Rennet and Alternative: A Review

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Abstract

Nowadays, the cheese market is growing larger as it shows a rising demand curve. Rennin is the most widely used proteolytic enzyme for cheese production which is extracted from the gastric mucous membrane of bovines. To meet rising demand with required quality attributes, the industry needs an efficient source of milk curdling enzyme which meets quality parameters, standards of industries as well as consumer satisfaction. There is a number of animal rennet substitutes available like vegetable rennet, microbial rennet, etc. but, these substitutes having some disadvantages mainly high proteolytic activity which leads to the bitter taste of the final product. The best alternative of animal rennet is Recombinant chymosin which is trending now. Recombinant chymosin is prepared with the help of genetic modification of microorganisms which ensures total control on production as well as quality attributes. E. coli used to purify recombinant chymosin, which was used to ensure suitability to manufacture Cheddar cheese and Colby cheese which shows non-significant differences between the yields of Colby cheese manufactured using calf rennet and recombinant chymosin. Recombinant chymosin which is produced by Kluyveromyceslactis and calf rennet which having 70% chymosin exhibited analogous thromboelastographic characteristics, and coagulate milk more quickly than calf rennet with 55% chymosin.

Keywords: - *Cheese, Chymosin, Proteolytic Enzyme, Genetic Modification;.*

INTRODUCTION

Cheese production using rennet is one of the ancient practices. The production of countless different types of cheese use protein hydrolysing enzymes, of which rennin is the most widely used. Rennin is extracted from the gastric mucous membrane of bovines. Milk coagulation is irreversible and has two phases. In the first enzymatic phase, the micellar colloid that protects the casein (K-casein) separates the glycomacropeptides (part of K-casein, integrated by non-protein nitrogen), in that way making the protective effect reduce or disappear. The second coagulation stage involves the formation of salt ion bridges at favourable temperatures between the calcium-sensitive casein micelles, thereby quickly

producing coagulation. Rennet, used in the cheese-making process, can be originated from a vegetable sources, animal sources, or microbial sources.

A. Animal Rennet

Animal origin proteolytic enzyme can beacquired from the stomach of a suckling mammal, usually a calf of a lamb. The traditional rennet used to make cheese is the animal rennet. Most European cheese use animal rennet for coagulating milk.

B. Vegetable Rennet

Vegetable origin rennet is obtained from the plant or parts of the plant. The proteolytic enzymes are extracted from the plants and modified into a form similar to that of animal rennet. This type of rennet is acceptable for a lactovegetarian individual.

C. Microbial Rennet

Microbial based rennetis a common enzyme made from microorganisms through fermentation. Commonly fungi and bacteria are used for producing microbial rennet. This type of rennet is also suitable for a lacto-vegetarian individual. The increasing global cheese production (4% a year) in the last two decades, in association with a decline in bovine rennet production, has elevated the price of the ethnicanimal based rennet. This situation has encouraged the search for milk clotting enzymes from alternative sources. Microorganisms such as *Parasitica, Mucorpussilus, Endothia, Bacillus cereus, MucormieheiCryptococcus albidus,*are identified to produce rennet, such as proteinases, which can substitute calf rennet.

Among these microorganisms, it was discovered that the *Mucormiehei* and *Mucorpussilus*yield a coagulating enzyme with characteristics that are very comparable to commercial rennet, producing great coagulation and production rates. Commercial provisions based on the filamentous fungus *Mucormiehei* are presently replacing animal rennin in the production of more popular cheeses and by now represent about 33% of the production process. Different cheese made using microbial rennet is mentioned in Table I.

TABLE I: DIFFERENT CHEESES MADE USING MICROBIAL RENNET

Type of cheese	Type of cheese
Brie	Crumbled Goat Cheese
Shredded Parmesan	Reduced or low Fat Sharp Celtic Cheddar
Light String Cheese	Organic Light Whipped Cream Cheese
Sliced Colby Jack	Sliced Swiss

