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Study on the Development of Mozzarella Cheese Adding Lipase Enzyme and Its Quality Evaluation

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Abstract - *The study was conducted for the development of mozzarella cheese by use of rennet and lipase enzyme. For the development of the mozzarella cheese, four types of treatments have been taken in which T_0, as a control sample in which 2.5g of rennet enzyme used alone. Now this rennet enzyme would be replaced by lipase enzyme by 0.25 g for three sample e.g. T_1(2.25g vs 0.25g), T_2 (2.00g vs 0.50g), T_3 (1.75g vs 0.75g). After the production of the mozzarella cheese, physio-chemical properties such as stretch ability, hardness, moisture, fat, protein, acidicity and total solid content of the mozzarella cheese were evaluated. Organoleptic properties such as flavor, tast, body and texure and colour appearances were also evaluated by sensory team for evaluation of the quality of the cheese. Microbiological analysis (SPC) was conducted for the fresh cheese and stored cheese (10 days) for evaluation of the shelf life of the cheese. After the evolution of the quality and shelf life, it was concluded that the stretch ability, fat, protein, total solid content and acidicity of produced Mozzarella cheese were increases and hardness and moisture of product decreases from sample T_0 to T_3. While sensory properties such as taste, appearance, and flavour of T_3 was good in compression of T_0 but colour of T_0 is better than T_3. The overall perception and acceptability by sensory members was positive. The shelf life of the developed cheese was found more than 10 days.*

Key Words*: *Mozzarella cheese, Rennet enzyme, Lipase enzyme, Microbiology, Pasta cheese

1. INTRODUCTION

Cheese is one of the most important products of the dairy world. It is a bright star in the dairy products heaven and is considered nature's versatile food. Converting milk to cheese is means of conserving the major constituents, namely fat casein in most nutritious from by removing whey cheese contains high level of protein which are rich source of essential amino acids. It is also a good source of certain vitamins and essential minerals (calcium and Phosphates), the figure peaked at 31% in 1955 and the annual world cheese production is 12.823 million tons [23, 19].

Originally mozzarella is the name of cheese made from high fat water buffalo milk found in South Italy. Also for many decades, however, Italians have made mozzarella cheese from cow and the cheese is highly accepted [18]. Mozzarella cheese is defined by [27]as a member of pasta – filate family (pulled curd, soft cheese). Mozzarella cheese has various shapes like round cake, oval, egg and rectangular shapes. This cheese is not aged like most cheeses and is actually best when eaten within hours of its making. The process of making mozzarella is called pasta filata, which means the curds are heated in water or whey until they form strings (hence the term "string cheese") and become elastic in texture. The curds are stretched, kneaded until smooth, and then formed into round balls to make fresh mozzarella cheese. The annual production of mozzarella cheese in the United States is about 312 million kg. Which accounts for 17 percent of the total cheese produced and utilizes about 6 percent of total milk production of mozzarella cheese about 15723600 tonnes in 2012 which accounts for the 35 percent of the nation's total cheese production.

In the India, dairy industry, the manufacturing of dairy desserts from mozzarella cheese with different variety is minimal. According to [15],mozzarella cheese is the most popular soft cheese produced locally but, however, as a savoury product it is available in a smooth or chunky texture, plain or with added savoury condiments such as ham, biltong, onions, chives, garlic, enzymes and a variety of herbs. Mozzarella cheese has better shelf life than other cheese. It can preserve for long duration with better quality. When lipase enzyme will be added, the flavour, texture, colour and taste of the mozzarella cheese will be improved in positive manner.

In this paper we are discussed about the historical background of the mozzarella cheese, methodologies used

for development of mozzarella cheese with the addition of the lipase enzyme and discussion about how will be the physio- chemical properties and organolyptic properties change due to addition of lipase enzyme

2. MATERIALS AND METHEDOLOGY

In this section we discussed about the materials and methodology had used for the development and quality testing of mozzarella cheese.

