Characteristics of Cheeses Manufactured Using Pepsin from Adult Cattle Abomasa

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Abstract

The cheese making quality of pepsin extracted from adult cattle abomasa using dilute HCl (HP) and commercial vinegar (VP) was assessed using three types of cheeses namely Alpine, Tilister and Pastafilata (Pasta). The level of each extract was substituted with commercial calf rennet (CR) at 0, 25, 50, 75, 100%. Renneting time (RT) for cheese milk, butterfat (%BF) and total nitrogen (%TN) losses in whey were assessed. Chemical composition and microbial quality of cheese samples obtained at different storage time intervals and their sensory quality at maturity were determined. RT and TN losses in Alpine cheeses increased with increasing levels of pepsin from 41min and 0.49% in 25% pepsin to 58 min and 0.55% in 100% pepsin (HP) respectively. Chemical composition of all cheese types with different levels of HP and VP were significantly different (P<.01) and were not related to the levels of pepsin. The pH in Pasta and Alpine and % TN in Tilster cheese were not significantly different for the different levels of HP and VP. Microbial counts in Alpine cheeses were not significantly different(P>0.05). Coliform and yeast/mould counts in Tilster and Pasta cheeses were not significantly different while the Standard Plate Counts (SPC) in Tilster and Pasta cheeses were significantly different. Sensory quality was not significantly different for smell and taste. However, bitterness in Pasta and appearance in Alpine and Pasta were significantly different (P<0.05) between enzyme combinations. Bitterness and taste in Alpine and bitterness in Tilister cheeses were significant different (P<.001) between levels of HP and VP. Overall quality assessment of all cheeses did not show significant differences (P>0.05) between levels of pepsin and types of extraction media for pepsin. HCl and vinegar extracted pepsin was an appropriate rennet substitute for the small scale cheese processors.

Keywords: pepsin, rennet, cheese

Cheese making and milk powder manufacturing are some of the most efficient ways used in many countries to preserve milk. In cheese making, 10 – 12 L of fresh milk are used to produce one kg of cheese which can be stored up to one year and is easy to transport to other parts of the country. Cheese processing has been successfully introduced in Kagera (De Wolf, 1990), Hai district (the Losaa Women Group) (Ulicky, 1989) and at Mulala village (Arumeru) (Kurwijila, 1990). Some commercial dairy farmers in Tanga, Njombe, Mbeya, Iringa, Arusha and Kilimanjaro process some fresh milk into cheese to enhance their income (Kurwijila, 1990). A major problem facing cheese making enterprises in different regions of Tanzania is lack of locally produced rennet, a coagulant necessary to produce any type of ripening cheese. Most producers rely on donations from abroad which is not a very sustainable way of developing a dairy industry. A good example was that facing the Losaa Women Group in Hai district who were

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stranded for lack of rennent after the termination of FAO support at the end of 1990 (Kurwijila, 1990). The small size of the cheese production industry in Tanzania makes the importation of even small quantities of rennet commercially difficult to justify and is rather uneconomical.

Calf rennet is usually preferred by cheese manufacturers to other milk coagulants because of its high chymosin content which results in more specific milk coagulating activity (Andren and Reedtz, 1990). Rennet is usually extracted from the 4th stomach of the young calf of less than 4 weeks old. However, there is a wide shortage of calf rennet due to increases in cheese production and a decrease in the number of veal calves slaughtered (Barbano and Rasmussen, 1992). Male calves are highly valued by farmers in Tanzania and hence it would not be practical to depend on calves for chymosin. The shortage of calves even in developed countries has led to use of rennet substitutes such as microbial proteinases, porcine pepsin, bovine pepsin and recently genetically recombined microbial rennet (Farmakalids, 1995; Dajnowiec *et al.* 1997) but they are more proteolytic than calf rennet. Rennet substitutes from molecular cloned chymosin, and extraction of pepsin from older cattle are the main approaches being adopted in order to overcome the shortage of calf rennet (Visser, 1993). Production of microbial coagulants requires a very high technical know how and requires sophisticated equipment and initial costs are extremely high (Webb *et al.* 1974). Porcine pepsin from pig cannot be accepted in some parts of Tanzania due to religious considerations especially the Seventh Adventists Church and Muslims. The only alternative is to use bovine pepsin which has been used successfully in other parts of Davide *et al.* 1982). Chri Hansen's Laboratory produce a milk coagulant containing 50% bovine pepsin, 30% porcine pepsin and 20% calf rennet under the trade name B-P TM (Chri. Hansen laboratory Inc, 1990). Bovine pepsin is extracted from the world (Green, 1972; adult cattle abomasa. In Tanzania, adult cattle are slaughtered daily in thousands, this could provide readily and abundant supply of pepsin.

