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RESEARCH ARTICLE

STUDIES ON THE PRODUCTION AND QUALITATIVE CHARACTERISTICS EVALUATION OF CHEESE USING PINEAPPLE [*ANANAS COMOSUS (L) MERR.*] FRUIT JUICE AS A MILK CLOTTING AGENT

Akanksha Soni and *Ram Rakha

State Institute of Food Processing Technology, Lucknow, India

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ABSTRACT

The present study is aimed to innovate and standardize technique in cheese making by whole pineapple juice used as a milk coagulant and incorporation of nutrients in cheese. It is also aimed to reduce the cost of rennet enzyme, easy availability and for those who are vegetarian and prohibited due to religious factor. Full cream milk was used to produce cheese by rennet enzyme in T1 & T2 while pineapple juice was incorporated as clotting agent in T3, T4 & T5. Ascorbic acid retention in T3, T4 & T5 was 1.16, 2.52 and 4.46 mg/100gm of cheese. It is a bioactive compound and is very essential to regulate various biochemical processes in the body. Presence of ascorbic acid in dairy product is an attractive feature of this study. The other chemical constituent viz protein, fat, SNF and moisture were found slightly lesser in juice treated cheese than rennet treated cheese (control) but there was no significant difference found in chemical constituents of cheese samples. Pineapple juice was found suitable for soft cheese production. The softness of cheese increased with the increase in quantity of juice in the treatments. The extra soft character of cheese in juice treated Bromelain enzyme present in juice as reported by Roseiro, *et al.,* (2003) and Ilany and Netzer, (1969). The product was organoleptically analyzed for colour, flavour, texture and taste. Application of 2.5% juice scored 94, which was found highest than other treatments. The calculated Critical Difference of all treatments is 1.3618. It was found that T1 differs with T4 by 2.25, T2 differs with T4 by 2.25 and T3 differs with T4 by 1.75 significantly.

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INTRODUCTION

Milk coagulation is the main step for producing cheese and coagulating enzymes, which are preparations of proteolytic enzymes, have been used in cheese making for thousands of years, and they seem to be the oldest known application of enzymes. The earliest indication of cheese making descends from cave paintings around 5000 BC; Harboe *et al.,* (2010). Historically, most enzyme preparations used for cheese have been extracts from the stomachs of ruminants, but coagulants from microbes and plants were also used at very early dates (Harboe *et al.,* 2010 and Jacob *et al.,* 2011). Ruminant stomach, especially, that of the calf, is the source of rennet. It contains chymosin as the main enzyme component and has been the most widely used in cheese making. The cheese production increased by a factor of approximately 3.5 since 1961 but the rennet supply decreased due to the limited availability of ruminant stomachs (Jacob *et al.,* 2011). Various factors such as high price of rennet, religious concerns (e.g., Islam and Judaism), diet (vegetarianism) or ban on recombinant calf rennet (in France, Germany and The Netherlands) have encouraged the search for alternative milk-clotting sources (Roseiro *et al.,* 2003). The research has been directed towards discovering milk-clotting enzymes which would satisfactory replace calf rennet in cheese making, including microbial, recombinant, and plant-based enzymes (Jacob *et al.,* 2011). The most important substitutes which fulfill the requirements of cheese manufacture include microbial, recombinant, and plant-based enzymes which have been isolated and studied. Rennet substitutes produced by micro-organisms and genetically engineered microorganisms have proven to be suitable substitutes for animal rennet, but increasing interest has been directed toward vegetable coagulants i.e., the milk-clotting enzymes extracted from plants. Manzoor Ahmad, *et al.,* (2013) Plant proteases have been used as milk coagulants in cheese making for centuries either as crude extracts or in purified form. These coagulants are an alternative to the calf rennet due to the limited availability and high price of rennet, religious factors, diet or ban on recombinant calf rennet in some countries. These enzymes are found in almost all kinds of plant tissues and can be obtained from their natural source or through in vitro culture to ensure a continuous supply of plant proteases.

****Corresponding author:*** **Ram Rakha,**
State Institute of Food Processing Technology, Lucknow, India.

Almost all the enzymes used as milk coagulants belong to aspartic proteases, but enzymes from other groups such as cysteine and serine proteases have also been reported and possess the ability to clot milk under proper conditions. The excessive proteolytic nature of most plant coagulants has limited their use in cheese manufacturing due to lower yields of cheese, bitter flavors and texture defects. Rosiero, *et al.,* (2003) Plant extracts have been used as milk coagulants in cheese making since ancient times. Cheeses made with vegetable coagulant can be found mainly in Mediterranean, West African, and southern European countries. Spain and Portugal have the largest variety and production of cheeses using *Cynara spp.* as the vegetable coagulant. The extracts of *Cynara spp.* have been used in the making of Portuguese Serra and Serpa cheeses (Mace do, *et al.,* 1993) and Spanish Los Pedroches, La Serena (Roa, *et al.,* 1999) and Torta del Casar cheeses (from ewes' milk) as well as Los Ibores cheese (from goats' milk) and Flor de Guía cheese (from a mixture of ewes' and cows' milk) (Fernandez-Salguero, *et al.,* 1991; Fernandez-Salguero, 1999; Sanjuan, *et al.,* 2002). In West African countries like Nigeria and the republic of Benin, extracts from *Calotropis procera* (Sodom apple) have been used in traditional cheese making (Rosiero, *et al.,* 2003). However, the excessive proteolytic nature of most vegetable coagulants has limited their use in cheese manufacturing due to lower cheese yield and defects in flavor and texture (Lo Piero, *et al.,* 2002). Therefore, the search for new potential milk-clotting enzymes from plants is in continuous process, so as to make them industrially useful and go with the increasing global demand for diversified and high quality cheese production (Hashim *et al.,* 2011).

