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Mozzarella Production with an Addition of Passion Fruit *(Passiflora edulis)* Juice

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ABSTRACT

This research aimed to evaluate the effect of passion fruit juice on the mozzarella properties, determine suitable temperature and rennet concentration for casein coagulation and evaluate some quality properties of mozzarella products. The analytical results showed that the passion fruit mozzarella (PM) made with 3% (v:v) passion fruit juice (PJ), rennet concentration of 0.025% (v:v) at coagulation temperature of 37 ℃ had the cheese yield efficiency of 9.47%, total solid content of 55.86% and cheese solid yield of 45.94%. Furthermore, the protein, lipid and dry matter contents of the PM samples were 24.10%, 21.30% and 52.20%, respectively. The microbiological analysis showed that harmful pathogens including *Escherichia coli, Salmonella spp, Listeria monocytogenes, Staphylococci* were not detected in the samples. The microstructure images of PM samples had many sharp edges in a form of clusters. Moreover, the average acceptance score of PM samples (6.8/9.0) was higher than that of the first reference sample (5.9) which used citric acid for acidification process and Emborg mozzarella (RS2) (6.0). Thus, adding passion fruit juice to the mozzarella making-process positively affected quality properties of final product, especially the sensory properties (sense of smell). Therefore, applications of passion fruit juice in the dairy product industry are potential.

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1. Introduction

Cheese is a high nutritional value food with a wide variety of flavors and textures [1]. Among them, Mozzarella is one of the most consumed cheeses worldwide [2]. With the development of the economy and the cultural exchange between the West and the East, cheese has become popular all over the world, including in Vietnam.

Cheese are products rich in essential nutrients, especially protein and minerals [3]. However, in Vietnam the natural cheese has a small share (2% of total sales in 2019). Meanwhile, processed cheese accounted for 98%, and most of all are imported mainly from New Zealand, Germany, France and Australia [4]. Currently, the demand for mozzarella cheese is increasing not only because of its health benefits but also its capacity to combine with other dishes such as pizza, hotdogs, and hamburgers. Therefore, the customers' demand for cheese, particular for mozzarella, is constantly increasing, requiring cheese manufacturers to make appropriate changes to meet the demand.

In mozzarella making process, the micelle casein is often coagulated by rennet – a complex set of enzymes produced in the stomachs of ruminant mammals with 95% of chymosin [1]. Casein coagulation in cheese manufacturing is directly affected by the type of enzyme used, the ratio and composition of substances present in milk fat, protein, whey and casein, the presence of calcium ions and pH [1]. To create appropriate pH condition for enzyme activation, an acid is often added into the milk mixture [1]. Citric acid is a typical ingredient, a flavor enhancer to food products, also a natural preservative, inhibiting the growth of bacteria and preventing oxidation in food products. Furthermore, citric acid is abundant in citrus and passion fruits. Passion fruit *(Passiflora edulis)* is popular in Vietnam with a characteristic flavor and aroma. Passion fruit juice contains citric acid (20-50 g/L), iso-citric acid (170-380 g/L), L-malic acid (1.3-5.0 g/L) and other organic acids [5].

In this study, the passion fruit juice was added to fresh cow's milk to create the appropriate pH condition for rennet activation in mozzarella making process. The influence of rennet concentration and temperature of coagulation process on the quality of mozzarella was investigated. Besides, chemical composition, microbiological criteria, sensory evaluation and surface microstructure properties of studied mozzarella products were also determined.

2. Materials and Methods

2.1. Materials

Fresh cow's milk was purchased from Nonglam Milk retail store at Nong Lam University in Ho Chi Minh City, Vietnam. Passion fruit *(Passiflora edulis)* was purchased at Co.op mart supermarket system at 191 Quang Trung, Hiep Phu, Thu Duc City, Ho Chi Minh City. The selected passion fruits had a glossy dark purple rind with the same size of about 13 fruits/kg.

Naturen Extra 220 rennet manufactured by Chr Company Hansen, Denmark was purchased from Russian Federation. This enzyme was extracted from animals with the ratio chymosin:pepsin = 95:5. The product is a yellowish liquid with characteristic odor. The product has pH of 5.8 and density of 1.14 kg/m^3. Citric acid (99,5% purity) was purchased at Anh San Trading Service Co., Ltd, address 270A Ly Thuong Kiet, Ward 14, District 10, HCMC. In this study, citric acid was used for production of the reference sample 1 (RS1).