Studies on extraction of bovine pepsin from adult cattle abomasa as a rennet substitute for small scale cheese processors in Tanzania has successfully been done at Sokoine University of Agriculture, using dilute HCl (HP) and Vinegar (VP) (Ryoba, 1999). However, no studies have been done on the use of pepsin extracted using HCl and vinegar for cheese making. The aim of this study was to investigate the suitability of HCl and vinegar extracted pepsin for Alpine, Tilisiter and Paster cheeses manufacture when used alone or mixed with commercial rennet at different levels. Cheese characteristics were compared to determine the best enzyme combination for each type of cheese.

Materials and Methods

Materials

Preparation of different combinations of pepsin/rennet for cheese makingPepsin was extracted by using either HCl (HP) or vinegar (VP) according to the method described by Ryoba (1999). HP and VP extracts as well as calf rennet (CR) were analysed for their milk clotting activities according to International Dairy Federation (IDF) Standard 110 (1987). Extracts HP and VP were then mixed with CR at different ratios and amounts of enzymes were measured according to their pepsin units (PU) (Table 1). The 100 CR was used as the rennet control. The coding given in the first column of Table 1 was used throughout the text to represent the enzyme combination and cheese.

Alpine and Pasta cheese was made as described by FAO-RDDTT (1990). Duplicate cheeses of 2 kg each were made from each combination for 9 days. Since Alpine and Tilisiter mature for 42 days while Pasta mature for 14-21 days, the main interest in maturing time was centered on 42 and 14 days for Alpine/Tilisiter and Pasta respectively. Samples were taken from Alpine and Tilisiter after 2, 21, 42 and 56 days while for Pasta samples were taken after 2, 14 and 28 days.

Table 1: Percentages and amounts of rennet and pepsin extract combinationas for cheese making

Level of HP or VP	Enzyme Type	% HP or VP	%CR	Amount sHP/VP (ml)	Amounts CR(g)
100CR	CR	0	100	0.0	1.2
25HP	HP	25	75	16.	0.9
50HP	HP	50	50	32.	0.6
75HP	HP	75	25	48	0.3
100HP	HP	100	0	64	0.0
25VP	VP	25	75	6	0.9
50VP	VP	50	50	13	0.6
75VP	VP	75	25	19	0.3
100VP	VP	100	00	25	0.0

Key: RC=rennect control; HP=pepsin extracted using dilute. HCI; VP = pepsin extracted using vinegar
Source: Ryoba (1999)
These enzyme combination s were used to coagulate 40 L of milk.

Methods

The pH, moisture, fat, salt and acid contents in cheese were determined as described by Egan *et al.* (1981). Total nitrogen was determined by the Kjeldahl method according to AOAC standard (1975).

Water soluble nitrogen (WSN) was analysed according to the method described by Ling (1963). The results of TN, BF, salt and % WSN were presented on dry matter basis. Ripening index as a measure of ripening or proteolysis was calculated by dividing water soluble nitrogen by total nitrogen (TN) and then multiplying by 100.

Total bacterial count was determined as described by IDF Standard 100B (1991), counts of coliform bacteria was determined by IDF Standard 73A (1985) and yeast and mould according to IDF Standard 94B (1990). Sensory evaluation of cheese was done by 15-20 semi-trained panellists of 25-50 years old of which 5-10 were women. They were asked to rank the coded samples for intensity of taste, smell and appearance (Table 2).

Renneting time, TN and % BF were determined for Alpine and Pasta cheeses only. RT was time taken from when rennet was put into cheese milk until cheese milk became firm enough for cutting. Total nitrogen and BF losses in whey of Alpine and Pasta cheeses were determined by Kjeldahl method according to AOAC (1975) and Egan *et al.* (1981) method respectively. A complete randomised design was used in the analysis of obtained cheese data.

