation of cheese adhesiveness (Bulut-Solak and Akin, 2019; Zheng *et al.*, 2016). In this study, cheese from the microbial rennet has better water retention than animal rennet. It suggested a higher interaction between water and protein, resulting in a higher adhesiveness value. From Table 7, it can be implied that the control cheese had adhesiveness around 0.56 ± 0.01 mJ and 0.67 ± 0.01 mJ. Tomato paste increment reduced the cheese adhesiveness. As previously explained, low pH promotes syneresis and lower cheese moisture content. Due to weak water and protein content, the adhesiveness declines with the tomato paste insertion. ANOVA two-way test (Table 9) confirmed the significant influence of rennet type and tomato paste on adhesiveness value ($p < 0.05$).

Cohesiveness is the degree of strength of the internal bonds of the product or the degree of chewed mass held together. According to (Meletharayil *et al.*, 2015), altered protein influences cohesiveness value. The decrease in moisture and calcium content also correlates to cheese’s cohesiveness. According to Table 8, the cheese cohesiveness increased with the addition of animal rennet and tomato paste. It is proposed that chymosin selectively reduces casein molecules’ electrostatic interactions. Along with this occurrence, the inclination for micelles hydrophobic interaction increases, and pH drops to the isoelectric point. Around this point, the solid-like behavior surges and forms stronger and less permeable gels (Sadeghi *et al.*, 2014). During the ripening process, a thin fibrous casein matrix transforms into thicker and stronger structures. This strong matrix creates sturdy internal bonds and improves cohesiveness. Decreasing pH value due to tomato paste increased cohesiveness to around 0.65 and 0.74. It is indicated that syneresis encourages the tight protein matrix, so it improves the cohesiveness value. It was confirmed through the ANOVA test that rennet type, tomato paste, and interaction of both variables play an essential role in the cohesiveness value ($p < 0.05$).

Chewiness is the energy required to masticate the product to the ready-to-swallow state, while gumminess is the force required to disintegrate the product to the state of ready-to-swallow. Chewiness and gumminess are correlated to hardness, cohesiveness, and elasticity. In this study, the chewiness value is similar to gumminess (as seen in Table 8). It can be observed that the addition of tomato pastes decreases cheese chewiness and gumminess. The decrease of pH could increase the interaction between proteins and strengthen the chewiness and gumminess value. However, further pH reduction increases proteolytic activity and syneresis, resulting in loss of protein network and free oil, making the structure less elastic (Ong *et al.*, 2012b). According to ANOVA two-way test (Table 9), rennet type, tomato paste, and interaction of both variables contribute to the value of chewiness and gumminess ($p < 0.05$).

Springiness is the rate of deformed product return to its undeformed condition. Its value is related to chewiness and gumminess. Like chewiness and gumminess, springiness is reduced when tomato pastes increase or lower pH. This decline might be due to the loss of elasticity. Similar to gumminess and chewiness' ANOVA test, rennet type, tomato paste, and interaction of both variables are significant in determining the springiness value ($p < 0.05$).

The statistical analysis (PLSR)

Partial Least Square Regression (PLSR) was applied to determine the effect of rennet type, tomato paste, and cheese composition on the mechanical qualities of cheese. The correlation of cheese composition to mechanical properties is displayed in Figure 2. Variables highly influence factors 1 and 2 located in the inner and outer circles. Factor 1 consists of 61% data input (X) and 66% data output (Y), while factor 2 consists of 14% data input (X) and 17% data output (Y). Cheese composition (moisture, fat, and protein) is the data input, and all mechanical properties are the data output. The response (Y) variables are utilized to interpret the relationships between X and Y variables. When predictors (X) are projected in the same direction as a response from the center, it suggests the predictors are positively related to the response. Predictors have a negative relationship if they are projected in the other direction. Predictors projected near the center are poorly displayed in the model and are therefore difficult to comprehend.

Figure 2 showed that fat, protein, and moisture were firmly represented by factor 1. Protein, moisture, and fat position opposite of firmness, hardness, cohesiveness, and gumminess. Meanwhile, adhesiveness, chewiness, and springiness were positioned in the same direction as protein, moisture, and fat. Hence, fat was less significant than protein and moisture due to its place in the inner circle. Opposite placement indicated that protein, moisture, and fat were negatively correlated to those mechanical properties. In comparison, the same direction implied a positive correlation to mechanical properties.