According to ancient records passed down through the centuries, the making of cheese dates back more than 4,000 years. No one really knows who made the first cheese. According to an ancient legend, it was made accidentally by an Arabian merchant who put his supply of milk into a pouch made from a sheep's stomach, as he set out on a day's journey across the desert. The rennet in the lining of the pouch, combined with the heat of the sun, caused the milk to separate into curd and whey. That night he found that the whey satisfied his thirst, and the cheese (curd) had a delightful flavor which satisfied his hunger. Travelers from Asia are believed to have brought the art of cheesemaking to Europe. In fact, cheese was made in many parts of the Roman Empire when it was at its height. The Romans, in turn, introduced cheesemaking to England. During the middle Ages-from the decline of the Roman Empire until the discovery of America-cheese was made and improved by the monks in the monasteries of Europe. For example, Gorgonzola was made in the Po Valley in Italy in 879 A.D., and Italy became the cheesemaking center of Europe during the 10th Century. Roquefort was also mentioned in the ancient records of the monastery at Conques, France as early as 1070. Cheese is the ripened or unripened soft, semi-hard, hard or extra hard product, which may be coated, and in which the whey protein/casein ratio does not exceed that of milk, obtained by:

(a) Coagulating wholly or partly the protein of milk, skimmed milk, partly skimmed milk, cream, whey cream or buttermilk, or any combination of these materials, through the action of rennet or other suitable coagulating agents, and by partially
(b) Draining the whey resulting from the coagulation, while respecting the principle that cheese-making results in a concentration of milk protein (in particular, the casein portion), and that consequently, the protein content of the cheese will be distinctly higher than the protein level of the blend of the above milk materials from which the cheese was made; and/or
(c) Processing techniques involving coagulation of the protein of milk and/or products obtained from milk which give an end-product with similar
(d) Physical, chemical and organoleptic characteristics as the product defined under(a)

Repined cheese is cheese which is not ready for consumption shortly after manufacture but which must be held for such time, at such temperature, and under such other conditions as will result in the necessary biochemical and physical changes characterizing the cheese in question. Mould ripened cheese is a ripened cheese in which the ripening has been accomplished primarily by the development of characteristic mould growth throughout the interior and/or on the surface of the cheese.
Unripened cheese including fresh cheese is cheese which is ready for consumption shortly after manufacturing.

MATERIALS AND METHODS

All raw materials carried to the laboratory on the same day and stored in refrigerators. All the raw materials as well as finished product were analyze for the physical, chemical and sensory parameter prior to production of cheese. The screw type juice extractor was used for the extraction of juice. Pasteurization of milk carried out in aluminum vessel over LPG furnace. Thereafter; quality of milk and pineapple juice were calculate according to trial T_1, T_2, T_3, T_4 and T_5. Blending of juice into milk as per specification of each treatment for clotting then it put for ½ hour to proceed enzymatic reaction. When coagulation of milk completed then precipitated milk was separated out from whey with the help of muslin cloth by continuous squeezing with hands. Clotted milk is then pressed by wooden plate making it too heavy to remove excess moisture. Now coagulated material was place in desired mould and set it for maturation in BOD incubator for 60 days.

Flow Chart:

Receiving of milk
↓
Pre-heating (35- 40^{0}C)
↓
Filtration / Clarification
↓
Standardization
↓
Boiling for (63^{0}C /30 mins)
↓
Addition of pineapple juice / starter as per technical program in each treatment
↓
Coagulation / Setting
↓
Cutting
↓
Drainage of whey
↓
Salting
↓
Hooping

↓
Dressing
↓
Pressing
↓
Curing / Maturing

Physico-chemical analysis of Milk, pineapple juice and Cheese

The raw material as well as finished product were analysed for following physical and chemical characteristics:

Colour: Colour, vision and appearance are two different things. Vision can be regarded as the process of seeing whereas appearance is the recognition and assessment of the properties such as surface structure, opacity, colour etc. associated with the seen object. Colour is used as an index of quality of the product. Therefore, the colour of food is measured to identify its property.

Flavour: The flavour of a food was determined by the way that certain molecules in the food interact with receptors in the mouth (taste) and nose (smell) of human beings. The perceived flavor of a food product depends on the type and concentration of flavor constituents within it, the nature of the food matrix, as well as how quickly the flavor molecules can move from the food to the sensors in the mouth and nose. Analytically, the flavor of a food is often characterized by measuring the concentration, type and release of flavor molecules within a food or in the headspace above the food.

Boiling Point: It was determined by using high temperature thermometer during boiling of milk.

Recovery %: To avoid water content errors, the cheese yield was estimated in relation to the dry matter by the use of the following expression as given by Michalski *et al.,* (2003).

Melting Point: It was recorded according to Stanford Research Systems. The pharmacopeias regard the capillary method as the standard technique for melting point determination.

pH: It was measured by the method described as in FSSAI Manual (2016).