DESIRABLE CHARACTERISTICS OF MICROBIAL

I. RENNET

Some of the desirable characteristics of rennet substitutes (which replace calf-chymosin) [1] are specified below:

a) It should cause sweet curdling at a temperature used for cheese milk (i.e. 30^0C) accompanied by very low proteolysis to cause sufficiently firm gel with elasticity to facilitate manipulation.
b) It should not be inhibitory to starter cultures especially acid development should be normal.
c) It should form a coagulum which is physically and chemically similar to animal rennet.
d) Yield of cheese should be the same or superior.
e) Loss of fat in whey should be minimum.
f) It should not impart off flavour in the product.
g) It should be cheaper than animal rennet.
h) It should be available easily.
i) It should have higher stability during storage.
j) It should be non-toxic and non- pathogenic.

k) It should show similar residual enzyme behaviour as calf-rennet, and Properties such as the influence of pH, Ca^{2+}ions, presence of other enzymes, temperature, etc. should be as calf rennet.

l) It should not be having undesirable enzymes.

A. Bacteria source of rennet

Among the genera *Alkaligenes, Streptococcus, Corynebacterium, Streptomyces, Escherichia, Thermoactinomyces, Lactobacillus, Seratia, Bacillus (Bacillus substilis, Bacillus polymyxa, Bacillus mesemtrucus)* and *Pseudomonas* have been investigated. The preparations derived from below sources have been tested in cheese making :

- Milkrozyme (*Bacillus substilis*)
- Milcozyme (*Bacillus polymyxa*)

But it is unsuitable due to very high proteolytic activity that in turn causes bitterness and body defects in hard and semi hard varieties.

B. Fungi source of rennet

The extracellular enzymes of various species of fungi belonging to genera, *Absidia, Ascochyta, Ascomycetes, Aspergillus, Basiliobolus, Bisidiomycetes, Chaetomium, Colletotrichum, Conidiobolus, Endothia, Entomophthora, Panus, Rhizomucor, Rhizopus*, etc. have been studied as a rennet substitute. However, extra cellular enzymes from *Rhizomucorpusillus, Rhizomucormiehei and Endothiaparasitica*(Renamed as *Cryphonectriaparasitica*) only have proved most suitable rennet substitutes. The enzymes derived from these moulds belong to the group of aspartic proteases (preciously called acid proteases). In general, microbial proteases used in cheese making consist of about 325-360 amino acid residues, and depending on their amino acid composition, the molecular weight add up to between 33000 and 38000Da. The commercial preparation available from *Rizomucorpusillus, Rizomucormiehei* and *Endothiaparasitica* are depicted in TableII.

TABLE II: COMMERCIAL FUNGI RENNET

Source of Enzyme (Microorganism)	Trade Name
Rhizomucormiehei	Hannilase, Rennilase, Fromase, Miki, Marzyme
Rhizomucorpusillus	Noury, Meito, Emporase, Novadel
Endothiaparasitica /Cryphonecteriaparasitica	Suparen, Surecurd

The important cheese making characteristics of fungal rennet derived from *Rizomucorpusillus* (MP), *Rizomucormiehei* (MM), *Endothiaparacitica* (EP), are collated in Table III, Table IV and Table V [2]. It is evident that the MM is most sensitive to change in temperature, where MP rennet is most sensitive to change in the pH and calcium concentration. EP rennet on the other hand is least affected by these three variables. As regards to the photolytic activity of the fungal rennet, EP rennet have greater photolytic activity than calf rennet (CF) as well as MP and MM rennet, the latter rennet being similar to calf rennet. The residual rennet activity of MM and MP rennet in cheese curd is independent of the pH; Whereas it is not so in case of calf rennet.

TableIII Sensitivity of Fungal Rennet to Changes in P_H, Temperature and Ca^{+2} In Milk [2]

Order of Sensitivity	Temperature	pH	Ca^{+2}
1. (Most Sensitive)	MM	MP	MP
2. +++	MP	CR, MM	MM
3. ++	CR	-	CR
4. (Least Sensitive)	EP	EP	EP

Where,

MM: Rhizomucormiehei

EP: Endothiaparasitica

MP: Rhizomucorpusillus

CR: Calf Rennet

Table IV: Proteolytic Activity of Different Fungal Rennet [2]

Enzyme	Casein		
	α_s-casein	***B-casein***	***K-casein***
Endothiaparasitica	+++	++	+
Mucorpusillus	++	++	++
Mucormiehei	++	+	++
Calf rennet	++	+	++

***The number of (+) sign indicates extent of sensitivity.**

TABLE V: Residual Coagulant Activity (%) In The Coagulum at Different pH [2]