The correlation between rennet type and tomato paste is exhibited in Figure 3. Factor 1 represented 50% of the data input (X) and 90% of the data output (Y), whereas factor 2 represented 50% of the data input (X) and 2% of the data output (Y). Rennet type and tomato paste were the input data or predictors. The outputs or responses were firmness, hardness, adhesiveness, cohesion, chewiness, gumminess, and springiness. It can be seen in Figure 3 that tomato paste and all mechanical properties were strongly represented by factor 1. The increase of tomato paste positively enhanced cheese's firmness, hardness, cohesiveness, and gumminess. On the contrary, adding tomato paste negatively correlated to adhesiveness, chewiness, and springiness. Rennet type was a less significant variable than tomato paste in the mechanical properties value.

CONCLUSION

Tomato pastes fortified functional cheese was successfully obtained by varying rennet types and tomato paste. Animal rennet was more effective in milk clotting and curd formation than microbial rennet. Tomato paste fortification during cheddar cheese improved the lycopene content in the cheese. Nevertheless, tomato paste addition also encouraged the faster rate of cleaving of the casein peptide bond due to the low pH. The cheddar cheese obtained in this study was still listed as extra hard and low-fat cheese. The cheese's protein, moisture, and fat interaction created a unique cheese texture.

Due to its low-fat and high protein breakdown, the cheddar cheese had high firmness and hardness; and low chewiness, gumminess, and springiness.

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Table 1. The tomato paste composition

Parameters	Value
°Brix	25.24 ± 0.55
pH	4.45 ± 0.07
Lycopene (mg/100 g)	6.97 ± 0.34

Table 2. Milk Clotting Activity (MCA) and Curd Yield

No	Tomato Paste (g/L)	Rennet Type	MCA (U/mL)	Curd yield (%)
S1	Control	Animal	737 ± 136	17.50 ± 0.02
S2	5			17.24 ± 0.04
S3	10			16.84 ± 0.02
S4	15			16.35 ± 0.01
S5	Control	Microbial	1224 ± 364	16.63 ± 0.04
S6	5			16.41 ± 0.04
S7	10			16.07 ± 0.02
S8	15			15.53 ± 0.09

Table 3. The pH value in curding, cheddaring, and ripening process

No	Rennet Type	Tomato Paste (g/L)	pH		
			Curding	Cheddaring	Ripening
S1	Animal	Control	6.60 ± 0.02	6.42 ± 0.09	6.06 ± 0.04
S2		5	6.67 ± 0.02	6.35 ± 0.17	6.03 ± 0.03
S3		10	6.50 ± 0.06	6.33 ± 0.18	5.90 ± 0.03
S4		15	6.42 ± 0.11	6.27 ± 0.11	5.56 ± 0.01
S5	Microbial	Control	6.43 ± 0.03	6.05 ± 0.04	5.95 ± 0.03
S6		5	6.38 ± 0.00	6.02 ± 0.07	5.83 ± 0.07
S7		10	6.37 ± 0.00	6.00 ± 0.01	5.73 ± 0.06
S8		15	6.34 ± 0.01	5.91 ± 0.11	5.47 ± 0.06

Table 4. The cheese composition and color

No	Moisture (%-w)	Fat (%-w)	Protein (%-w)	Lycopene (mg/100 g)	L*	a*	b*
S1	31.83 ± 0.24	15.50 ± 0.35	20.19 ± 0.06	0.000 ± 0.000	78.25 ± 2.71	0.43 ± 0.21	19.51 ± 4.26
S2	30.50 ± 0.71	15.62 ± 0.18	19.84 ± 0.06	0.081 ± 0.004	76.38 ± 2.99	4.27 ± 0.38	41.60 ± 3.22
S3	30.33 ± 0.47	15.13 ± 0.18	19.62 ± 0.12	0.168 ± 0.022	77.07 ± 1.41	8.49 ± 0.70	61.75 ± 1.27
S4	28.67 ± 0.00	15.50 ± 0.00	19.49 ± 0.06	0.249 ± 0.015	63.66 ± 1.00	17.88 ± 2.37	64.75 ± 1.66
S5	32.17 ± 0.24	15.63 ± 0.18	19.62 ± 0.12	0.000 ± 0.000	75.34 ± 2.52	1.38 ± 2.12	28.60 ± 1.74
S6	30.83 ± 0.24	15.50 ± 0.00	19.67 ± 0.06	0.082 ± 0.003	65.61 ± 0.35	5.36 ± 0.51	41.73 ± 4.98
S7	30.67 ± 0.00	15.25 ± 0.35	19.41 ± 0.06	0.170 ± 0.006	64.97 ± 1.26	15.84 ± 0.79	58.54 ± 3.51
S8	29.17 ± 0.24	15.25 ± 0.00	19.19 ± 0.12	0.248 ± 0.027	57.37 ± 0.32	20.92 ± 1.02	51.73 ± 2.53