2.1 Raw Materials

For the development of the cheese, the most important thing was milk from buffalo or cow. In this research, 20 liters of buffalo milk was used as main raw material. Rennet tablet were used to coagulate milk using enzyme .In other words they turn milk in liquid form into curd for cheese making. Lipase microbial based lipased used for cheese making 1\8th of a tea spoon for 4 liters of milk. Other raw materials were calcium chloride, salt and packaging materials (food grade polyethylene pouches of 300 gauge thickness)

2.2 Chemicals

Most of the chemicals used in this investigation were of analytical grade. They were obtained from the laboratory of food science and technology .These chemicals were Sulphuric acids, Sodium Hydroxide, Neutral Solvent (KOH), Phenolphthalein Indicator (0.5 g of phenolphthalein powder in 50% of ethanol) and Starch Indicator.

2.3 Experimental Pla

The study was conducted for the development of mozzarella cheese by use of *rennet* and *lipase* enzyme. For the development of the mozzarella cheese, four types of treatments have been taken in which T_0, as a control sample in which 2.5g of rennet enzyme used alone. Now this rennet enzyme would be replaced by lipase enzyme by 0.25 g for three sample e.g. T_1(2.25g vs 0.25g), T_2 (2.00g vs 0.50g), T_3 (1.75g vs 0.75g). These samples were developed with five replications for find out better results.

Table -1: Experimental Plan

Variables/ Parameters	Levels	Descriptions	Quality parameters
Product	1	The development of mozzarella cheese adding lipase enzyme and its quality evaluation	A. Physico-chemical characteristics Moisture, fat, Protein, Ash
Ingredients	6	Milk, acid, lipase, rennin, salt and water.	B. Rheological properties Strechability, Meltability and Textural characteristics
Treatments	4	2.5, 2.25, 2.0 and 1.75g (Rennet). 0, 0.25, 0.5 and 0.75, (Lipase).	C. Sensory evaluation 9 Point Hedonic scale
Storage condition	1	8-10°C	
Packaging material	1	LDPE	
Sample size	1	100 g	