2.2. Manufacturing of passion fruit mozzarella

Passion fruit mozzarella was obtained following the flowchart (shown in Figure 1). Passion fruit was selected, rinsed with water 3 times and cut in a half. Finally, a table spoon was used to scrape the flesh and juice. The PJ was filtered through a sieve (Ø = 1 mm) and the table spoon was used to rub and squeeze to the seeds to collect more passion fruit juice (PJ). The above PJ was then centrifuged (1310 ×g, 15 min.) to collect the PJ without impurities. After that, PJ was added to the milk with different volume (6,9,12,15,18 mL) at 8 - 10°C [6] (***Experiment 1***). Milk solution (300 mL) after adding passion fruit juice was heated up to the investigated temperature ranges (15, 32, 37, 42, 45°C) (***Experiment 2***). Then, rennet was added in different volumes (50, 75, 100, 125, 175 µL) (***Experiment 3***). After that, the coagulation process was conducted in 15 minutes. The curds were occurred (Figure 2A) and then were cut into small cubes (about 3×3×4 cm) (Figure 2B). The container then was gently shaked so that the curds do not stick to the container wall. Further, the curds were rested for 6 minutes at an ambient temperature (28-32°C) to let whey drain out. After that, cutting curds were put onto a plastic sieve and let whey separate for another hour (Figure 2C). The separated whey – a by-product – is rich in lactose, serum protein, calcium and a few other nutritional ingredients. The cheese curds were regularly dipped in hot water at 80°C for 20 seconds, then were folded and kneaded to receive the texture characteristic properties of mozzarella (Figure 2D). This process was standardized with the same handled force in 20 seconds and a number of repeated times (5 times). The cheese at this time was evenly heated and became to stick together. The cheese curds were shaped in a square mold (5×5×2 cm) by regularly dipping it in hot water (80°C) for 20 seconds (Figure 2E). After molding, cheese block was soaked in NaCl solution (20%) at 8 – 10 °C in 15 minutes [7]. Finally, PM cheese samples were obtained by cutting the cheese block into small pieces (2×2×1 cm) (Figure 2F) for further analysis.

Experiment 1: Investigate the effect of the passion fruit juice volume on the properties of mozzarella. The volume of milk was 300 mL, the volume of rennet solution was 100 µL, temperature of coagulation: 32 ± 1°C, draining & kneading at 80 ºC. The adding volumes of passion juice were 6, 9, 12, 15, 18 (mL) corresponding to the cheese samples PJ6, PJ9, PJ12, PJ15, PJ18, respectively.

Experiment 2: Investigate the effect of temperature in coagulation process on the properties of mozzarella. The volume of PJ was 9mL obtained from experiment 1. Coagulation temperature: 15, 32, 37, 42, 47°C corresponding to the cheese samples T15, T32, T37, T42, T47, respectively. The other parameters were unchanged as in experiment1.

Experiment 3: Investigate the effect of rennet concentration on the properties of mozzarella. The volume of PJ was 9mL obtained from experiment 1. Coagulation temperature was 37± 1°C obtained from

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experiment 2. Rennet concentration was 50, 75, 100, 125, 150 µL corresponding to the cheese samples R50, R75, R100, R125, R150, respectively. The other parameters were unchanged.

In all the above experiments, two reference samples were used. The first reference sample (RS1) was made following the same technological process illustrated in figure 2 but instead of PJ, the citric acid solution (6%, 6 mL) was added to create the initial pH value (pH 5.9) for the casein coagulation with rennet. The second reference sample (RS2) was Emborg mozzarella (Denmark) which bought in the supermarket.