Principle: When substances are dissolved in water depending on their degree of dissociation and their chemical nature can accept $H^{+\,Ion}$ of water and make it either acidic or basic pH is negative logarithm concentration of H^+. It extends from 0 to 14 taking the hydrogen ion concentration of 1M to 14M and can cover the range from 1 N Hcl to 1N NaOH. For every unit change in pH, there will be 10 times change in the H^+ ion. The glass electrode pH meters are widely used at present. It has been found that thin membrane of proper glass is selectively permeable to H^+. The potential across a glass membrane is a concentration potential due to differences in H^+. The instrument is calibrated against a buffer of known pH and pH of unknown is determined.

Acidity: Acidity % as citric acid and lactic acid were analysed according to method given by Miller (1950).

Fat: It was determined by Soxhlet Apparatus, the method described in AOAC (1970).

Principle: Fat and Oil are soluble in solvent such as-petroleum ether, hexane etc. Ether soluble materials are extracted using a soxhlet extraction apparatus using a soxhlet extraction apparatus and then residue is weighed.

Protein: Percent nitrogen content of the cheese products were determined using the micro Kjeldahl method ([18]AOAC., 1990) and crude protein content calculated using the factor 6.38.

Ash: Percentage of ash in cheese was calculated accordingly method given in AOAC (1960).

Principle: This method determines as ash the residue remaining after incineration of the sample and as acid insoluble ash the residue remaining after incineration of sample and acid digestion of the ash constituents under specified conditions for test.

SNF: It was estimated as per method of AOAC (2012).

Principle: Total solids content is the entire residue left after complete evaporation of water from milk. This includes fat protein, lactose and mineral matter. These solid constituents exist in milk in a mechanical mixture.

Ascorbic Acid: Ascorbic acid was estimated according to method described by Bessey and King (1933).

Sugars

[A] Reducing sugar %: Sugars were estimated according to method described by [22]**Lane** and **Eynon** (1923).
Estimation of reducing sugars was done by using formula:
[B] Non- Reducing Sugar and Total Sugar %:
Non Reducing Sugars % = (Total invert sugars % - Reducing sugars %) x 0.95
Salt: Salt was estimated according to the method described as in NCAL (1954).

Principle: An aliquot taken from a neutralized solution containing sodium chloride is titrated with a standardized solution of silver nitrate using potassium chromate as an indicator.

Moisture: Moisture was estimated by the method described in AOAC (1960).

Principle: With increase in temperature, moisture evaporates.

Boiling temperature – 100^0 C

Energy: The Energy value in foods is defined as in [24]**FAO** (2002). The unit of energy in the International System of Units (SI) is the joule (J). A joule is the energy expended when 1 kg is moved 1 m by a force of 1 Newton. This is the accepted standard unit of energy used in human. The SI (from the French System International d'Unités) is the modern metric system of measurement. It was established in 1960 by the 11^{th} General Conference on Weights and Measures (CGPM – Conference General Des Poids et Mesures), which is the international authority that ensures wide dissemination of the SI and modifies it, as necessary, to reflect the latest advances in science and technology. The SI is founded on seven SI base units, which are assumed to be mutually independent. There are 22 derived SI units defined in terms of the seven base quantities. The SI derived unit for energy, as work or quantity of heat, is the joule (m2 ·kg·s-2), the symbol for which is J. The calorific value of cheese was calculated using formula:

Total energy in (k.cal) = 4 x (CHO + protein + 9 x fat)

TSS: TSS of pineapple juice as Brix was measured by using hand refractometer as per method of Rangana (1986).

Microbiological investigation: This parameter was carried out for yeast and mold count in milk and cheese adopting standard procedure described in FSSAI Manual (2012).

Sensory Analysis

Cheese acceptability tests were performed in each treatment (T_1, T_2, T_3, T_4 and T_5). Five untrained panelists were used for the organoleptic (sensory) evaluation of experimental cheese based on their free will. Prior to evaluation, the panelists washed their mouth in order to remove the palates and after taste they washed it again. A four quality attributes like, colour, flavour, texture and taste were evaluated according to the Composite Scoring Test described by Rangana (1977).

RESULTS AND DISCUSSION

Data of physical characteristics of raw milk is given in Table 1 reveals white color, milky flavor and boiling point of milk was investigated 103.50^{0}C. Physical parameters are very close to Sukumar De (1991). Whereas, chemical parameters of raw milk is given in Table 2. The protein was estimated 3.3g/100g, Fat 6.0g/100g; SNF 27.50%, calculated energy 87 k.cal/100g and pH 6.6. The results confirm description provided by Sukumar De (1991). It is clear from table no.3 showed chemical constituents of pineapple juice. The pH of Juice was recorded 3.17. Acidity % as (citric acid) analysis 0.52. Total sugar was found 9.65. The product was also analyzed for ascorbic acid. The ascorbic acid was estimated 46.75 mg/100gm of juice. Total Soluble Solids present in juice was observed 10.5 B$^{0.}$ The results of current study accompanied with Daniel Simoes *et al.,* (2011). The table no.6 containing regarding physical properties like recovery % 24.50, 24.75, 22.50, 20.45 and 18.25 in T_1, T_2, T_3, T_4 and T_5 respectively. Maximum recovery was reported in 20.45% followed by 18.25% in T_5 in comparison to T_1& T_2 where rennet is used only, more recovery were there. The results are accordance with Ramet JP (1997) as cited. Melting Point was recorded maximum 60^{0}C in control trial white minimum 58^{0}C. It is due to hardness and softness of product. Pineapple juice added treatments produce soft cheese. Soft cheese has lesser melting point than hard one as mentioned by Davis (1965 and 1976).