Enzyme	pH			
	5.2	***6.0***	***6.4***	***6.6***
Calf rennet	83	70	47	30
Mucormiehei	19	19	18	19
Mucorpusillus	11	12	13	14

NATURAL RENNET V/S MICROBIAL RENNET

No significant differences in yield, recovery of milk constituents and composition of fresh cheese.Faster protein breakdown and release of TVFA. Consequently, rapid body and flavour development at the temperature of curing studied (seeTable VI, [2])

Table VI: Influence of the Various Rennet on Cheddar Cheese from Buffalo Milk [2]

Particular	Rennet Type					
	Calf (C)	***Meito (M)***	***Rennilase (R)***	***CM***	***CR***	***MR***
	Composition of whey					
Fat (%)	0.25	0.27	0.27	0.27	0.27	0.27
Protein (%)	0.99	1.01	1.01	1.02	1.01	1.02
	Composition of cheese					
Moisture (%)	36.92	37.12	36.78	37.20	37.05	37.35
Fat (%)	32.28	32.58	32.40	32.43	32.30	32.40
Protein (%)	24.50	24.78	24.89	24.75	25.01	25.01
Salt (%)	1.52	1.48	1.53	1.56	1.53	1.54
pH (%)	5.37	5.33	5.31	5.30	5.33	5.36
	Yield and Recovery of cheese					
Yield (%)	12.86	12.59	12.50	12.59	12.50	12.41
Fat recovery (%)	92.32	91.15	90.00	90.69	89.75	89.40
Protein recovery (%)	78.74	77.97	77.75	77.83	78.09	77.52
	Ripening changes at 180 days (5-6^{0}C)					
Maturity Index (%)	14.43	21.94	25.30	21.33	24.45	24.65
TVFA *	12.08	14.58	15.97	13.60	13.23	17.35

A. Properties of Commercial Microbial Rennet

i) Ratio of Milk coagulating to proteolytic activity:

Most of the rennet substitutes have very low ratios compared to calf rennet. However, the properties are influenced by temperature, buffering capacity, pH, type of milk, calcium content, acidity development, etc. As the methods used for measuring milk coagulating and proteolytic activity vary considerably of various reports is not possible.

ii) Influence of pH:

Animal rennet which contained chymosin and pepsin (coming as an impurity or mixed) is inactive at pH 7.0 while coagulating activity though disturbed in microbial rennet substitutes does show proteolytic activity. However, pH 7.0 or above is possible only with mastitic milk or abnormal milk. So, not of commercial value. The influence of pH between 5.0 to 6.5 is of commercial significance.

iii) Influence of Temperature:

The coagulating ability of chymosin seems to increase with a temperature higher than 15^{0}C, below which though it cleaves the k-casein, does not show coagulum or gel formation. However, above 30^{0}C there is a tremendous influence in chymosins and this rate of increase in coagulating activity is known to vary greatly among various rennet substitutes. A temperature of 30^{0}C to 40^{0}C used in gel formation has a great commercial significance in the quantity of enzyme needed and behaviour of coagulum. Chymosin seems to be

inactivated at more than 42^0C. So, coagulating activity though considerably higher, the enzyme also inactivated much faster. Microbial rennets seem to be more resistant to temperature fluctuations. However, the increasing rate in milk coagulating activity even upto 60^0C is observed in microbial rennet. However, except for identification or inactivation of residual enzymes, it may not have much commercial value in cheese making.

iv) Influence of Ca^{+2}ions:

Increasing the Ca^{+2} ions in milk increases the coagulating ability without influencing proteolytic activity. In this regard a milk coagulant of microbial origin seems to show varied influence, in terms of increase in coagulating ability that is observed.

v) Presence of other enzymes:

Accept for the presence of pepsin, the calf-rennet seems to possess no other enzymes. While in the case of microbial rennet it's the reverse situation. Un-purified commercial microbial preparation shows considerable variation in this content. Enzymes like other proteases, beta-galactosidase, lipase may present which may spoil the cheese if not selected properly.

vi) Influence on curd tension:

Microbial rennet preparation forms softer curd than animal rennet. Addition of Ca^{+2} though increases curd strength to use commercially slightly more holding period than animal rennet is necessary for achieving the required firmness at the time of cutting, which otherwise can affect yield due to formation of fines (small bits of curd). The curd tension seems to be 1.5 to 2 times more in animal rennet than that of microbial rennet.

vii) Quality of cheese and quantity of enzyme:

The quality of Cheese made by using Chymosin is unquestionably superior then the same made by using the microbial rennet. However, due to the demand of cheese for the vegetarian people, the requirement of microbial rennet has been increased in a larger proportion.