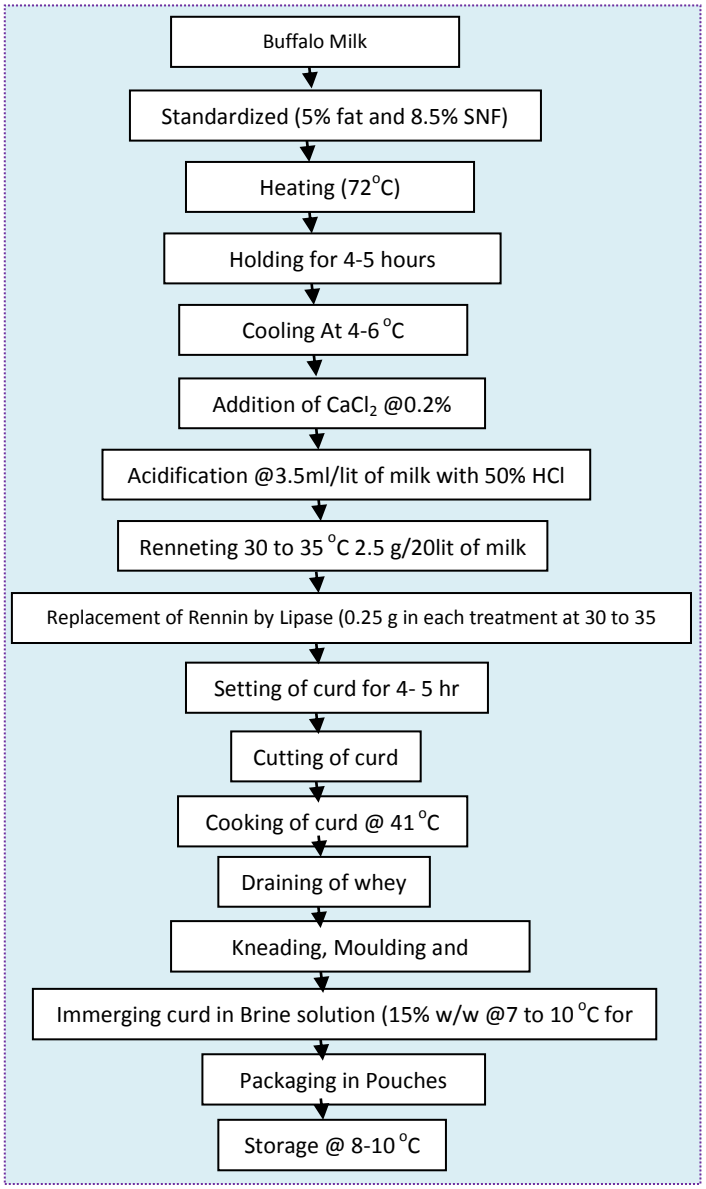

Figure-1: Layout of the Development of mozzarella cheese

2.4 Physicochemical Analysis

2.4.1Moisture content [10, 14 and 27]

It was analyzed in Hot air oven at 103C and was calculated by formula.

$$M.C. = \frac{(\text{Wt.of the sample at desired time})-(\text{wt.of bone dry material})}{(\text{Wt.of sample at any time})} \times 100 \quad (1)$$

2.4.2 Determination of ash content by Muffle furnace [10, 14 and 27]

$$\text{Ash } \% = \frac{W_2 - W_1}{W} \times 100 \quad (2)$$

Where,

W_2 = Final weight of dish + Ash

W_1 = Weight of dish

W = Weight of sample

2.4.3 Determination of fat content by Soxhlet method [10, 14 and 27]

$$\% \text{ Fat content } = \frac{W_1 - W_2}{W} X100 \quad (3)$$

Where,

W_1 = Initial weight of round flask

W_2 = Final weight of flask + fat

W = Weight of sample

2.4.4 Determination of protein content by Micro-kjeldhal method [10, 14 and 27]

$$\% N = \frac{\text{Sample}-\text{blank X N of HCl X vol.of digest X0.014}}{\text{Aliquot taken X Wt.of sample}} \times 100 \quad (4)$$

Aliquot taken × Wt. of sample Protein =Nitrogen % × 6.25.

Where,

S = Sample Titrate Reading

B = Blank Titrate Reading

2.4.5 Titratable acidity [10, 14 and 27]

$$\% \text{ Titratable acidity} = \frac{V \text{ x } N \text{ x ME of lactic acid x } 100}{\text{weight of sample}} \quad (5)$$

Where: V = volume of sodium hydroxide used to titrate

N = normality of the sodium hydroxide solution

ME = milli-equivalents of lactic acid (0.09008)

2.4.6 Cheese yield

The cheese yield was calculated as the cheese mass per equiv alent volume of
the initial milk [16]

$$\% \text{ Yield} = \frac{\text{mass (g) of produced cheese x } 100}{\text{initial mass (g) of the milk}} \quad (8)$$

2.5 Shelf life of Mozzarella cheese [10, 23, 28]

Shelf life is the length of time that a commodity may be stored without becoming unfit for use or consumption. It applies to foods, beverages, pharmaceutical drugs, chemicals, and many other perishable items. In some regions, an advisory best before, mandatory use by, or freshness date is required on packaged perishable foods. Shelf life of cheese was done by incubating the sample and counting the colonies for total plate count, and yeast and mold. Shelf life depends on the degradation mechanism of the specific product. Most can be influenced by several factors: exposure to light, heat, and moisture, transmission of gases, mechanical stresses, and contamination by things such as micro-organisms. Product quality is often mathematically modelled around a parameter (concentration of a chemical compound, a microbiological index, or moisture content).

2.6 Statistical Analysis

Data obtained from the chemical analysis and physicochemical properties was subjected to analysis of variance technology two way classification, and critical different was used to determine best treatment. Completely randomized design (CRD) was used to know the significant different between treatment of product regarding the attributes. Calculated a value was compound with a table value of F at 5% level of signification.