The Table No. 5 reveals chemical characteristics of cheese. It is clear that acidity was estimated in maximum value 0.17% in T_4 and T_5 followed by 0.15% in T_3, 0.10% in T_2 and 0.9% in T_1. Acidity shows increasing trend as quantity of pineapple juice increases. Added juice hastens lactic acid formation from sugar, which was present in milk as well as in juice. The results are closed to Ferial Aziza, *et al.,* (2016) also reported in their work. The protein content of cheese were analysed in range from 38.75 to 40.50%. Pineapple juice added treatment showed slight decrease, this is due to high proteolytic activity of Bromelain Enzyme present in juice, which disrupt peptide bond and drained out with whey. The results are accompanied with J. Ilany and A. Natzer (1969) as reported in their studies. The product was also analysed for quantity of fat. It was observed lowest 17.01% in T_2 and highest 18.47 in T_4. The results are same as reported by Maxhuni and Kukaj (2015). Ash content in cheese was estimated minimum 3.15% in T_1 whereas 3.86% maximum in T_4. Ash quantity showed increased amount in those treatments in which juice used as clotting material. Current findings agreement with the result by Salwa Putra, *et al.,* (2015) in their study. SNF was found detected between the ranges of 32.70% - 48.18%. The SNF exhibits raising trend. It is due to addition of salt and rest part came from pineapple juice. The ascorbic acid was found 1.16, 2.52 and 4.46 in T_3, T_4 and T_5 respectively. SNF content of the cheese samples of current study resemble with the results of Raphaelides, *et al.,* (2006) reported that there is significant effect on the SNF content of the cheese during storage period. This is probably due to the summation of protein and ash content.

Table 1. Physical parameters of raw milk

Parameters	Value				
Treatments	T_1	T_2	T_3	T_4	T_5
Recovery %	24.50	24.75	22.50	20.45	18.25
Melting Point ^{0}C	60	60	59	58	58

Table 2. Chemical parameters of raw milk

Parameters	Value
Colour	White
Flavour	Milky
Boiling point	101.7

Table 3. Chemical parameters of pineapple juice

Parameters	Value
Fat (gm)	6.0
Protein (gm)	3.3
SNF %	27.50
Energy K.cal	87
pH	6.6

Table4. Physical parameters of finished product

Parameters	Value
pH	3.7
Acidity % (citric acid)	0.52
Sugars %	9.65
Ascorbic acid (mg/100gm)	46.75
Total Soluble Solids (B^0)	10.50

Ascorbic acid was detected null in T_1 and T_2 as milk contains negligible quantity of ascorbic acid. The findings regarding vitamin C had very positive feature of the product. It is bioactive compound which has antioxidant property. Very little quantity of sugar was found in cheese as compared to milk. It was 0.95% minimum in T_1 and 1.85% maximum in T_5. It is clear that most of the sugar converted into lactic acid during storage and drained out with whey. The results are similar as reported by Milci, *et al.,* (2005) that the decrease in total solid content with increasing periods. Results of current study are also supported as lactose is one of the basic nutrients consumed by lactic acid producing micro-organisms. Lactose remaining in the curd is converted into lactic acid. Lactic acid inhibits the growth of undesirable micro-organisms. It is very important in production of flavour in the cheese. It determines the smoothness of the body of the cheese Le Jaouen, *et al.,* (1987).

Physico-chemical parameters of finished product (cheese)

Table 5. Chemical parameters of finished product

Parameters	Treatments				
pH	5.02	5.02	5.13	4.96	5.00
Acidity % (lactic acid)	0.9	0.10	0.15	0.17	0.17
Fat %	17.25	17.01	17.40	18.47	18.25
Protein	44.50	45.05	42.75	40.12	38.75
Ash %	3.15	3.67	3.75	3.86	3.70
SNF %	32.75	32.70	48.19	46.14	45.00
Ascorbic acid (mg/100gm)	0.00	0.00	1.16	2.52	4.46
Sugars %	0.95	0.99	1.04	1.50	1.85
Salt %	0.81	0.69	0.65	0.66	0.70
Moisture %	54.15	54.10	55.25	56.10	57.10
Energy (k.cal)	337.05	337.25	331.76	232.71	326.65

Table 6. Yeast and Mold Count in Cheese

Parameter	Treatment				
	T1	T2	T3	T4	T5
Yeast and mold count/g	10.80	10.50	10.75	10.38	10.90

Table 7. Sensory Evaluation of finished product

Treatment	Sensory Characteristics				
	Colour (25)	Flavour (25)	Texture (25)	Taste (25)	Total (100)
T_1	23	22	22	22	89
T_2	22	23	23	21	89
T_3	24	23	21	23	91
T_4	25	25	24	24	98
T_5	24	24	22	24	94