Table 2:Scores for ranking the characteristics of cheeses.

Characteristics	Rank	Interpretation
Appearance/taste/smell	1	Poor
	2	Good
	3	Very Good
Bitterness	1	Very bitter
	2	Slight bitter
	3	No bitter

Source: Ryoba (1999)

Chemical composition, microbial quality was performed on the SAS/1992 (proc. GLM) package, ASA Institute INC., Cary, NC, USA. The enzyme combinations and age of cheese were the main effects. Sensory evaluation was analysed by one way analysis of variance for comparing the means using Bonferroni test.

Overall cheese quality assessment for chemical composition, microbial quality and sensory evaluation for the cheeses, were done by comparing average grades of cheese characteristics with their respective controls. Cheese grades that fell within the acceptable range of the controls were arbitrary assigned 2 points while those that fell out of the range were assigned 1 point. The points were added and analysed statistically by Bonifferoni test to give differences between pepsin levels suitable for each type of cheese.

Results and Discussion

Renneting times (RT) of Alpine cheese milk were within the acceptable range of 40 –60 minutes except for Pasta cheese milk. Renneting times were higher in cheeses with high pepsin levels although the RT was not directly related to level of pepsin. Similar findings by Andren and Reedtz, (1990) indicated that chymosin had shorter gelling times than pepsin. TN loss in whey of 0.49 to 0.66% from cheese made with different pepsin levels (Table 3) was within the level reported by Bank *et al.* (1993). The TN loss in Alpine whey was directly related to the level of pepsin showing that pepsin was more proteolytic than the VP combinations.

Higher losses of TN in Pasta whey agrees with results of Hill (1989). Butterfat losses in whey for Alpine 100VP, Alpine 25HP, Pasta 100VP, 25VP were quite high (Table 3).

al.(1992). Under practical conditions, establishment of correct fat content in cheese and borer samples cause specific problems due to the inhomogenousity of cheese.

Table 3: Renneting times of cheese milk (min), total nitrogen (%) and butterfat (%) in whey for the production of alpine, and pasta.

Name of Cheeses	Vari-able	Rennet/ pepsin combinations									SED	P Value
		100CR	100HP	25HP	50HP	75HP	100VP	25VP	50VP	75VP		
Alpine	RT	40^{b}	58^{a}	41^{b}	43^{b}	45^{b}	55^{a}	43^{b}	45^{b}	60^{a}	1	*
Pasta	RT	45	45	46	50	45	45	44	45	50	1	NS
Alpine	TN	0.52^{b}	0.55^{b}	0.49^{b}	0.50^{b}	0.52^{b}	0.66^{a}	0.55^{b}	0.62^{a}	0.64^{a}	0.00	*
Pasta	TN	0.48^{b}	0.56^{c}	0.59^{c}	0.59^{c}	0.48^{b}	0.92^{a}	0.69^{c}	0.54^{c}	0.66^{c}	0.01	**

Key: RT = Renneting time, BF=butterfat, TN= Total nitrogen: NS- no significant difference; *Significant difference; **highly significant difference

Within the row, means followed by the same superscript do not differ signifacanlty at P>0.05 according to DMRT

Barbano *et al.* (1992) reported that the use of bovine pepsin was conducive to more BF loses in whey than other coagulants

Results of chemical composition of Alpine, Tilisiter and Pasta cheeses shown in Table 4, 5, 6, varied and were significantly different but were not directly related to the levels of pepsin except BF content in Alpine. High pH differences observed in some cheeses, especially those with high levels of pepsin, may have been partly due to the longer coagulation time of milk (Medina *et al.* 1992)and high microbial metaboities as reported by Walstra *et al.* (1993). The trend of moisture decreasing in most cheese with ripening time agreed with reports by Fox *et al.* (1995) and Walstra *et al.* (1993). Report by Holsinger *et al.* (1995) showed that the amount of moisture retained in cheese is influenced by the extent and combination of steps used in cheese making as well as the method of moisture determination. In Alpine, Tilisiter and Pasta, the BF content in control cheeses was on the higher side while it was on the lower side in cheeses with highlevels of pepsin. These results showed that the level of pepsin had some influence on BF content and are in agreement with findings by Barbano *et*