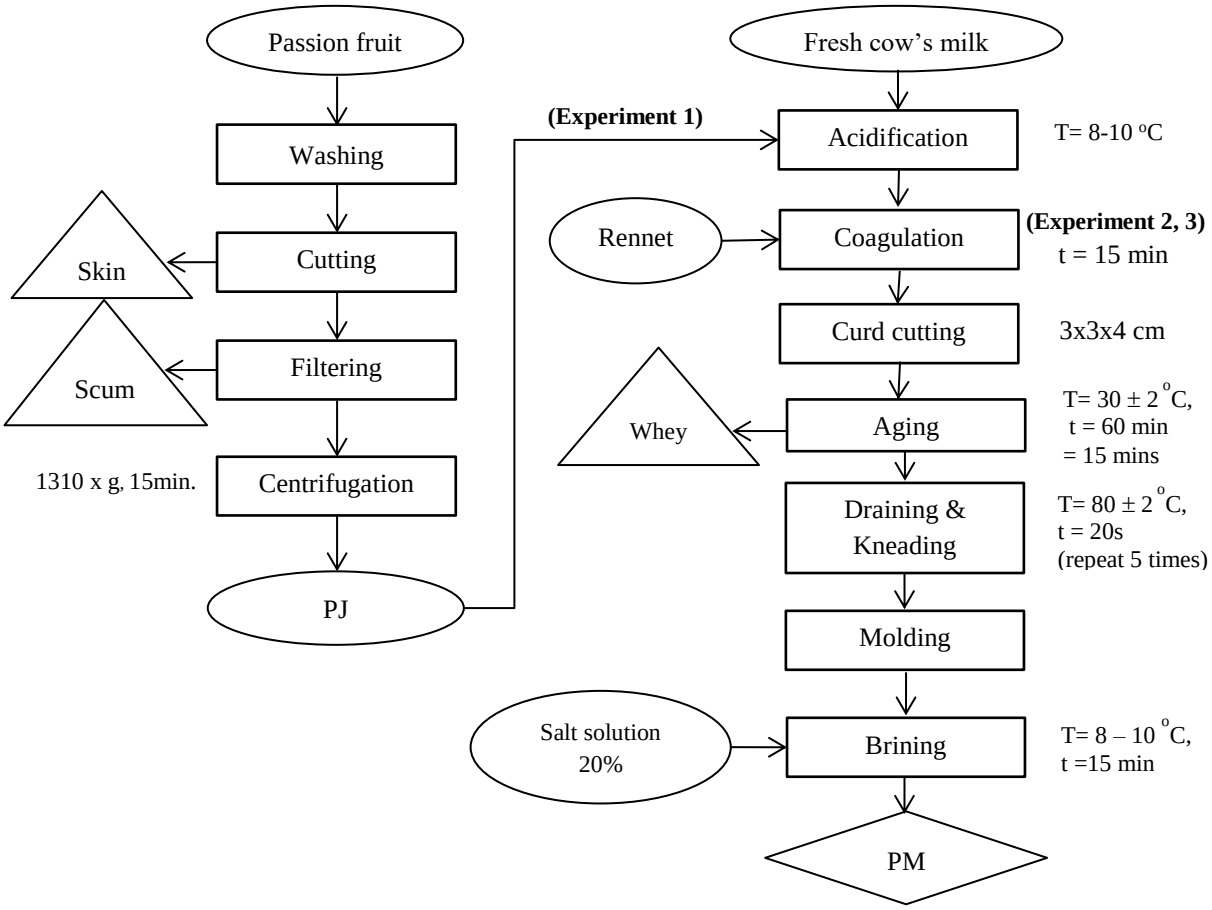

Figure 1. *Flowchart of Passion fruit mozzarella (PM) production*

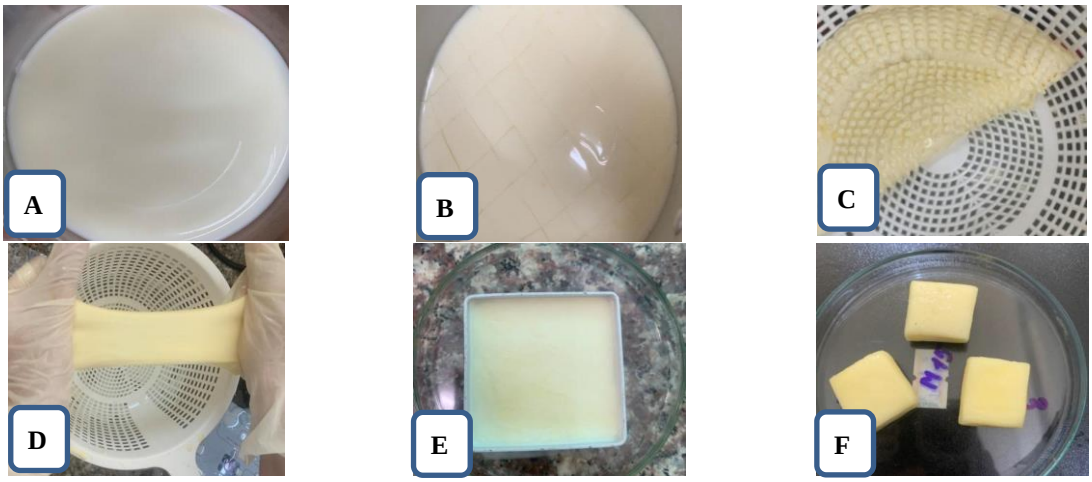

Figure 2. *The cheese curds before cutting (A), after cutting (B), after aging (C) during kneading (D), during molding (E) and the final cheese sample (F)*

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2.3. Analytical methods

The yield of cheese (H, %) was determined by formula (1):

$$H = \frac{m_1}{m_0} \times 100 \quad (1)$$

Where, m_1 was the mass of obtained cheese (g); m_o was the initial mass of fresh milk (g).

The solid cheese yield (CSY, %) was calculated as the ratio between the total dry matter of the obtained product and the total dry matter of raw milk [8]. CSY was calculated according to the formula:

$$\%CSY = H \times \frac{m_{TScheese}}{m_{TSmilk}} \quad (2)$$

Where, H was the yield of cheese (%); $m_{TScheese}$ was the total solid in cheese (g); m_{TSmilk} was total solid in milk (g).

Titratable acidity (TA, mmol/100g) of cheese samples was carried out according to ISO 11869:2012. Acidity is the amount, in milliliters, of 0.1 mol/L sodium hydroxide solution required to titrate 100 grams of product to pH 8.3±0.1. The titratable acidity (TA) was determined as following:

$$TA = \frac{V \times 10}{m} \quad (3)$$

Where, V was the volume of NaOH solution used for titration (mL); m was the mass of the titrated cheese sample (g). For liquid sample, such as milk and passion fruit juice, instead of sample's weight, we used the sample's volume (mL). So that, the TA will have a unit in mmol/100 mL.

Texture profile analysis (TPA). The texture characteristics of PM were analyzed by a Brookfield (USA) CT3 texture analyzer. The cheese samples (2×2×1 cm^3) were covered with food wrap, then put in a plastic box, stored in a refrigerator at a temperature of 4±1°C until analyzed. The samples kept in the container were taken out of the refrigerator about 30 minutes before being analyzed. The temperature of samples was 25°C. Values of hardness, resilience, adhesiveness were measured. Parameters for measurement were: a cylinder force (TA-AACC36) with diameter of 3.6 cm; TA-BT-KIT fixture; test speed of 1.0 mm/s; pretest speed of 2.0 mm/s; recovery time of 5.0 s; Trigger load of 5.0 g and target distance of 3.0 mm [9].

Microstructure observation. Scanning electron micrograph (SEM) was taken to compare the differences in microstructural images in mozzarella products made from fresh milk with and without PJ addition. The cheese samples (2×2×1 cm^3) were frozen for at -50ºC for 24h. They were then freeze-dried by equipment Yamato DC-401, Japan (30±2 ºC, 10-20 Pa, 24h). The cheese samples were mounted on a stub and were taken photographs with TM4000PLUS (Japan). Observation conditions were selected: Accelerating Voltage (Standard 10 kV Mode 2), Vacuum level (Standard (H), Detector (BSE).