Statistical Figures

The chemical parameters of raw milk

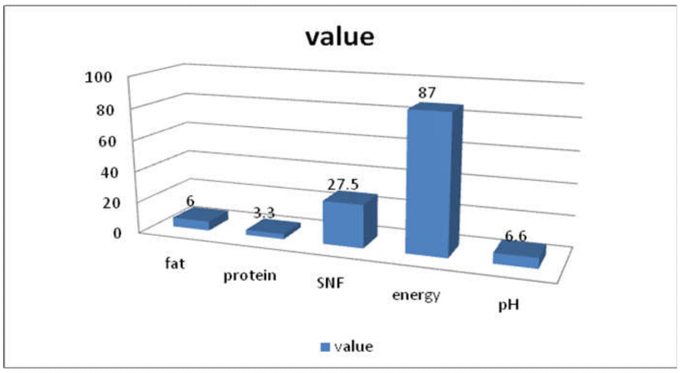


Figure 1.

The chemical parameters of pineapple juice

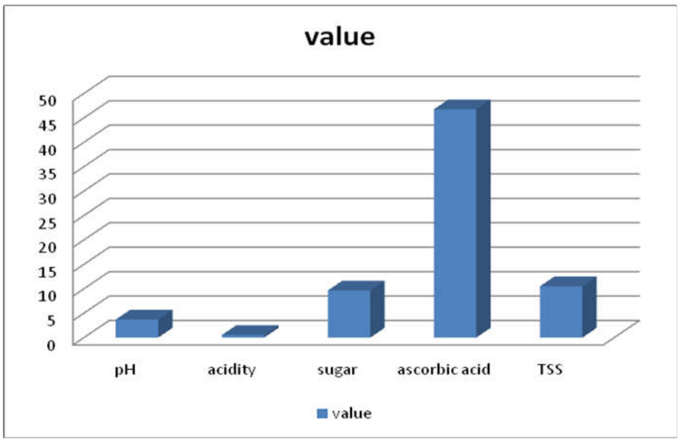


Figure 2.

The physical parameters of finished product

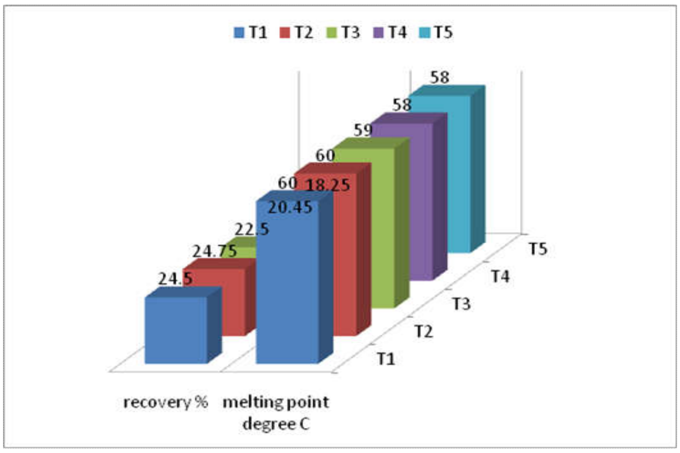


Figure 3.

The Chemical Parameters of Finished Product

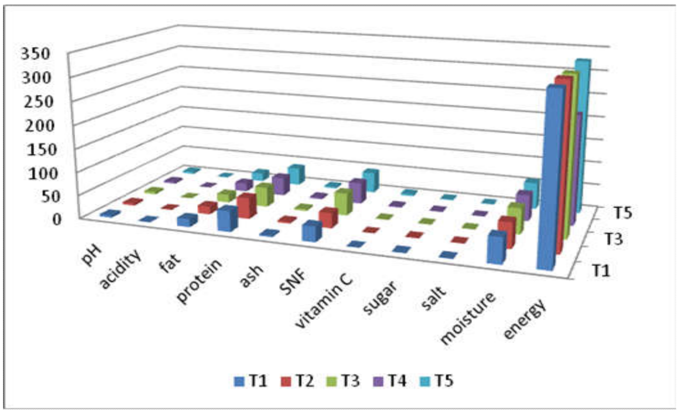


Figure 4.

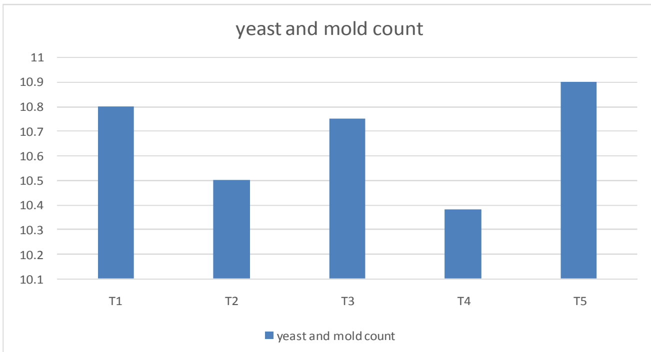


Figure 5.

The Sensory Evaluation of finished product

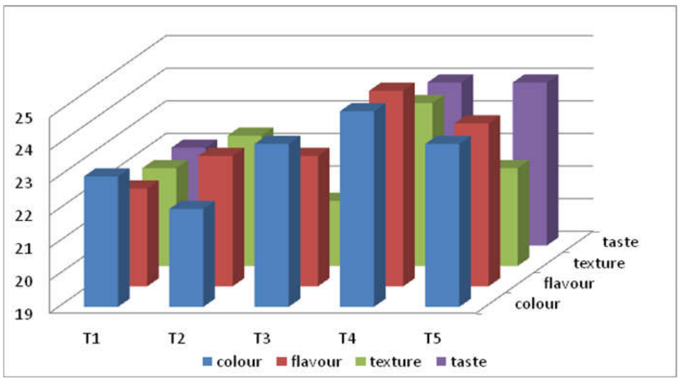


Figure 6.