3. RESULTS AND DISCUSSION

The research were conducted for "**A study on the development of mozzarella cheese adding lipase enzyme and its quality evaluation**." in the Department of Department of Food Science & Technology, Warner School of Food & Dairy Technology, Sam Higginbottom Institute of Agriculture, Technology and Sciences (Formerly Allahabad Agricultural Institute) (Deemed -to-be- University) Allahabad -211007,U.P. (India).

The data collected on different aspects, as per plan were tabulated and analysed statistically. The findings are also illustrated diagrammatically. The results obtained from the analysis during the course of investigation were presented in this section and discussed in detail, in the following sequences:

3.1 Development of Mozzarella Cheese

For the development of the mozzarella cheese with addition of lipase enzyme as a substitute or replication of rennet enzyme, 20 liters of fresh buffalo milk had taken from the local dairy at Allahabad, Uttar Pradesh. Collected milk was put for standardization which was 5% fat and 8.5% SNF. After the standardization of milk, sample put on the gas stove at 72°C for 15 minutes. After heating of the sample it put on holding state for 4 to 5 hour. After the holding of the standardize milk sample, it was put in the

freezer at 4-6 °C for the purpose of cooling of the sample. For the making of curd from the milk, first add calcium chloride of 2% and then add acid (HCl with 50% concentration) at the rate of 3.5ml/ liter of milk sample. Addition of enzymes was the most important part of this research, in this process 2.5 g of rennet enzyme was added in the sample of 20 liter for the development of the mozzarella cheese at 30 to 35 °C. For the purpose of making more flavoured and improve shelf life of the cheese, 0.25 g of rennet enzyme was replaced by lipase enzyme in each sample but always remember that did not make add lipase more than 50:50 proportions. After the rennetfication process, the sample was settled for 2 to 4 hr. After the settlement of the curd, the sample was cut in horizontal and vertical form by knife. After the making of small pieces by cutting, it cooked for 15 minutes at 41°C. When cheese was formed from the milk it produced lots of whey which always affected the cheese if it present in it. So after cooking maximum whey would be drained by a clean silk cloth. After drainage of the whey these process were used for making drained and shaped cheese. For the salting of the cheese samples, curd was immerging in Brine solution (15% w/w @7 to 10 °C for 2 hrs). Pieces of the curds were packed in air packed plastic pouches. After packaging of the cheese, the pouches were stored at 8 to 10°C temperature.

3.2 Physio- Chemical Analyses

3.2.1Physical analysis of the developed mozzarella cheese

Physical properties of developed mozzarella cheese were studied on the basis of stretchability and hardness of it. During present investigation it was observed that the stretchability and hardness have considerably affected by the different treatment due to the addition of lipase enzyme. The results obtained from the five replicates had been explained further.

Stretchability of developed mozzarella cheese

The data regarding stretchability in mozzarella cheese sample of different treatments were percentage in Table-2. From the perusal of data on stretch ability in mozzarella cheese addition with Lipase enzyme of different treatments and control the highest mean total stretch ability was recorded in the mozzarella cheese sample of T_3(171.2 cm), followed by T_2 (170.4), T_1 (169.6) and T_0(168.2). The difference the mean values of T_0 - T_1 (1.4) was lower than the C.D. value, 3.407773019. Therefore, the difference was non-significant. The difference the mean values of T_0 - T_2 (2.2) was lower than the C.D. value, 3.407773019. Therefore, the difference was non-significant. The difference the mean values of T_0 - T_3 (3.0) was lower than the C.D. value, 3.407773019. Therefore, the difference was non-significant. The difference the mean values of T_1 - T_2 (0.8) was lower than the C.D. value, 3.407773019. Therefore, the difference was non-significant. The difference the mean values of T_1 - T_3 (1.6) was lower than the C.D. value, 3.407773019. Therefore, the difference was non-significant. The difference the mean values of T_2 - T_3 (0.8) was lower than the C.D. value, 3.407773019. Therefore, the difference was non-significant. Findings of authors; [2, 5, 31, 32]have supported above results.