Sensory evaluation. The sensory evaluation of cheese samples was performed according to TCVN 10565-3:2015 (ISO 22935-3:2009) with the scale of 0 to 5 points using a commercial product as reference samples. The test was evaluated by a panel of five members (2 males and 3 females, age of 19-21) who were trained in the organoleptic descriptions of Mozzarella products. Evaluated attributes were appearance, texture, smell, taste. The sensory evaluation was carried out in a clean room without strange odors. The accessors took the test in a separate test booth under fluorescence light. Moreover, the other hedonic test was done in order to determine the acceptance of the passion fruit mozzarella samples. The level of liking was rated by 30 testers/consumers (10 males and 20 females, age of 19-21) who often consume cheese products. The testers evaluated the samples on a nine-point scale (hedonic scale) with 1 was "Extremely dislike" and 9 was "Extremely like".

Colorspace measurements. The colorspace for the PM cheese samples was determined by a CR-400 colorimeter (Minolta, Japan) according to Tatol and Mokrzycki, 2011 [10]. The color parameters of passion fruit mozzarella samples were compared with those of the reference samples (RS1 and RS2). The L* value represents the the lightness on the surface from black (0) to white (100), a* value is relative to the tint from green (-120) to red (120) and b* value expresses the hue from blue (-120) to yellow (120). The difference in color (△E) was calculated using the formula (4):

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (4)$$

Where, ΔL^*, Δa^*, Δb^* were the deviations of L*, a* and b* values between cheese samples, respectively. Based on the △E value, the difference in color between the samples was expressed as: 0 < △E < 1 (the observer did not notice the difference in color); 1 < △E < 2 (only experienced observers were able to notice the difference in color); 2<△E<3.5 (inexperienced observers might notice color differences); and △E > 3.5 (there was a clear color difference between the two samples).

Other analysis. pH was measured by means of a penetration pH meter (Hanna HI 9124, USA). Total solid content, ash, fat and protein contents were estimated by ISO 5534:2004 [11], ISO/CD 9877|IDF 258[12], ISO 1211:2010 [13] and Kjeldahl method [14], respectively. Microbiological analysis of PM was determined following specified criteria of QCVN 5-3:2010/BYT [15].

Statistical analysis. Each experiment was done in triplicate. Data were expressed as means ± standard deviation. Experimental data was statistically analyzed by ANOVA (Duncan test) that was used to study the difference between means with a significance level of α = 0.05 in SPSS software program.

3. Results and Discussion

3.1. Chemical composition and some properties of raw materials

The analytical results (Table 1) showed that the protein, lipid, dry matter and ash contents of fresh cow's milk were 2.93%, 3.41%, 11.50% and 0.69%, respectively. The titratable acidity and pH with the chemical composition of the raw milk using in this study met the Vietnamese standards (TCVN 7405:2009) for the milk ingredient. The sensory properties showed that the milk had an ivory white color, a characteristic smell and taste of milk, no strange odors without impurities. The analytical results also showed that the fresh PJ had pH value of 3.04 and titratable acidity of 3.05 mmol/100mL. PJ was light yellow, clear with a characteristic aroma and sour taste.

Table 1. *Chemical composition of fresh cow's milk*

	Chemical composition (%)				Titratable acidity (mmol/100mL)	pH
	Protein	Lipid	Dry matter	Ash		
Fresh cow's milk	2.93 ± 0.74	3.41 ± 0.41	11.50 ± 1.37	0.69 ± 0.04	1.80±0.02	6.74±0.01
TCVN 7405:2009	≥ 2.8	≥ 3.2	≥ 11.5	-	16-21	-
Passion fruit juice					3.05±0.04	3.04±0.01

"-" not detected.

3.2. The effect of the passion fruit juice volume on the quality properties of mozzarella

Some quality properties of mozzarella samples with different volume of passion fruit juice in casein coagulation process were analyzed and shown in Table 2.

Table 2. *The effect of passion fruit juice volume on mozzarella quality properties*

Property	Sample				
	PJ6	PJ9	PJ12	PJ15	PJ18
V_{PJ}	6	9	12	15	18
pH	6.1 ± 0.1^{e}	5.9 ± 0.1^{d}	5.7 ± 0.1^{c}	5.5 ± 0.1^{b}	5.3 ± 0.1^{a}
H (%)	5.20 ± 0.15^{a}	8.23 ± 0.24^{d}	8.41 ± 0.16^{d}	7.87 ± 0.3^{c}	7.59 ± 0.11^{b}
CSY (%)	25.17 ± 0.57^{a}	39.85 ± 0.77^{d}	39.00 ± 0.20^{d}	35.29 ± 0.42^{c}	31.98 ± 0.86^{b}

Different superscript letters show significant differences among the means within each row ($p < 0.05$)