Although most cheeses had low salt content at the beginning, this increased with ripening time because of the diffusion of salt across the curd (Steffen *et al.* 1993). The salt content of cheese was not directly related to the level of enzyme. The amount of nitrogen in all cheeses was not influenced by the level of pepsin. Hardy

1987) showed that TN in most cases was influenced by moisture changes during these ripening. The WSN values varied with levels of pepsin but there was no apparent direct relationship with the level pepsin in this study. Similar conclusions were drawn by McSweeney *et al.*(1993), who pointed out that proteolysis measured by WSN might not be sufficiently discriminating as an index of quality.

Data on microbial quality of chesses is also shown in Table 4, 5 and 6. The number of microbial counts were not significantly different in

Alpine as well as icantly different and wee not related to the level of pepsin. In this study strict hygienic conditions were closely observed to avoid contamination of the cheese. However, Zottola and Smith (1993) stated that in-spite of the hygienic standards, contamination of milk and curd with undesirable organisms cannot be fully prevented.

Table 4: Least square means of chemical and microbiological composition of Alpine cheeses at 42 of maturity

Variable	Rennet/pepsin enzyme combination										
	100CR	100HP	25HP	50HP	75HP	100VP	25VP	50VP	75VP	SED	P Value
pH	5.35[a]	5.58[b]	5.31[a]	5.32[a]	5.22[c]	5.25[c]	5.20[c]	5.36[a]	5.50[b]	0.001	**
Acid	3.50[a]	2.00[c]	3.65[a]	3.20[a]	2.85[b]	3.55[a]	3.35[a]	2.65[b]	2.05[c]	0.001	**
MC	41.6[a]	40.1[a]	32.4[b]	33.9[b]	38.3[a]	40.1[a]	33.7[b]	37.9[a]	43.6[a]	0.03	*
BF	54.8[a]	53.4[a]	46.2[b]	49.2[a]	50.2[a]	51.8[a]	43.7[b]	54.8[a]	60.3[a]	0.28	*
Salt	4.54[d]	4.00[b]	2.88[b]	2.72[a]	7.13[a]	4.42[d]	3.03[b]	1.28[c]	2.83[b]	0.04	***
TN	6.87[d]	6.49[a]	6.42[a]	7.15[b]	5.98[a]	6.56[a]	6.26[a]	6.43[a]	7.86[b]	0.001	*
WSN	0.81[a]	0.67[b]	0.64[b]	0.71[a]	1.17[a]	0.85[a]	0.98[a]	0.79[a]	0.97[a]	0.11	*
RI	11.8[a]	10.3[b]	10.0[a]	9.9[a]	19.56[b]	13.0[a]	15.7[b]	12.3[a]	12.3[a]	1.7	*
SPC[5]	0.9	2.3[a]	1.3	6.2	1.5	1.1	2.3	4.0	1.4	0.004	NS
Coli[3]	0	1	1	0	0	0	0	0	0	0.01	NS
YM[5]	0.4	1.2	2	4	1.8	0.5	1.7	1	0.8	0.1	NS

Key: BF=butterflat. MC=moisture, TN=total nitrogen, WSN= Water solube nitrogen, RI=ripening index, DRMT=Duncan's Multiple Range Test, SPC=standard plate count, Coli=coliform count, YM=yeast and mould count; NS-Not significantly difference *significant difference ** highly signficant difference Within the row, means followed by the same superscript do not differ significantly at P>0.05 according to DMRT

Table 5: Least square means of chemical and microbiological composition of Tilisiter cheeses at 4 days of maturity