Hardness Analysis of developed mozzarella cheese

After the observation of Table-3, the average hardness of five replicate of different samples of the cheese were 4.74, 4.60, 3.407773019 and 4.28 g/cm^2 for T_0 , T_1 , T_2 and T_3 respectively. This was happened due to presence of lactose enzyme which increases the value of strength of the bonds between the atoms. Above results have been supported by findings of authors; [3, 7, 11, 30, 32] Data for average hardness of different cheese samples were statistically analyzed to find out significant difference between treatments with regard to the hardness in cheese samples.

3.2.2 Chemical Analysis of the Mozzarella Cheese

During the present observation on the development of the lipase added cheese, it was observed that there were a significant change in the values of protein, ash (total solid), moistures, fat and acidic contents. This was due to the addition of lipase enzyme during the development of the cheese.

Moisture content in mozzarella cheese

The data regarding moisture present in mozzarella cheese sample to different treatment are presented in Table-4, the perusal of data of moisture percentage in mozzarella cheese sample of different treatment and control the highest mean moisture percentage was recorded in the mozzarella cheese sample T_0(56.54 %), followed by T_1 (55.74 %), T_2 (54.52 %) and T_3(52.88 %). The difference between the mean values of T_0 - T_1 (2.49) was lower than the C.D. value, 2.594Therefore, the difference was non-significant. The difference between the mean values of T_0 - T_2 (3.54) was greater than the C.D. value, 2.594. Therefore, the difference was significant. The difference between the mean values of T_0 - T_3 (3.66) was greater than the C.D. value 2.594. Therefore, the difference was significant. The difference between the mean value of T_1 -T_2 (1.22) was lower than the C.D. value 2.594. Therefore, the difference was significant. The difference between the mean value of T_1 - T_3 (2.86) was lower than the C.D. value, 2.594. Therefore, the difference was non-significant. Above results have been supported by findings of authors; [13,19,23,25].

Fat content in mozzarella cheese

The data regarding fat percentage in mozzarella cheese sample of different treatment are presented in Table-5. From the perusal of data on fat percentage in mozzarella cheese incorporation with mozzarella cheese sample of different treatment and control the highest mean fat percentage mozzarella cheese sample of T_0(24.88%), followed by T_1 (23.80%), T_2 (23.30%) and T_3(22.52%). Above results have been supported by findings of authors;

[5, 8, 15, 16]. Therefore, the difference was significant, indicating significant effect of treatment on fat percentage. The difference between the mean values of T_0 - T_1 (0.02) was smaller than the C.D. value, 0.97762; Therefore, the difference was significant. The difference between the mean values of T_0 - T_2 (1.09) was greater than the C.D. value, 0.97762; Therefore, the difference was significant. The difference between the mean values of T_0 - T_3 (1.17) was greater than the C.D. value, 0.97762;. Therefore, the difference was significant. [25],who reported 21–25, 18.79, 20.0 and 17.13% fat in mozzarella cheese, respectively.

Protein content in mozzarella cheese

The data regarding protein percentage in mozzarella cheese sample of different treatment are presented in Table-6. From the perusal of data on protien percentage in mozzarella cheese incorporation with mozzarella cheese sample of different treatment and control the highest mean fat percentage mozzarella cheese sample of T_3(24.08%), followed by T_2 (23.68%), T_1 (23.26%) and T_0(22.66%). Therefore, the difference was significant, indicating significant effect of treatment on fat percentage. The difference between the mean values of T_0 - T_1 (0.02) was smaller than the C.D. value, 0.30; Therefore, the difference was significant. The difference between the mean values of T_0 - T_2 (1.09) was greater than the C.D. value, 0.30; Therefore, the difference was significant. The difference between the mean values of T_0 - T_3 (1.17) was greater than the C.D. value, 0.30; Therefore, the difference was significant. The difference between the mean values of T_1 - T_2 (1.07) was greater than the C.D. value, 0.30; Therefore, the difference was significant.

The difference between the mean values of T_1 - T_3 (1.15) was greater than the C.D. value, 0.30; Therefore, the difference was significant. The difference between the mean values of T_2 - T_3 (0.08) was smaller than the C.D. value, 0.30; Therefore, the difference was significant.[25], who reported 21–25, 18.79, 20.0 and 17.13% protien in mozzarella cheese, respectively.