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The analytical results (Table 2) showed that when the volume of PJ adding to the milk increased from 6 to 18 (mL), the pH decreased gradually from 6.1 to 5.3 (p<0.05). This result could be explained by the effect of acidification on the balance among the casein micelle, the calcium in the curd and serum phase. Specifically, when adding acid, the dissolution of calcium phosphate in colloidal form promotes the activity of calcium ions in the serum phase and neutralizes the negative charge of the casein micelle [17]. Besides, when increasing from 6 to 9 mL of PJ volume, the cheese yield efficiency (H), and cheese solids yield (CSY) increased. When the PJ concentration continued to increase to 12mL, H% and CSY% tended to unchanged (p>0.05). As the pH decreased, the agglomeration of the micelles began to convert from κ - casein to para - κ - casein lower, and the rate of aggregation and gelation increased. In addition, some studies have shown that rennet was more active at low pH, in the range of 5.9 to 5.7, due to increased casein hydrolysis, which increases the release of hydrophilic macropeptides into the whey phase [17][6][1]. As the concentration was further increased from 12 to 18 mL, the cheese recovery and CSY% tended to decrease from 8.41 to 7.59% and from 39.00 to 31.89%, respectively. Especially, in the pH range from 5.3 to 5.5, many physicochemical properties of casein micelles had significant changes, including mass and dissociation of caseins (Figure 3). As the pH of milk decreases, calcium phosphate casein was dissolved, and the caseins were released into the milk serum phase [18]. All of these led to a calcium decrease in cheese, resulting in reduced H% and CSY%. In addition, the curd was also affected by different proportions of PJ with milk. Adding a high volume of PJ (18mL) caused the mixture to clump before adding the rennet. When coagulation occurred, the curd was not smooth and had many lumps that gave the surface of milk curds be crushed (Figure 3). With a low concentration of PJ (6mL), the curd was too soft which led to difficulties for draining and kneading process.

PJ6 PJ9 PJ12 PJ15 PJ18

Figure 3. *The surface of milk curds at different PJ volumes (from 6 mL to 18 mL) in the coagulation process*

The results of texture profile analysis (hardness, resilience, adhesiveness) of mozzarella samples made with different PJ volumes are presented in Table 3.

Table 3. *Effect of PJ on texture properties of mozzarella*

Sample	Hardness, g	Resilience, mm	Adhesiveness, mJ
PJ6	1412.67±79.06^{d}	0.50±0.01^{d}	0.05±0.05^{a}
PJ9	1088.33±69.12^{c}	0.44±0.03^{c}	0.06±0.02^{b}
PJ12	644.33±122.51^{b}	0.39±0.01^{b}	0.13±0.08^{a}
PJ15	593.00±88.29^{b}	0.32±0.01^{a}	0.24±0.11^{a}
PJ18	464.00±95.87^{ab}	0.31±0.02^{a}	0.49±0.26^{b}
RS1	2419.09±356.45^{e}	0.42±0.01^{c}	0.11±0.06^{a}
RS2	327.33±41.86^{a}	0.30±0.01^{a}	0.20±0.05^{ab}

In each column, different superscript letters show significant differences among the means (p < 0.05)

The analytical results (Table 3) showed a difference in the texture characteristics of the PM sample compared to RS1 and RS2 samples. Specially, when increasing PJ volume from 6 to 18mL, the hardness and resilience decreased from 1412.67g to 464g and from 0.50 to 0.31 mm, respectively. On the other

hand, the adhesiveness values were not statistically different. It could be explained by the fact that increasing PJ volume led to the acidification rate of milk increased. The speed of acidification strongly influenced the texture of cheese during production [19]. Furthermore, low pH value caused solubilization of calcium from casein aggregates that decreased interactions of proteins. Decreasing of pH probably facilitated structural rearrangements in the protein matrix. All of these then resulted in the weakening of the protein matrix that led to decreased hardness of cheese [6].

The color of mozzarella products made with different PJ volumes (6-18 mL) are presented in Table 4 and Figure 4. The analytical results showed that there had a difference in color between the PM with reference samples (ΔE_1 and $\Delta E_2 > 1.5$). The color difference between the PJ samples with RS1 is much smaller than the difference between them with RS2. This is explained by the fact that the PJ samples have the same milk ingredients as the RS1 samples. The difference increased when increasing passion fruit volume, which indicated that passion fruit has an effect on the color of Mozzarella. Based on the L* value, it could be seen that when increasing PJ volume in milk from 6mL to 18mL, the lightness decreased gradually from 82.43 to 65.32. Furthermore, the b* value was always positive, which means the samples were yellow tint. The intensity of yellowness depends on the volume of PJ.

Table 4. *Colorspace measurements of mozzarella samples made with different PJ volumes*

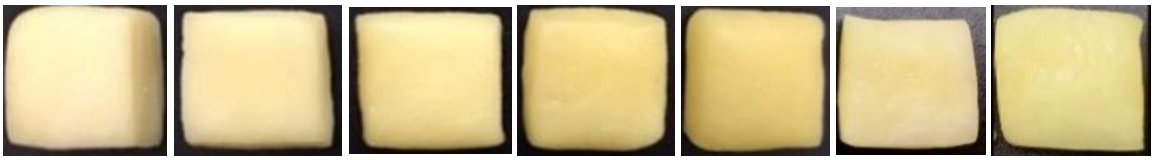
Sample	Value				
	L*	a*	b*	ΔE_1*	ΔE_2*
PJ6	82.43±0.68^{d}	-4.79±0.15^{a}	25.14±0.64^{b}	1.86	9.37
PJ9	82.06±2.26^{d}	-4.86±0.11^{a}	26.86±0.68^{cd}	2.18	9.54
PJ12	78.75±1.08^{c}	-4.30±0.24^{b}	27.02±0.27^{cd}	5.33	10.08
PJ15	69.47±0.77^{b}	-4.68±0.10^{ab}	27.94±0.59^{de}	14.65	17.52
PJ18	65.32±1.95^{a}	-4.59±0.44^{ab}	28.67±0.92^{e}	18.86	21.36
RS1	83.99±0.77^{d}	-4.29±0.16^{b}	26.02±0.79^{ab}	0	-
RS2	84.29±0.82^{d}	1.85±0.07^{c}	21.26±0.71^{a}	-	0