Variable	Rennet/pepsin enzyme combination										
	100CR	100HP	25HP	50HP	75HP	100VP	25VP	50VP	75VP	SED	P Value
pH	51.51[a]	5.34[b]	5.02[c]	5.0[c]	5.03[c]	5.63[a]	5.51[a]	5.12[c]	5.16[a]	0.01	**
Acid	3.65[b]	3.75[b]	2.65[c]	4.95[a]	3.85[b]	4.75[a]	4.20[b]	3.05[c]	3.60[b]	0.01	**
MC	32.7[a]	30.1[a]	28.3[a]	26[a]	27.4[a]	29.3[a]	38.3[b]	27.8[c]	32.8[a]	0.18	*
BF	50.1[a]	48.7[a]	52.3[a]	48[a]	51.6[a]	43.1[b]	52.6[a]	46.4[a]	46.5[a]	0.16	*
Salt	0.74[b]	1.71[c]	2.58[c]	3.16[c]	2.06[c]	4.09[a]	2.43[c]	1.94[c]	1.79[c]	0.02	**
TN	8.03[a]	6.82[b]	9.07[a]	8.32[a]	8.06[a]	8.04[a]	7.83[a]	8.06[a]	6.96[b]	0.02	*
WSN	0.90[b]	0.98[b]	0.53[c]	0.93[b]	1.33[b]	1.44[b]	2.07[a]	0.96[b]	1.68[a]	0.03	**
RI	11[c]	14[c]	6[c]	11[c]	17[b]	17[b]	26[a]	12[c]	24[a]	0.35	**
SPC[5]	25[a]	8[b]	6[b]	4[b]	2[b]	7[b]	19[a]	10[b]	31[a]	0.003	*
Coli[3]	0	0	0	0	0	0	0	2	4	0.002	NS
YM[5]	58[a]	2[b]	1[b]	0[b]	0[b]	1[b]	1[b]	2[b]	1[b]	0.002	*

Key: BF=butterflat, MC=moisture, TN=total nitrogen, WSN= Water solube nitrogen, RI=ripening index, DRMT=Duncan's Multiple Range Test, SPC=standard plate count, Coli=coliform count, YM=yeast and mould count; NS-Not significantly difference *significant difference ** highly signficant difference Within the row, means followed by the same superscript do not differ significantly at P>0.05 according to DMRT

Table 6: Least square means of chemical and microbiological composition of Pasta cheeses at 14 days of maturity

Variables	Rennet/pepsin enzyme combinations										
	100CR	100HP	25HP	50HP	75HP	100VP	25VP	50VP	75VP	SED	P Value
pH	5.16^{a}	5.43^{b}	5.16^{a}	5.11^{a}	5.44^{b}	5.46^{b}	5.09^{a}	5.12a	5.08a	.01	*
Acid	2.85^{a}	2.6^{a}	2.20^{b}	3.00^{a}	1.60^{b}	2.65^{a}	2.30^{b}	2.65a	3.05a	.01	*
MC	44.1^{b}	46.4^{b}	45^{b}	49.19^{a}	43.7^{b}	44.3^{b}	42.3^{b}	43.7b	45.3b	.02	*
BF	44.2^{a}	38.2^{b}	45.5^{a}	48.1^{a}	43.5^{a}	32.8^{b}	43.8^{a}	45.2a	45.7a	.79	*
Salt	2.59^{c}	5.87^{b}	4.18^{b}	1.47^{a}	1.51^{a}	4.85^{b}	3.98^{c}	2.31c	2.93c	.08	**
TN	6.61^{b}	6.51^{b}	7.40^{a}	7.07^{a}	7.23^{a}	7.05^{a}	7.07^{a}	7.45a	7.40a	.11	*
WSN	0.65^{b}	0.64^{b}	0.58^{b}	0.71^{a}	0.57^{b}	0.59^{b}	0.57^{b}	0.60b	.60b	.02	*
RI	8.2^{a}	9.8^{b}	7.8^{a}	10b	7.8^{a}	8.3^{a}	8.0^{a}	7.9a	8.1a	.27	*
SPC[3]	9.1^{a}	22.7^{b}	9.0^{a}	18.9^{b}	4.5^{a}	9.0^{a}	22.9^{b}	13.6b	4.5a	.001	*
Coli[3]	0	0	0	0	0	0	0	0	0	.01	NS
YM[3]	2.2	6.8	18.1	13.6	11.3	11.3	11.3	11.4	4.5	.01	NS