Table 5. ANOVA statistical analyses on moisture, fat, protein, and lycopene (α = 0.05)

Variables	p-value			
	Moisture	Fat	Protein	Lycopene
Rennet Type (A)	1.37×10^{-10}	0.771	1.78×10^{-4}	0.949
Tomato Paste (B)	9.69×10^{-10}	0.095	5.60×10^{-5}	2.20×10^{-11}
Interaction (AB)	0.406	0.519	0.0481	0.999

Table 6. The MNFS and FDM of cheese

No	Rennet Type	Tomato Paste (g/L)	MNFS (%)	FDM (%)
S1	Animal	Control	37.67 ± 0.12	22.74 ± 0.44
S2		5	36.15 ± 0.91	22.48 ± 0.48
S3		10	35.74 ± 0.63	21.71 ± 0.40
S4		15	33.93 ± 0.00	21.73 ± 0.00
S5	Microbial	Control	38.12 ± 0.20	23.03 ± 0.18
S6		5	36.49 ± 0.28	22.41 ± 0.08
S7		10	36.19 ± 0.15	22.00 ± 0.51
S8		15	34.41 ± 0.28	21.53 ± 0.07

Table 7. The mechanical properties I

No	Rennet Type	Tomato Paste (g/L)	Firmness (N)	Hardness (N)	Adhesiveness (mJ)	Cohesiveness
S1	Animal	Control	33.48 ± 0.76	2.11 ± 0.01	0.56 ± 0.01	0.70 ± 0.02
S2		5	36.41 ± 0.83	2.18 ± 0.01	0.52 ± 0.02	0.71 ± 0.02
S3		10	38.28 ± 0.15	2.28 ± 0.01	0.45 ± 0.00	0.73 ± 0.02
S4		15	40.48 ± 0.41	2.52 ± 0.00	0.34 ± 0.04	0.74 ± 0.02
S5	Microbial	Control	31.51 ± 0.05	2.06 ± 0.00	0.67 ± 0.01	0.59 ± 0.01
S6		5	34.07 ± 0.42	2.15 ± 0.01	0.60 ± 0.04	0.65 ± 0.00
S7		10	36.76 ± 0.46	2.23 ± 0.00	0.50 ± 0.03	0.69 ± 0.00
S8		15	39.26 ± 0.13	2.48 ± 0.04	0.41 ± 0.00	0.73 ± 0.02

Table 8. The mechanical properties II

No	Rennet Type	Tomato Paste (g/L)	Chewiness (mJ)	Gumminess (N)	Springiness (mm)	Springiness (%)
S1	Animal	Control	42.62 ± 0.70	36.52 ± 0.50	1.17 ± 0.00	3.89 ± 0.01
S2		5	38.56 ± 1.91	33.61 ± 1.37	1.15 ± 0.01	3.81 ± 0.03
S3		10	30.60 ± 0.75	27.89 ± 0.54	1.10 ± 0.01	3.66 ± 0.02
S4		15	29.08 ± 0.08	26.80 ± 0.06	1.08 ± 0.00	3.62 ± 0.00
S5	Microbial	Control	54.51 ± 1.97	45.06 ± 1.41	1.21 ± 0.01	4.03 ± 0.02
S6		5	47.30 ± 0.83	39.88 ± 0.60	1.19 ± 0.00	3.95 ± 0.01
S7		10	38.47 ± 1.82	33.54 ± 1.31	1.15 ± 0.01	3.82 ± 0.03
S8		15	31.53 ± 1.30	28.56 ± 0.93	1.10 ± 0.01	3.68 ± 003

Table 9. ANOVA statistical analyses on mechanical properties (α = 0.05)