In each column, different superscript letters show significant differences among the means ($p < 0.05$)

ΔE_1 - Color difference between PJ samples with RS1;*

ΔE_2 - Color difference between PJ samples with RS2;*

"-" Not detected



PJ6 PJ9 PJ12 PJ15 PJ18 RS1 RS2

Figure 4. *Mozzarella samples at different volume of Passion fruit juice (PJ6-PJ18) and reference samples (RS1,RS2)*

In general, the color deviation of the studied cheese samples did not negatively affect the appearance of the product. Texture properties varied widely, however, within the product properties. Furthermore, according to Flavio Tidona et al (2021)[6], the milk pH should be reduced to 5.9 to facilitate the best coagulation process [6]. In addition, the PJ9 sample achieved a higher recovery efficiency and cheese solid yield. It's structure was smooth and clump-free. Therefore, the volume of passion fruit juice (9 mL) was chosen in order to add to the milk (300 mL) for the next experiments.

3.3. The effect of temperature in coagulation process on the quality properties of mozzarella

Some quality properties of mozzarella samples with different temperatures in casein coagulation process were analysed and shown in Table 5.

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Table 5. *The effect of coagulation temperature on mozzarella quality properties*

Property	Sample				
	T15	**T32**	**T37**	**T42**	**T47**
Coagulation temperature, ºC	15	32	37	42	47
H (%)	-	8.46 ± 0.45^{b}	9.29 ± 0.23^{c}	8.54 ± 0.16^{b}	7.75 ± 0.33^{a}
TS_{cheese} (%)	-	55.54 ± 0.16^{b}	56.46 ± 0.25^{c}	55.76 ± 0.37^{b}	52.46 ± 0.49^{a}
CSY (%)	-	40.20 ± 0.11^{b}	44.82 ± 0.20^{d}	43.51 ± 0.29^{c}	36.87 ± 0.34^{a}

Different superscript letters show significant differences among the means within each row ($p < 0.05$)
"-" Not dectected

The analytical results (Table 5) showed a difference in the quality properties of cheese samples at different temperatures of casein coagulation process (32 to 47°C). At 15°C, the cheese product could not obtain because the level of coagulation was low and the milk did not aggregate (Figure 4, sample T15). On the other hand, when the temperature increased from 32 to 37 °C, then H% increased from 8.46 to 9.29%, TScheese% increased from 55.54 to 56.46% and CSY% also grew 40.20 to 44.82%. It could be explained by the rennet activation under temperature condition during coagulation process [20].

Figure 5. *Surface of the curds at different coagulation temperatures*

The clumps of the cheese samples T42 and T47 (Figure 5) showed discontinuity of the gel system. This phenomenon led to a decrease in gel-forming and in dry matter holding capacity, which reduced product recovery and CSY. The results could be explained that the applied temperature was not suitable for rennet to work. Texture properties also changed accordingly (Table 6). Specifically, at 15ºC rennet did not work, resulting in not create product at this temperature. On the other hand, the hardness of cheese samples (T32 and T37) were unchanged (p>0.05). Increasing coagulation temperatures from 42ºC to 47ºC, the hardness was unchanged. This result showed a marked interactive of coagulation temperature on coagulation properties. Lowering of the coagulation temperature greatly altered the curd microstructure, with a tendency for less syneresis during cutting. Further research is required to quantify the changes in syneresis, in fat and protein losses to whey due to changes in the microstructure of curd particles arising from the different coagulation conditions.

Table 6. *Effect of coagulation temperature on texture properties of Mozzarella*

Sample	Hardness, g	Resilience, mm	Adhesiveness, mJ
T15	-	-	-
T32	922.17 ± 356.45^{b}	0.49 ± 0.02^{b}	0.02 ± 0.02^{a}
T37	859.08 ± 41.85^{b}	0.44 ± 0.01^{c}	0.08 ± 0.08^{ab}
T42	1376.33 ± 50.41^{c}	0.50 ± 0.04^{d}	0.04 ± 0.02^{a}
T47	1521.33 ± 74.83^{c}	0.39 ± 0.01^{d}	0.23 ± 0.11^{c}
RS1	2419.09 ± 356.45^{e}	0.42 ± 0.01^{bc}	0.11 ± 0.06^{abc}
RS2	327.33 ± 41.86^{a}	0.30 ± 0.01^{a}	0.20 ± 0.05^{a}

In each column, different superscript letters show significant differences among the means ($p < 0.05$)
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Table 7. *Color change results of PM and the reference samples*