Fresh Milk

Milk clotted with pineapple juice

Boiled Milk

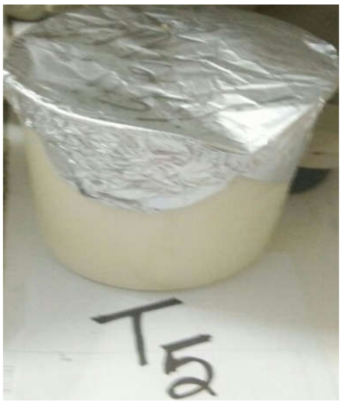
Draining of whey

Cutting of curd

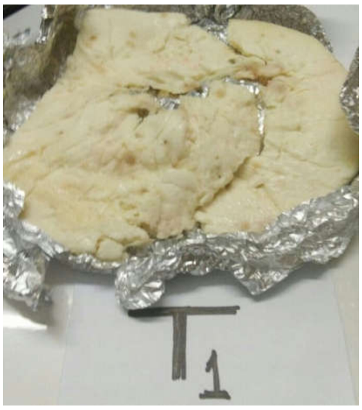
Pressing of cheese

Milk clotted by Rennet

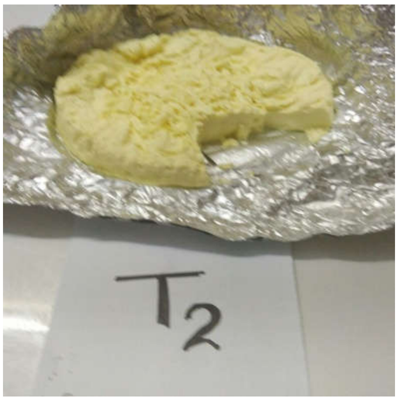
Whey drained out from pineapple flavoured Cheese



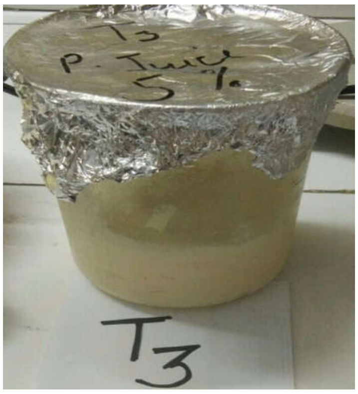
1.5% pineapple juice added to the milk



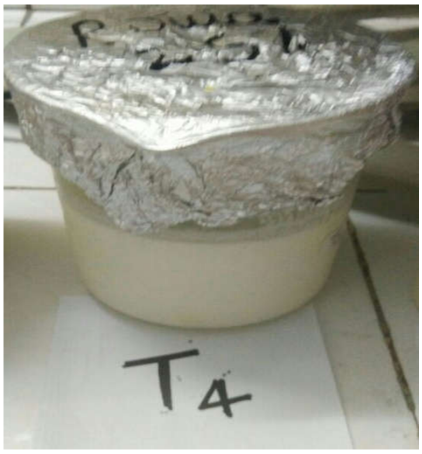
Cheese made by using rennet



Pineapple emulsion added cheese made by using rennet



5% pineapple juice added to the milk



2.5% pineapple juice added to the milk

Picture showing all samples in sequence

Arrangement of samples during sensory evaluation

Organoleptic test of all treatment

Salt was added in cheese to improve the quality. Salt was observed between the ranges of 0.65% - 0.81%. Salt raises quality attributes especially regarding colour, flavour and taste. This was the agreement with the reports of Schroder, *et al.,* (1988) and Mutang and Wilbey (2006). Moisture content showed increasing trend than control treatment. It was estimated 54.15, 54.10, 55.25, 56.10 and 57.10 in T_1, T_2, T_3, T_4 and T_5. In the current studies decrease in moisture level of the samples were found but it was under 45% according to USDA (2012) for low moisture Mozzarella Cheese moisture content must be greater than 45% but must be equal to or less than pH of 5.0 to 5.4. Energy values were calculated on the basis of carbohydrate, protein and fat. More confirm value 337.25 k.cal in flavoured cheese while low calorie were calculated in 331.76, 232.7, 326.65 k.cal in juice treated samples viz T_3, T_4 and T_5. The results of energy value in cheese were similar as described in Daily Value (2015). The statistical calculations also showed no significant difference among control T_1 and pineapple juice treated sample T_2, T_3, T_4 and T_5. The critical difference of all treatment was 72.9141. It is a very positive result regarding chemical constituents of rennet treated samples and pineapple juice treated samples. The Table No. 6 reveals the Microbial Characteristics of cheese. The Yeast and Mold Count was detected between the ranges of 10.38/g to 10.90/g in the cheese sample. The results of microbiological testing are within the legal limits of FSSAI (2006) where maximum yeast and mold count should be not more than 250/g in all other cheeses categories including fresh cheese, cheddar cheese, soft cheese, semi soft cheese etc.