Total solids content in mozzarella cheese

The data regarding total solid percentage in mozzarella cheese sample of different treatment are present in Table-7. From the perusal of data on total solid in mozzarella cheese incorporation with mozzarella cheese sample of different treatment and control the highest mean total solid percentage was recorded in the mozzarella cheese sample T_3 (49.12) followed by T_2 (45.64), T_1 (44.14), T_0 (43.54). The difference between the mean values of T_0 - T_1 (0.60) was lower than the C.D. value, 4.497. Therefore, the difference was non-significant. The difference between the mean values of T_0 - T_2 (1.10) was lower than the C.D. value, 4.497.

Therefore, the difference was non-significant. Above results have been supported by findings of authors;[26,28,30].

Acidicity of mozzarella cheese

The data regarding Acidity percentage in mozzarella cheese sample of different treatment are presented in Table-8. From the data perusal of data on acidity percentage in mozzarella cheese incorporation with same time and different temperature, of different treatment and control the highest mean acidity percentage was recorded in the mozzarella cheese sample of T_3 (0.758) followed by T_2 (0.742), T_1 (0.716) and T_0(0.698).

Therefore, the difference has significant effect of treatments on acidity percentage. Above results have been supported by findings of authors; [27,29]

Flavour and Taste Score of Mozzarella Cheese

From the perusal of data on flavor and taste score in mozzarella cheese sample of different treatment and control the highest mean flavour and taste score was recorded in the mozzarella cheese sample of T_3 (8.40) followed by T_2 (8.05), T_1 (7.95) and T_0(7.25). Therefore, the difference was significant, indicating significant effect of treatment of flavour and taste score. [1,16,20,29] have been supported the above results because it was similar as their findings.

Body and Texture Score of Mozzarella Cheese

The data regarding body and texture score in mozzarella cheese sample of different treatment are presented in Appendix A. From the perusal of data on body and texture score in mozzarella cheese sample of different treatment and control highest mean body and texture score was recorded in the mozzarella cheese sample of T_3 (8.55) followed by T_2 (8.30), T_1 (7.95) and T_0(7.90). [9, 17, 22, 28] have been supported the above results because it was similar as their findings.

3.3 Microbiological Quality Analysis

Microbial populations like bacteria and fungus were estimated by serial dilution followed by solidification in petriplate using nutrient agar and rose Bengal agar respectively. After solidification both bacteria and fungus colony containing plates were incubated at room temperature for 24 to 48 hours. Then formed colonies were counted and converted them as number of colony forming units (CFU) per gram of sample. Microbial load estimation for the cheese samples were carried out for fresh samples. **[9]**

Standard Plate Count (SPC)

Microbial quality was done by Standard plate count in fresh and stored samples. After the observation of the present research SPC was nil (0 cfu/g) at the 10^{-2} dilution factor and at room temperature for the fresh samples but for the stored samples due to the increment of the moisture bacteria grew easily but the increase value of the SPC is within the safe limit (10^3 cfu/g for bakery products) [10] up to 10 days for all samples. The results of the SPC showed in the following Table-8.

Self Life of Mozzarella Cheese

Consumers demand products that are minimally processed, nutritious, and safe, with longer shelf - life and good taste. Because of this reason, industry and researchers have studied and developed new processing and preserving technologies [10,13,23] For the measurement of the shelf life of the cheese, it has been stored at the temperature of 4° C for ten days and measured the total plate count (cfu/g) on current day, 2nd day, 5th day, 7th day and 10th day. After the observation of the data from the Table-9, it was found that the quality of the mozzarella cheese has decreases day by day but after 10 days it was still below than the standard limit and SPC was negative for all treatment. Findings of [23] supported the above findings.