Key: BF=butterflat, MC=moisture, TN=total nitrogen, WSN= Water solube nitrogen, RI=ripening index, DRMT=Duncan's Multiple Range Test, SPC=standard plate count, Coli=coliform count, YM=yeast and mould count; NS-Not significantly different *significantly different ** highly signficant difference

Within the row, means followed by the same superscript do not differ significantly at P>0.05 according to DMRT

Table 7: Mean squares of sensory evaluation scores of Alpine, Tilisiter and Pasta cheeses

Sensory Tests		N	100CR	100HP	25HP	50HP	75HP	100VP	25VP	50VP	75VP	SED	P Value
Taste	Alpine	17	1.9	2.1	2.5	2.5	2.4	1.8	2.1	1.8	2.7	0.14	NS
	Tilisiter	19	2.0	2.1	2.3	2.1	2.1	1.9	1.8	2.0	1.8	0.16	NS
	Pasta	15	1.9	2.1	2.1	2.0	1.9	1.7	1.5	1.9	1.7	0.18	NS
Smell	Alpine	17	1.95	2.0	2.1	1.9	2.1	1.8	1.9	1.8	2.1	0.17	NS
	Tilisiter	19	1.9	2.0	1.6	1.7	2.0	1.9	2.0	1.6	1.8	0.14	NS
	Pasta	15	1.7	1.8	2.1	1.9	1.9	1.9	1.7	1.9	1.9	0.15	NS
Appearance	Alpine	17	1.9^{a}	1.7^{a}	2.2	2.4^{b}	1.8^{a}	1.8^{a}	2.3^{b}	2.1^{b}	2.3^{b}	0.18	*
	Tilisiter	19	1.6	2.1	2.0	1.9	2.2	2.2	1.9	2.0	2.1	0.17	NS
	Pasta	15	1.4^{a}	2.1^{b}	2.2^{b}	2.3^{b}	2.1^{b}	1.7^{a}	1.5^{a}	1.9^{a}	1.7^{a}	0.17	*
Bitterness	Alpine	17	1.5^{a}	1.5^{a}	2.7^{b}	2.2^{a}	2.5^{b}	1.4^{a}	1.7^{a}	1.8^{a}	2.5^{b}	0.14	*
	Tilisiter	19	1.2^{a}	1.4$_{a}$	1.2^{a}	1.8^{b}	1.8^{b}	1.4^{b}	1.1^{a}	1.4^{a}	1.5^{a}	0.13	*
	Pasta	15	1.5^{a}	1.7^{a}	2.0^{a}	1.7^{a}	1.5^{a}	1.5^{a}	1.1^{b}	1.7^{a}	1.5^{a}	0.15	*

NS=not significantly different , *significant difference

Within the row, means followed by the same superscript do not differ significantly at P>0.05 according to DMRT

Table 8: Overall cheese assessment

Enzyme	100CR	100HP	25HP	50HP	75HP	100VP	25VP	50VP	75VP	SED	P Value
Alpine	2.73±0.15	2.27±0.18	2.47±0.21	2.33±0.23	2.67±0.16	2.53±0.19	2.27±0.21	2.27±0.21	2.47±0.24	0.2	NS
Tilisiter	2.47±0.23	2.73±0.15	2.27±0.22	2.66±0.18	2.63±0.12	2.53±0.19	2.47±0.21	2.66±0.16	2.20±0.24	0.19	NS
Pasta	2.80±0.14	2.53±0.21	2.60±0.19	2.53±0.19	2.73±0.12	2.73±0.15	2.4±0.19	2.87±0.10	2.87±0.10	0.15	NS

Key: NS - no significant difference

Coliforms are killed very fast during milk pasteurization but they easily re-contaminate milk and cheese during cheese making process and storage. Zottola and Smith (1993) indicated that yeasts and moulds could come from brine solution, manure and bedding materials.