If not manipulated the quantity of enzyme needed to bring about the coagulation of milk with microbial rennet is higher than animal rennet on basis of protein.

viii) Influence on starter culture:

Due to their higher proteolytic activity and higher release of smaller fractions of protein degradation products, the microbial rennet seems to be more stimulatory to starter growth and acid production. The degradation of bitter peptide formed occurs at a slower rate due to the high proteolytic activity.

ix) Influence during cheese making and ripening:

Softer curd seems to be formed by microbial rennet during coagulum formation faster or acid development and faster contraction of curd and higher proteolytic activity the green cheese formed by these enzymes seem to be crumblier and soft and show bitterness in the early or later stages of ripening. However, the ripening changes seem to be faster and the flavour development more pronounced.

x) Yield of cheese:

Microbial rennet due to higher loss of fat and protein and lesser moisture holding capacities show a lesser yield of cheese than animal rennet.

From the above points, it is evident that microbial rennets are available commercially and they are being used in cheese manufacture to fulfil the

requirement of milk coagulants as calf rennet is in short supply. In our country, cheese manufacturers use only rennet substitutes as there is a ban on the use of calf rennet. Mostly on the industrial level, cheese manufacturers use enzymes either from *Mucorpusillus*or *Mucormiehei*. Although, Maxiren (Chymosin from microbial origin) is available as it is immunologically similar to calf chymosin and is a bioengineered gene of the calf that is responsible for production [1, 2].

RECOMBINANT CHYMOSIN

The ascendingtotal world production of cheese, added with a decrease in the number of slaughtered calves, has stimulated anexploration for substitute sources of chymosin.

Recombinant chymosinembodies one of the successful applications of genetic engineering i.e., recombinant DNA technology in the dairy or food industry. Calf rennet (consists of ~ 90% chymosin), is generally used in cheese making for the coagulation of milk. Many animal, plant and microbial sources have been exploited as likelythe alternatives to calf rennet.

The clotting properties of the enzymatic coagulant from genetically modified sources varies in terms of their physicochemical factors.

The cheese making industry has continuously sought out novel and stable enzyme sources and recombinant chymosin has been found to be an effective alternative since it possesses multiple advantages than plant and microbial based milk-clotting enzymes.

A. Why recombinant rennet is needed?

- Worldwide Shortage of calf rennet for several decades.
- Fluctuation in supply the of rennet.
- Rennet Substitutes- Bovine pepsin, porcine pepsin and plant coagulants as they are not industrially feasible due to their very extensive protein hydrolytic nature and other inherent drawbacks.

A possible way out ofthis problem is the use of chymosin produced by genetically engineered microorganisms such as *Apergillusniger* and *Kluyveromyceslactis*. This ensures a constant quality of the coagulant and the amounts produced can be carefully controlled.

The recombinant enzyme has been proved to be identical to the main component of calf rennet, but it is difficult to conclude that the cheese will also be identical, because of the complex composition of the products [3].

B. Recombinant Chymosin

Successful enzyme preparations included mixtures of calf rennet with both bovine and porcine pepsin and the fungal enzymes derived from *Mucormiehei*, *Mucorpusilus*, and *Endothiaparasitica*. Several researchers have suggested that many of these proteolytic enzymes caused flavour, texture, and yield changes in certain types of cheese that are different than those produced by calf rennet.

Quality for calf chymosin was one of the primary attributes for mammalian enzymes that werecommunicated in microorganisms by cloning techniques. A wide range of research facilities have cloned the quality enzymes for calf prochymosin in *Escherichia coli*, and examined the structure of the quality attribute of the recombinant chymosin.

The communicated proenzyme in *E. coli*, is available primarily as insoluble consideration

bodies, included diminished prochymosin just as atoms that are interlinked by disulphide bonds. After breaking of the cells, inclusion bodies are collected by centrifugation.