The Table 7 reveals the Sensory Characteristics of cheese. Sensory Evaluation refers to the evaluation of recipe by sense organs. All the sense organs were used in the appraisal of food. Organoleptic evaluations of cheese for the five consecutive treatments during investigation are presented in table no.7. Overall mean score of quality attributes viz colour, flavour, texture and taste ranged from 22 to 25, 22 to 25, 21 to 24 and 21 to 24 respectively. Overall highest acceptability of product was found in T_4 (2.5% pineapple juice) getting highest score 98 followed by 94, 91, 89 and 89 T_5, T_3, T_1 and T_2 respectively.

The statistical analysis of the data was calculated as per method described by Steel and Torrie (1997) reveals that there was significant difference in colour, flavour, texture and taste. The calculated Critical Difference of all treatments is 1.3618. It was found that T_1 differs with T_4 by 2.25, T_2 differs with T_4 by 2.25 and T_3 differs with T_4 by 1.75 significantly.

Conclusion

On the basis of data after and during investigation concluded that the T_4 was found significant than others. In this treatment 2.5% pineapple juice was added to the milk for clotting. The quantity of juice was found enough to coagulate properly. Pineapple juice as a milk clotting agent can produce only soft cheese due to presence of high proteolytic Bromelain Enzyme and retained ascorbic acid in cheese was a positive result. Incorporation of pineapple juice in vegetarian cheese production also solves the problem of cheese-eaters due to religious factor as rennet is an animal derivative enzyme while Bromelain is not, as it is a plant derivative enzyme present in pineapple.

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REFERENCES

AOAC, 1970. Methods of analysis of the association of official agriculture chemist, 7^{th} edition P. box no. – 540 Benjamin Franklin station Washington 4, D.C.

AOAC, 1990. Association of Official Analytical Chemists Official Methods of Analysis, 15th Edn. Arlington, VA. pp. 840-850.

AOAC, 2012. Official Methods of Analysis of AOAC International. 19^{th} edition, volume II. Association of Official Analytical Chemists. Gaithersburg, Maryland 20877-2417, USA.

AOAC. 1960. Methods of analysis of the association of official agriculture chemist, 7^{th} edition P. box no. – 540 Benjamin Franklin station Washington 4, D.C.

Bessey D and King, G.C. 1933. The distribution of vit C in plant & animal tissues & its determination. *J. Biol Chem*, 103:687-698.

Daily Value. 2015. *www.healthyeating.org*

Daniel Simoes Couto, Lourdes Maria Correa Cabral, Virginia Martins da Matta, Rosires Deliza, Daniela de Grandi Castro Freitas, 2011. Concentration of pineapple juice by reverse osmosis: physicochemical characteristics and consumer acceptance. Food Science and Technology. Campinas), 31(4):1-9.

Davis, J.G. 1965 and 1976. *Cheese*, Vols. I & III, J & A Churchill Ltd., London.

Dr. Sc. Shurki Maxhuni, Prof. Assis, Dr. Sc. Halil Kukaj, Prof. Assis, 2015. The factors importance to economization produced cheese mozzarella from cow's milk. *International Journal of Engineering and Science,* 5(6):29-35.

FAO, 2002. Food Energy methods of Analysis and Conversion Factors. Report of a technical workshop Rome, 3-6 December.

Ferial Aziza Benyahia-Krid, Ouarda Aissaoui-Zitoun, Halima Boughellout, Faiza Adoui, Amani Harkati, Cocou Rogatein Bakou, Denis Wodo and Mohammed Nassereddine Zidoune, 2016. Fresh cheese "Peulh Type": Characterization and sensory aspects. *J Adv Dairy Res,* 4(4):1000163.

Fernandez- Salguero J, Sanjuan E. 1999. Influence of vegetable and animal rennet on proteolysis during ripening of ewe's milk cheese. *Food Chem,* 64:177-183.

Fernandez-Salguero J, Sanjuan E, Montero E. 1991. A preliminary study of the chemical composition of Guia cheese. *J. Food Compos Anal* 4:262-269.

Food Safety and Standard Authority of India, 2006. Ministry of Health and Family Welfare Government of India, New Delhi.

FSSAI Manual. 2016. Fruit and Vegetable. 5(2.3).

FSSAI, 2012. Manual of methods of analysis of Foods, Microbiological testing. Food Safety and Standard Authority of India. Ministry of Health and Family Welfare Government of India, New Delhi.

Harboe M, Broe ML, Qvist KB. 2010. In: Law BA, Tamime AY. eds. Technology of cheese making. Wiley-Blackwell, London.

Hashim MM, Mingsheng D, Iqbal MF, Xiaohong C. 2011. Ginger rhibozome as a potential source of milk coagulating cysteine protease. *Phytochem,* 72:458-464.

Ilany J. Feigenbaum and A. Netzer, 1969. Milk-Clotting Activity of Proteolytic Enzymes. *Journal of Dairy Science*, 52(6):43-46.

Jacob M, Jaros D, Rohm H. 2011. Recent advantages in milk clotting enzymes. *Int J Dairy Technol,* 64:14-33.

Lane, J.H. & L. Eynon, 1923. *J. Soc. Chem. Ind*., 42, 32 T.