Results on sensory quality of cheeses in Table 7 shows that the mean scores of cheeses were not influenced by enzyme combinations. The non significant differences in taste and smell of different cheeses was in agreement with the findings of Van de Berg and de Koning (1990). They reported that sometimes minor variations in composition could even occur between individual cheeses in the same batch even where two cheeses are being assessed as a pair. Such differences were mainly due to slight differences in intensity of the acid taste of the cheese which has no relation to the rennet used. Andren and Reedtz (1990) showed that using different ratios of chymosin/pepsin for cheese making was not a problem. High bacterial, yeast and mould counts which are undesirable organisms in cheeses, could have contaminated Pasta 25VP which scored relatively lower in appearance. The varying bitterness could have been caused by many factors in cheese (Fox *et al.* 1995). A report by Visser (1993) showed that the amount of rennet added in cheese is directly related to bitterness in cheese. This could be true for Alpine cheese with 100HP and 100VP which contained mostly pepsin a proteolytic enzyme that caused bitterness in cheese. The difficulty in identifying the bitter substances found in cheeses have been reported by many researchers (Walstra *et al.* 1993; Martely and Crow, 1993; Fox *et al.* 1995; Steele, 1995). No attempt was done to identify the bitter substances in this study.

Overall cheese quality assessment (Table 8) showed that cheeses produced with different levels of pepsin were not significantly different. Van de Berg and de Koning (1990) reported that sometimes minor variations in composition could occur between individual cheeses in the same batch even where two cheeses are being assessed as a pair. Such differences were mainly due to slight differences in intensity of the acid taste of the cheese which has no relation to the enzyme used.

Conclusion

It was concluded that all the enzyme combinations including 100% bovine pepsin were suitable for making all types of cheese studied. However, for the best cheese performance, 75HP for Alpine; 50HP and 50VP for Tilisiter and 50VP and 75VP enzyme combinations could be used in making these cheeses. This means that 50-75% pepsin could be blended with calf rennet. Therefore, dilute HCl and vinegar were appropriate local materials which can be used by small scale cheese processors in Tanzania to extract pepsin.

Acknowledgement

The authors wish to thank the Norwegian Agency for Development Co-operation (NORAD) for financing this study.

References

Andren, A. and Reedtz, C., 1990. The effect of chromatographically pure bovine chymosin and pepsin A on cheese curd firmness. *Journal of Dairy Research* 57, 109-117.

AOAC Standard,1975. Official Methods of analysis. Association of Official Analytical Chemists. Washington, D.C. pg 1008.

Bank, J.M.; Law, A.J.R.; Leaver, J and Horned., 1993. The inclution of whey proteins in the cheese- an overview. *Cheese Yield and Factors Affecting its Control, IDF Seminar*, Cork pp 387-401.

Barbano, D.M. and Rasmussen, R.R., 1992. Cheese yield performance of fermentation produced chymosin and other coagulants. *Journal Dairy Science* 75, 1-11.

Dajnowiec,F.; Reps,A.; Wasilewsk,R. and Kolakowsk,P., 1997. Coagulation pf milk proteins by proteolytic enzymes produced by genetic engineering. *Milchwissenchaft* 52(3),150-153.

Davide,C.; Peralta,C. and Cruz,L., 1982. Preparation of milk coagulants from adult carabao cattle and goat abomasa. *Phillippine Agriculture* 65, 131-145.

De-Wolf, 1990. Small holder dairy production in Kagera region. *Proceedings of Tanzania Society of Animal Production*, Arusha international Conference Centre, 17 pg 87.

Egan, H.; Kirk, R.S. and Sawyer, R.,1981. *Pearson's Chemical Analysis of Foods*. 8th Edition. Longman Scientific and Technical Publishers. London pp 433-506.

FAO-RDDTT, 1990. Rural milk processing. *A Practical Guide to Small Scale Cheese Making in E. Africa*. Prepared by FAO Reginal Dairy Development and Training Team, Naivasha. Kenya. 6-29.

Farmakalidis. E., 1995. Cheese and chymosin gaining acceptance: *Food Australia* 47(12),572-573.

Fox, P.F.; McSweeney, P.L.H. and Singh, T.K., 1995. Methods of assessing proteolysis in cheese during maturation. *Chemistry of Structure-Function Relationships in Cheese*. Edited by E.L. Malin and Tunick, Plenum Press, New York, pp 161-187.

Green, M.L.;1972. Assesment of swine bovine pepsin, chicken pepsin as rennet substitute for cheddar cheese making. *Journal of Dairy Research* 41,261-273.