The individual research facilities have detailed a few contrasts in the methodology for renaturation of prochymosin from the incorporation bodies, however, all have followed a similar method. The enzymatic properties of recombinant *E. coli*chymosin are vague from those of local calf chymosin. The proteins were indistinguishable when seen by immunodiffusion in gels, yet a slight contrast was seen by compound connected immunosorbent examine (ELISA) [4]. Table VII shows the recombinant chymosin arrangements at/or moving toward lawful and business acknowledgment.

The prochymosin gene is also derived from *Saccharomyces cerevisiae*; production levels have been reported to be 0.5 to 2.0% of total yeast protein. The gene of *R. Miehei* protease is expressed in *A. oryzae*. Prochymosin is expressed in *Trichodermareesei, Kluyveromyceslactis, A. niger,* and *A. nidulans*. In most cases, the reported yields of the model systems were about 10-40 mg of the enzyme/litre of medium culture. However, 3.3 g of the enzyme was obtained per litre.

The yeast, *Kluyveromyceslactis*, has recently been used as a secret storage agent for chymosin synthesis, which has led to the formation of a large chymosin production process. If produced at the industrial level, the yield will probably be at the final level.

Chymosins are close to or have official marketing and acceptance, and are generally considered safe (GRAS). Most companies reproduce cow's rennet in a variety of pathogens, however, in India; a large source of buffalo milk, showing a different texture than that of a cow.

The rennet from the buffalo's source has a natural consistency to shut down the buffalo's milk. Therefore, the National Dairy Research Institute, Karnal, has taken the lead in compiling buffalo genetic chymosin. Buffalo chymosincDNA is genetically engineered in *E.coli* and the N-terminal component of purified buffalo chymosin shows that it is highly compatible with bovine chymosin.

Commercially preparations are available in liquid, powder or tablet forms. There has been widespread acceptance of recombinant chymosin as milk coagulating enzyme for cheese making in USA and UK. Currently in USA, recombinant calf chymosin is the principal milk coagulant for cheese production (see Table VII; Figure I)**.**

Table VII: Recombinant Chymosin Preparations At/or Approaching Legal and Commercial Acceptance

DNA Source	Producing microorganisms	Manufacturing Company
Calf abomasum	*Kluyveromyceslactis*	Gist Brocades (Maxiren)
Calf abomasum	*Apergillusniger*	Chr.Hansen (Chymogen)
Synthetic	*Escherichia coli*	Pfizer (Chymax)