Le Jaouen, J. C. 1987. The making of Farmstead Goat Cheese. *Cheesemaker's Journal*. Ashfield, MA. Pp. 15-74.

Lo Piero AR, Puglisi I, Peptrone G. 2002. Characterization of "lettucine", a serine-like protease from *Lactuca sativa*leaves, as a novel enzyme for milk clotting. *J Agric Food Chem,* 50:2439-2443.

Macedo IQ, Faro CJ, Pires EM. 1993. Specificity and kinetics of the milk-clotting enzymes from cardoon. *Cynara cardunculus L.*. toward bovine k-casein. *J Agric Food Chem,* 41:1537-1540.

Manzoor Shah, Shabir Mir, Mohd Paray. 2013. Plant protease as milk-clotting enzymes in cheese-making: A review. *Dairy Science & Technology*, 94(1):5-16.

Michalski M., Gassi J., Famelart M, Leconte N., Camier B. 2003. The size of native milk fat globules affects physico-chemical and sensory properties of Camembert cheese. *Lait* 83:131-143.

Milci, S., Z. Goncu, Z. Alpkent, and H. Yaygin, 2005. Chemical, microbiological and sensory characterization of Halloumi cheese produced from ovine, capine and bovine milk. *International Dairy Journal*, 15:625–630.

Miller, C.D. 1950. Enamel corrosive properties of fruit and fruit juice. *J. of Nutri*, 41:63-71.

Multag MA, Wilbey RA. 2006. Effect of Chymosin reduction and salt substitution on the properties of white salted cheese. *International Dairy Journal,* 16:903-909.

NCAL, 1954. National Canners Association Laboratory Manual for the canning industry. Section 21, p.13. National Canners Association, Washington, D.C.

Ramet JP. 1997. Technology Comparee des different types de caille. In: Eck A, Gillis JC. Le fromage, de la science a I'assurance de qualite. 3eme edition. Lavoisier Tec and Doc Pp 349-415.

Rangana, 1977. A Manual of analysis of fruit and vegetable products. II reprint, Tata McGraw-Hill Publishing Company Limited, 12/4 Asaf Ali Road, New Delhi-110002.

Rangana, S. 1986. Hand book of analysis and quality control for fruit and vegetable products. 2nd edn. Tata McGraw Hill Publishing Co. Ltd., New Delhi.

Raphaelides S.N., Antoniou K.D., Vasilliadou S., Georgaki C. & Gravanis A. 2006. Ripening effect on the rheological behavior of Halloumi cheese. *J. Food Engineering*, 76:321-326.

Roa I, Lopez MB, Mendiola FJ. 1999. Residual clotting activity and ripening properties of vegetable rennet from *Cynara cardunculus* in La Serena cheese. *Food Res Int,* 32:413-419.

Roseiro LB, Barbosa M, Ames JM, Wilbey RA. 2003. Cheese making with vegetable coagulants–the use of *cynara L*. For the production of ovine cheeses. *Int J. Dairy Technol,* 56:76-85.

Salwa Putra, Bagus Priyo Purwanto, Evy Damayanthi, Yopi, Heni Rizqiati, 2015. Characteristics of Mozzarella Cheese from Water Buffalo Milk in North Sumara. Applied Research Journal, 1(4):216-221.

Sanjuan E, Millan R, Saavedra P, Carmona MA, Gomez R, Fernandez-Salguero J. 2002. Influence of animal and vegetable rennet on the physicochemical characteristics of Los Pedroches cheese during ripening. Food Chem, 78:281-289.

Schroeder CL, Bodyfelt FW, Wyatt CJ, McDaniel MR. 1988. Reduction of sodium chloride in Cheddar Cheese: Effect on Sensory, Microbiological and Chemical Properties. *Journal of Dairy Science, 71:2010-2020.*

Stanford Research System, *www.thinkSRS.com*

Steel R.G.D., J.H. Torrie, 1997. Principles and Procedures of statistics. A Biometrical approach.. 3rd Edn. McGraw Hill book Co. NY. USA.

Sukumar De, 1991. *Outline of Dairy Technology*, 33 Impression Oxford University- Press, YMCA Library Building 1, Jai Singh Road, New Delhi– 110001 India.

USDA, United States Department of Agriculture. 2012. Specifications for Mozzarella Cheese, Lite Mozzarella. The U.S. Department of Agriculture. September 24. United State. US): Agriculture Marketing Science.

APPENDIX

Appendix-1. Specimen Evaluation Test
Composite scoring Test

Name Date
Product - cheese

Characteristics	Treatments					
	T_1	T_2	T_3	T_4	T_5	Total
Colour						
flavour						
texture						
taste						
Total						

Comments- Signature

Appendix-2. Completely Randomized Design of Data on sensory evaluation of cheese sample

Parameters	Values
G	461
CF	10626.05
RSS	10653
TSS	26.95
SSE	12.25
SST	14.7
MST	3.675
MSE	0.8166
F_{CAL}	4.500
F_{TAB}	3.06
CD	1.3618

Appendix 3. Completely Randomized Design of Data on chemical characteristics of cheese sample

Parameters	Values
G	2409.18
CF	105529.9685
RSS	52940.7005
TSS	427410.732
SSE	294529.574
SST	132881.158
MST	33220.2895
MSE	5890.59148
F_{CAL}	5.6395
F_{TAB}	2.56
CD	72.9241

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