Hardy, J., 1987. Water activity and the salting of cheese. *Cheesemaking Science and Technology*, Edited by A. Eck. Lavoisier Publishing Inc. New York, pp 37-58.

Hill, R.D., 1989. The ß-Lactogloblin k-casein complex. *Cannadian Institute of Food Science and Technology Journal* 22:120-125.

Holsinger, V.H.; Smith, P.W. and Tunick, M.H., 1995. Overview: Cheese chemistry and rheology, In: *Chemistry of Structure-Function Relationships in Cheese*. Edited by E.L. Malin and Tunick. Plenum Press. New York. pp 1-6.

IDF Standard 110A. 1987. Calf Rennet and Bovine Rennet: *Determination of Chymosin and Bovine Pepsin Content* (chromatographic method).

IDF Standard 73A, 1985. Enumeration of coliform: Colony count technique and most probable number technique at 30 ^{0}C. *International Dairy Federation*, Brussels.

IDF Standard 94B, 1990. Enumeration of yeast and mould, Colony count technique at 25 ^{0}C. *International Dairy Federation*, Brussels.

IDF Standard 100B, 1991. Enumeration standard colony count. Colony count techniqueat 30 ^{0}C. *International Dairy Federation*, Brussels.

Kurwijila. R.L., 1990 Rural Dairy Technology Apropriate to rural small scale holder production. *The Tanzania Experience, paper presented at Dairy Marketing Symposium* 26-30 November. 1990. ILCA. Addis Ababa. Ethiopia.

Ling, E.R., 1963. A Textbook of Dairy Chemistry. Volume 1 Chapman and Hall Ltd. UK pp126-131.

Martley, F.G. and Crow, L.L., 1993. Interaction between non-starter organisms during cheese manufacture and ripening. *International Dairy Journal* 3, 461-483.

McSweeney, P.L.H.; Oslon, N.F.; Fox, P.F.; Healy and Hojrup, P., 1993. Proteolytic specificity of chymosin on bovine si-casein. *Journal of Dairy Research* 60, 401-412.

Medina, L.M.; Barrios, M.J.; Serrano. C.E.; Jordano. R., 1992. *Alimentaria* 34 (274), 61-64.

Ryoba, R.N.Z., 1999. Studies on preparation and use of bovine pepsin from adult cattle abomasa as rennet substitute for small scale cheese processors. PhD Thesis at Sokoine Univerisity of Agriculture, Morogoro. Tanzania.

Steele, J.L., 1995. Contribution of lactic acid bacteria to cheese ripening. In: *Chemistry of Structure-Function Relationships in Cheese*. Edited by E.L. Malin and Tunick, Plenum Press, New York, pp 209-219.

Steffen, C.; Eberhard, P.; Bosset, J.O. and Ruegg, M., 1993. Swiss-Type Varieties. In:*Cheese Chemistry, Physics and Microbiology*, Edited by P.F. Fox, 2nd Edition. Chapman and Hall, London, pp 83.

Ulicky, E., 1989. A case study of three women cooperative for milk collectionin Tanzania. *A Paper Presented at FAO Regional Seminar on Dairy Extension for Rural Smallholders*, Zimbabwe 1989.

Van den Berg, G and de Koning, P.J., 1990. Gouda cheesemaking with purified calf chymosin and microbiologically produced chymosin *Netherland Milk Dairy Journal* 44, 189.

Visser. P.M.W., 1993. Proteolytic enzymes and their relation to cheese ripening and flavour: an overview. *Journal of Dairy Science* 76:329-180.

Walstra, P.; Noomen, A. and Geurts, T.J., 1993. Dutch-Type Varieties, Cheese: *Cheese Chemistry, Physics and Microbiology*, Edited by P.F. Fox, 2nd Edition. Chapman and Hall, London, pp 39-41.

Webb,H.B.; Johson,A.H. and Alford, J.A.,1974. *Fundamentals of Dairy Chemistry*. AVI Publishing CO Inc. Westport, Connecticut, pp 603-662.

Zottola, E.A. and Smith, L.B., 1993. Growth and survival of undesirable bacteria in cheese. Cheese Chemistry, Physics and Microbiology, Edited by P.F. Fox, 1st Edition. Chapman and Hall, London, pp 471