Table-2: Stretchability analysis

Sr. No.	Treatments	Replications (cm)					Total	Mean
		R_1	R_2	R_3	R_4	R_5		
1	T_0	170	165	168	171	167	841	168.2
2	T_1	168	172	169	170	169	848	169.6
3	T_2	173	168	169	172	170	852	170.4
4	T_3	169	174	172	170	171	856	171.2

Table-3: Hardness Analysis

Sr. No.	Treatments	Replications (g/cm²)					Total	Mean
		R_1	R_2	R_3	R_4	R_5		
1	T_0	4.5	4.9	4.6	5.0	4.7	23.7	4.74
2	T_1	4.6	4.5	4.4	4.7	4.8	23	4.6
3	T_2	4.3	4.2	4.6	4.4	4.7	22.2	4.44
4	T_3	4.4	4.0	4.5	4.3	4.2	21.4	4.28

Table-4: Moisture Analysis of mozzarella cheese

Sr. No.	Treatments	Replications (%)					Total	Mean
		R_1	R_2	R_3	R_4	R_5		
1	T_0	56.7	59.2	55.4	54.3	57.1	282.7	56.54
2	T_1	54.3	56.8	55.6	58.2	53.8	278.7	55.74
3	T_2	51.9	56.4	54.8	53.6	55.9	272.6	54.52
4	T_3	52.1	54.6	50.8	51.2	55.7	264.4	52.88

Table-5: Fat Analysis of mozzarella cheese

Sr. No.	Treatments	Replications (%)					Total	Mean
		R_1	R_2	R_3	R_4	R_5		
1	T_0	23.8	24.8	25.3	24.8	25.7	124.4	24.88
2	T_1	23.2	23.8	24.5	24.7	22.8	119.0	23.80
3	T_2	23.6	22.9	23.9	23.5	22.6	116.5	23.30
4	T_3	22.4	22.8	23.2	21.7	22.5	112.6	22.52

Table-6: Protein Analysis of mozzarella cheese

Sr. No.	Treatments	Replications (%)					Total	Mean
		R_1	R_2	R_3	R_4	R_5		
1	T_0	22.4	21.6	23.5	22.8	23.0	113.3	22.66
2	T_1	23.6	22.8	23.2	23.0	23.7	116.3	23.26
3	T_2	22.9	23.7	23.8	24.1	23.9	118.4	23.68
4	T_3	24.0	24.5	23.8	24.2	23.9	120.4	24.08

Table-7: Total solids Analysis

Sr. No.	Treatments	Replications (%)					Total	Mean
		R_1	R_2	R_3	R_4	R_5		
1	T_0	43.7	40.8	44.6	45.7	42.9	217.7	43.54
2	T_1	45.7	43.2	44.4	41.2	46.2	220.7	44.14
3	T_2	48.9	43.6	45.2	46.4	44.1	228.2	45.64
4	T_3	47.9	55.4	49.2	48.8	44.3	245.6	49.12

Table-8: Acidic value in mozzarella cheese

Sr. No.	Treatments	Replications (%)					Total	Mean
		R_1	R_2	R_3	R_4	R_5		
1	T_0	0.70	0.74	0.68	0.66	0.71	3.49	0.698
2	T_1	0.72	0.69	0.74	0.75	0.68	3.58	0.716
3	T_2	0.69	0.74	0.75	0.77	0.76	3.71	0.742
4	T_3	0.72	0.75	0.77	0.79	0.76	3.79	0.758

4. CONCLUSIONS

After the observation of the experimented data of the mozzarella cheese, it concluded that when the lipase enzyme was added in the place of rennet enzyme partially, it found that physio- chemical properties such as stretch ability, hardness moisture, fat, protein, total solid and acidity were improved in acceptable platform because the stretch ability of the cheese increases from sample T_0 (168.2) to T_3 (171.2 cm) and the hardness of the cheese was decreased from sample T_0 (4.74 g/cm²) to T_3 (4.28 g/cm²), and the moisture of the cheese samples were decreases from sample T_0(56.54 %) to T_3(52.88 %) while fat, protein, total solid content and acidicity of the cheese sample were increased due to addition of lipase enzyme from sample $T_0$to T_3. The sensory evaluation of the cheese sample such as body texture, colour, taste and flavor were changed but in positive manner. By this addition the shelf life of the mozzarella cheese also increased and it would be stored for more than control sample.

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