Variables	p-value						
	Firm.	Hard.	Adhes.	Cohes.	Chew.	Gumm.	Spring.
Rennet Type (A)	$8.3x10^{-5}$	$5.75x10^{-5}$	$1.2x10^{-4}$	$4.1x10^{-4}$	$2.80x10^{-6}$	$2.80x10^{-6}$	$4.27x10^{-7}$
Tomato Paste (B)	$1.4x10^{-7}$	$1.38x10^{-4}$	0.00	$6.6x10^{-4}$	$2.21x10^{-7}$	$2.21x10^{-7}$	$1.69x10^{-8}$
Interaction (AB)	0.428	0.887	0.249	0.0338	0.0069	0.0069	$9.70x10^{-4}$

Figure 1. The cheddar cheese with variation of rennet type (animal and microbial) and tomato paste

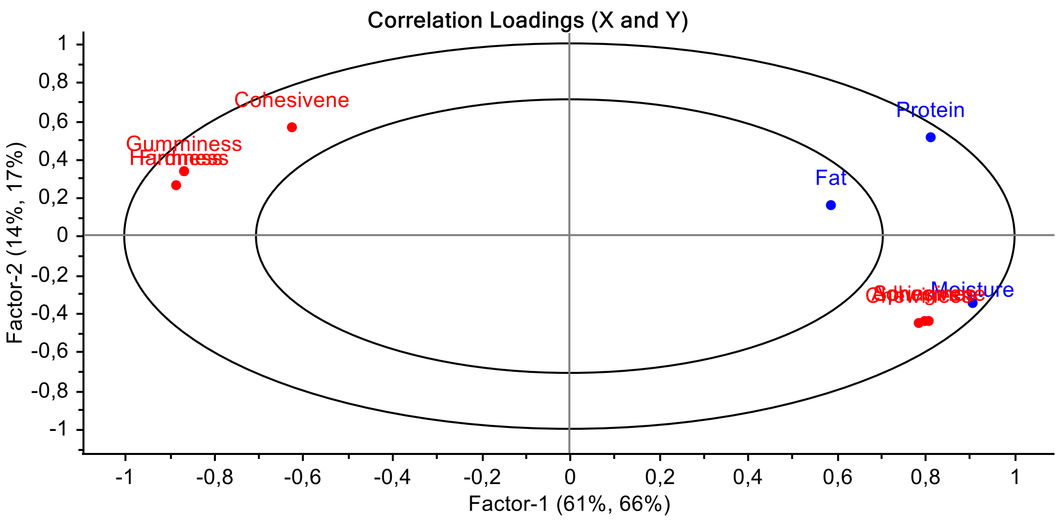

Figure 2. The Partial Least Square Regression (PLSR) of mechanical properties and cheese composition

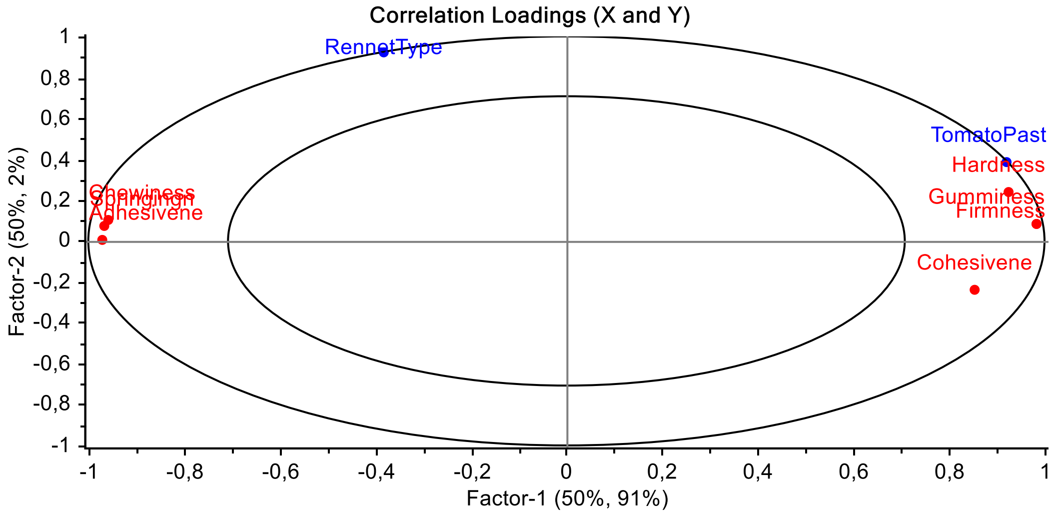

Figure 3. The Partial Least Square Regression (PLSR) of mechanical properties and variation