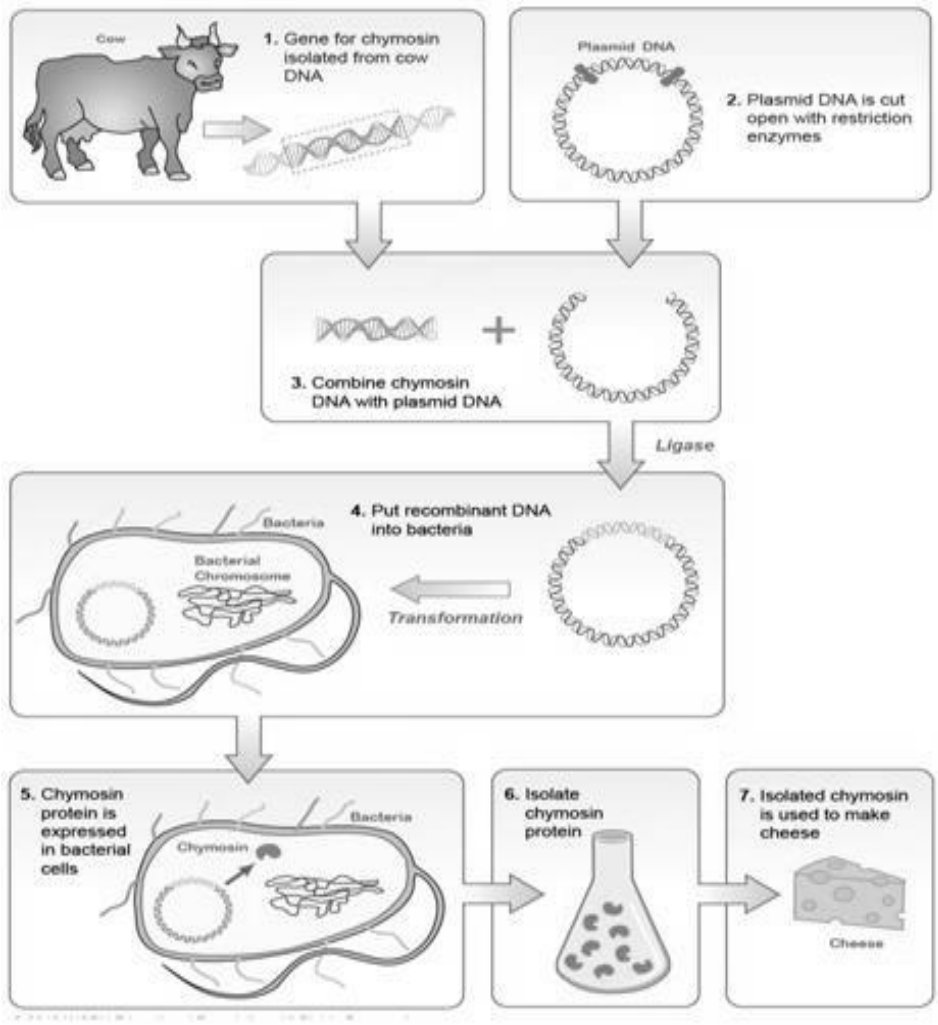

Figure I: Cheese Production Using Recombinant Chymosin

C. Difference in microbial and calf chymosin

Despite efforts to reduce the difference between calf and microbialchymosin, diversity still exists, making the latter the preferred enzyme for cheese making. Some of these differences include:

(I) Proteolytic activity and milk density the function is slightly higher in microbial rennets, leading to greater hydrolysis of cheese proteins during ripening and leading to a more lean body and inclination. (II) Microbial rennets are more thermostable, cannot be inactivated at normal pasteurization temperature, and therefore present problems in the processing of cheese whey, (III) Change in milk-clotting activity from calf to microbial rennet will require adjustment of the process parameters such as temperature, pH, calcium concentration, etc., [4].

D. Application of recombinant rennet in cheese

A number of cheese making investigations have been completed with recombinant chymosin and since the vast majority of the rennet (> 90%) added to cheese milk is lost in the whey, immobilization would significantly expand its reactant life. A few rennets have been immobilized, however, their effectiveness as milk coagulants has been addressed. Thus, there is a genuinely broad help for the view that immobilized catalysts can't coagulate milk appropriately attributable to detachment of the Phe–Met peptide obligation of k-casein, and that

the clear coagulating movement of immobilized rennets is because of the filtering of the compound from the help.

Various sorts of traditional cheeses have been effectively made by utilizing recombinant rennet on an exploratory or pilot scale. No significant changes have been distinguished between cheeses made with recombinant chymosins or normal catalysts, with respect to cheese yield, smell, taste, aging and surface. The recombinant chymosins are indistinguishable with calf rennet as indicated by the report on biochemical and hereditary confirmations.

i) Cheddar cheese and Colby cheese: Recombinant chymosin extracted from *E. coli* was inspected for its appropriateness to produce cheddar and Colby cheese. No major differences were noted between the yields of Colby cheese produced with recombinant chymosin and calf rennet. Beginning Cheddar cheeses made with recombinant chymosin were much bitter, cheese found with more amount of unnecessary proteases. Reducing the strength of purified prochymosin preparations increased total enzymatic activity upto 2.7 times. Clearly, the purified recombinant arrangements contained some oligomers that got dynamic subsequent to being joined into the cheese mass, making unpleasant flavors create during maturing. Diluted enzymatic compound arrangements were utilized effectively in the production of cheese [5].

ii) Manchego cheese: Ewes' milk coagulation was majorlyaffected by the different type of rennet;genetically modifiedchymosin produced by *Kluyveromyceslactis* was compared with two commercially available calf rennets with knownchymosin contents of 55 and 70%. TheCalf rennet and recombinant chymosin with 70% chymosindisplayed similar thrombel as to graphic at tributes, and coagulate the milk very speedily than calf rennet with 55% chymosin. The type and source of rennet had no effect on Manchego cheese dry matter, recovery of dry matter or pH in the final cheese. Nitrogen soluble at pH 4·6 was the only N-fraction, which is affected by the type/source of milk clotting enzyme (rennet), with slightly elevatedthe proportion for cheese made with recombinant chymosin or with calf rennet with 70% chymosinas that of made with rennet containing 55% chymosin. Very little differences in sensory or rheological characteristics between Manchego cheese made with recombinant chymosin and that made with the calf rennets were detected[6].

iii) The use of recombinant chymosin in making domiati cheese: Domiati cheese was made from buffalo milk using recombinant chymosin (RC) or calf rennet (CR). The attained results revealed that the differences in clotting time, curd tension and curd syneresis were not significant whether RC or CR was used. This was true with respect to the yield of fresh cheese and the loss in cheese weight after pickling. Acidity and pH values significantly changed during pickling, whereas the coagulants had no significant effects.