Sample	Value				
	L*	a*	b*	ΔE_1*	ΔE_2*
T15	-	-	-	-	-
T32	79.33±0.53^{a}	-5.35±0.1^{a}	30.94±1.02^{c}	6.86	13.03
T37	82.39±0.65^{b}	-4.23±0.61^{b}	28.27±1.21^{b}	2.76	9.45
T42	82.93±1.29^{bc}	-4.16±0.24^{b}	27.78±2.16^{b}	2.05	8.95
T47	83.21±0.47^{bc}	-4.40±0.05^{b}	29.67±0.58b^{bc}	3.73	10.52
RS1	83.99±0.77^{c}	-4.29±0.16^{c}	26.02±0.79^{a}	0	-
RS2	84.29±0.82^{d}	1.85±0.07^{c}	21.26±0.71^{a}	-	0

In each column, different superscript letters show significant differences among the means ($p < 0.05$)
"-" Not detected
ΔE_1 - Color difference between T32 – T47 with RS1*
ΔE_2 - Color difference between T32 – T47 with RS2*

Colorspace measurements of mozzarella products made with different coagulation temperatures are presented in Table 7. The results showed that there was no significant change in color between the cheese samples (T32-T47). But these samples still showed color difference between them and the control samples (RS1 and RS2) because of the difference in raw materials and ingredients.

In summary, the temperature for casein coagulation process was 37°C. This value was appropriate with the product information report by manufacturer that the product has an optimal operating temperature about 36-40°C. At this temperature the PM product had a higher value of H%, CSY%, TS cheese%, and also texture and color. Moreover, the energy power consumption was saved.

3.4. The effect of rennet concentration on the quality of mozzarella

Some quality properties of mozzarella samples with different rennet concentration in casein coagulation process were analysed and shown in Table 8.

Table 8. *The effect of rennet concentration on the mozzarella quality properties*

Property	Sample					
	R25	R50	R75	R100	R125	R150
Rennet (µL)	25	50	75	100	125	150
H (%)	-	9.37 ± 0.06^{a}	9.47 ± 0.11^{a}	9.33 ± 0.03^{a}	9.35 ± 0.09^{a}	9.39 ± 0.05^{a}
TS_{cheese} (%)	-	52.73 ±0.94^{a}	55.86±0.08^{c}	54.25±0.61^{b}	52.29±0.89^{a}	51.70±0.70^{a}
CYS (%)	-	42.97±0.76^{a}	45.94±0.06^{c}	44.15±0.49^{b}	42.56 ±0.82^{a}	43.59±1.41^{a}

In each column, different superscript letters show significant differences among the means ($p < 0.05$)
"-" Not detected

The analytical results (Table 8) showed that at rennet concentration of 25 µL, the curd was not created because of low rennet activity. When increasing the concentrations of rennet from 50 to 150 µL did not affect the recovery efficiency H% ($p>0.05$). Otherwise, the TScheese% and CSY% showed the higher values at the rennet concentration of 75 µL. This result could be explained that increasing rennet concentration increased the hydrolysis rate of the enzyme, so that decreased rennet coagulation time [21]. Therefore, R75 was a preferred concentration of rennet used to manufacture mozzarella product with commercial benefits and time savings.

The analytical results (Table 9) indicated that when changing the rennet concentration (50 to 150 µL) during the coagulation process there were differences in the texture properties of the Mozzarella

samples. Specifically, when increasing the concentration of rennet (50 to 150 μL), the hardness of PM also increased significantly (812.08g to 2035.67g) (p<0.05). This could be explained that gel synthesis increased strongly with increasing rennet concentration. Besides, more the volume of rennet increased more the aggregation of the casein micelle occurred. The increased rate of aggregation may have an effect on the initial mode of aggregation of casein particles, which will ultimately result in a denser network and hardness. Otherwise, resilience, and adhesiveness were not significantly different (p>0.05).

Table 9. *Effect of rennet concentration on texture properties of Mozzarella*

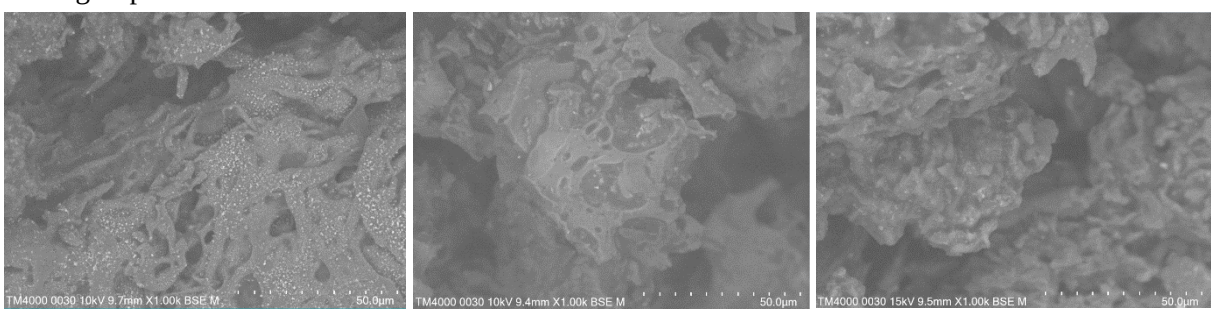
Sample	Volume of rennet (μL)	Hardness, g	Resilience, mm	Adhesiveness, mJ
R25	25	-	-	-
R50	50	812.08±85.43^{b}	0.44±0.01^{b}	0.04±0.02^{ab}
R75	75	1129.83±152.41^{c}	0.44±0.02^{b}	0.06±0.05^{ab}
R100	100	1079.00±129.22^{c}	0.42±0.01^{b}	0.03±0.02^{a}
R125	125	1590.41±195.69^{d}	0.44±0.02^{b}	0.04±0.04^{ab}
R150	150	2035.67±235.98^{e}	0.43±0.04^{b}	0.04±0.03^{ab}
RS1	-	2419.09±356.45^{f}	0,42±0,01^{b}	0,11±0,06^{b}
RS2	-	327.33±41.86^{a}	0.30±0.01^{a}	0.20±0.05^{c}