The values of non-protein nitrogen (NPN)/total nitrogen (TN), soluble nitrogen (SN)/TN and total volatile fatty acids (TVFA) were nearly similar in fresh cheese; however, values increased during pickling for all cheese samples. The rate of increase was not affected by the coagulant used. The pickled cheese made with RC had lower total free amino acids and amino acid pattern than CR-cheese. The organoleptic properties of the resultant

cheese were identical and the coagulant used seemed to have no effect with this respect [7].

*vi) Cheddar cheese using Recombinant camel chymosin*Cheddar cheese was manufactured using fermentation-producedcalf or camel chymosin as a coagulating agent with levels chosen to producea similar curd strength in the same time. There was no significant variation in the nutrient/composition and pH of the 60 days aged cheeses or the percent yield of the cheeses manufactured with either clotting agent although there was apresence of comparable high moisturecontent of the cheeses made using cameluschymosin.

The final sensory analysis represents that the CAF-C cheeses were categorised by higher concentrations of brothy and sulphur flavours and bitter taste, had more breakdown of texture, were more cohesive and adhesive, increased smoothness and mouth coatingas characterised with CAM-C. These findingsconclude that there was less initial proteolysis in CAM-C during ripening as compared with CAF-C due to the fact that, although added at a level to give same curd strength at the same time, the proportion of cameluschymosin used to make cheeses was low than that of calf chymosin and also that the cameluschymosinhas only 20% of the general proteolytic activity of calf chymosin[8].

The conclusion of this investigationgives an idea thatcameluschymosin can be imparted successfully to manufacture Cheddar cheese with less levels of proteolysis but with palatable flavour, which may be of significance in cases where there is a propensity to bitterness.

E. Recombinant Lamb Chymosin as an Alternative Coagulating Enzyme in Cheese Production

Recombinant lamb chymosin (RLC) was prepared andanalysed for its possible use in cheese making. The proteolytic activity and milk clotting activity of RLC were assessed in comparison with commercial cow rennet (CR),recombinant calf chymosin (RCC), and microbial coagulant (MC). RCC, MC and RLC showed very comparable responses to pH, with a sharp increase in time of the coagulation at pH 6.6 to 6.8 and decline in curd firmness at the pH 6.5 to 6.6. In the case of CR, there was ascend in the time of coagulation and decrease in the firmness of curd, at pH 6.4 to 6.5 and 6.6 to 6.8 respectively. Optimum clotting activity can be obtained for RLC at 40°C, for MC at 60°C and for both CR and RCC at 45°C. The temperature instability of RLC at temperatures more than 45°C could constitute a positive effect in making hard type cheese varieties. The addition of $CaCl_2$ to milk prior to cheese making resulted in enhanced clotting activity of all coagulating agent, most effective for CR. The proteolytic activity of RLC was significantly lower as compared that of CR but not significantly varied from the activity of RCC. The lesser proteolytic activity in the cheese manufactured with RLC did not have any undesirable effect on organoleptic properties. The inclusive quality of the cheese manufactured with RLC was very little comparable to that of the cheese made with RCC, and both cheeses were better scored than the cheese made with CR [9].

CONCLUSION

If we use microbial rennet then there are many disadvantages than advantages. The mainly high proteolytic activity givesa bitter taste which

doesnot accept by the consumer. Latest improvements in hereditary designing innovation have given recombinant chymosin as an option in contrast to calf rennet. The utilization of recombinant chymosin in cheddar produce instead of more conventional calf rennet seems to give the cheese business an elevated level of predictable quality cheese. Regardless of the promising reports in the exchange and logical writing during the previous quite a long while, cloned chymosin still can't seem to show up in the commercial center.

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