In each column, different superscript letters show significant differences among the means (p < 0.05)
"-" Not detected

3.5. The quality properties of passion fruit mozzarella

Thus, the cheese samples were processed according to the technological process (Figure 2) with a volume of passion fruit juice of 9 mL to 300 mL fresh milk (3% v:v). The additional rennet concentration was 75 μL (0.025% v:v) at 37°C in 15 min. Passion fruit mozzarella samples were analyzed the chemical composition and microbiological criteria. The results showed that, PM had protein, fat and dry matter content of 24.10%, 21.30% and 52.20 %, respectively. *Escherichia coli*, *Salmonella*, *Listeria monocytogenes*, *Coagulase-positive Staphylococci* were not detected in this product, which is consistent with QCVN 5-3:2010/BYT for fresh cheese production.

The microstructure of three mozzarella samples (RS1, RS2 and PM) was observed (Figure 6). SEM image results showed that there was difference in the microstructure of the products. We observed the surface of PM samples had many sharp edges in a form of clusters. Meanwhile, RS1 samples showed more voids than PM samples that might lead to low product yield efficiency of RS1. On the other hand, the RS2 sample had many non-pointed rounded corners with large voids resulted in the low value of hardness. Both PM and RS1 samples had many salt crystals in their structure because we performed the brining step with 20% NaCl solution.



RS1 RS2 PM

Figure 6. *Microstructure of the reference samples (RS1, RS2) and passion fruit mozzarella (PM)*

The sensory evaluation results of description scores (Figure 7A) and hedonic score (Figure 7B) of mozzarella samples are shown. The results indicated that PM samples had higher sensory scores in terms

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of appearance, texture and smell compared to RS1 and RS2 samples. Besides, the taste of PM and RS2 samples were better than that of RS1 sample. Moreover, the hedonic scores from 30 customers showed their acceptance level to the PM samples higher than that of reference samples (RS1, RS2). It could be explained by the contribution of the passion fruit juice's aroma on the acceptability of PM samples. Thus, the organoleptic evaluation results showed the positive effect of passion fruit juice's addition into mozzarella production, especially on the smell perception.

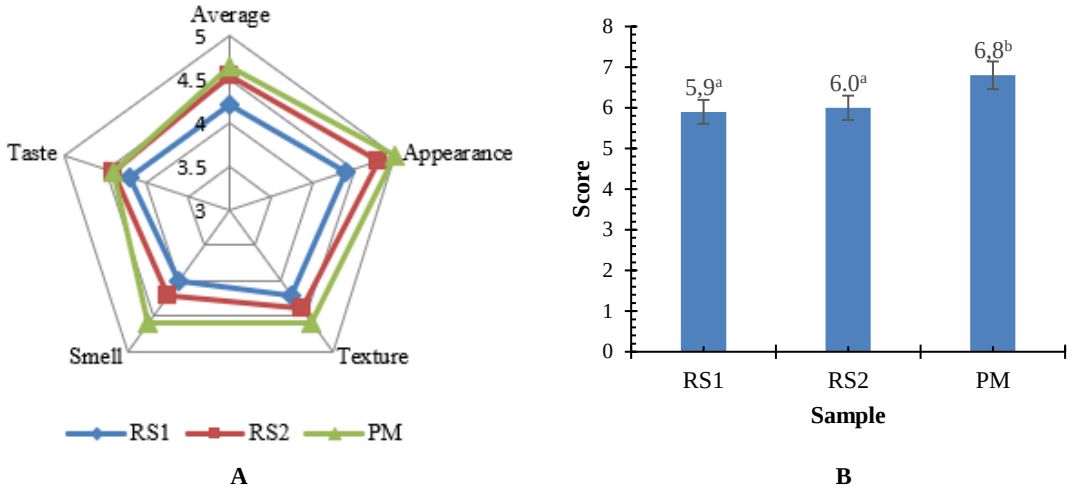

Figure 7. *The results of description scores (A) and hedonic scores (B) of mozzarella samples*

4. Conclusions

Adding the volume of passion fruit juice of 3% (v:v) created the appropriate pH condition for rennet "Naturen Extra 220" to activate. The mozzarella cheese product made with 0.025% (v:v) rennet concentration at 37 ^{o}C had the cheese yield efficiency, total solids cheese, cheese solids yield of 9.47%; 55.86%; 45.94%, respectively. The protein, lipid and dry matter contents of passion fruit mozzarella were 24.10%, 21.30% and 52.20%, respectively. The sensory evaluation results showed the positive effect of passion fruit juice on organoleptic properties of PM product, especially sense of smell. So that, applications of passion fruit juice in the dairy product industry are potential. The limitation of the study is that the product quality has not been evaluated during the storage period, and the product recovery efficiency is not high compared to other studies. These results will be implemented in our next study.

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