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Effect of Proteases on Meltability and Stretchability of Nabulsi Cheese

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Abstract: Problem statement: Boiled white brined cheese (Nabulsi cheese) is the mostly consumed cheese in Jordan; this cheese should show meltability and high stretchability in order to fit in the production of high quality Kunafa and other popular local sweets and pastries. However, these characteristics are rarely available when usual processing and preservation methods were used. **Approach:** This study was based on the hypothesis that it would be possible to imply meltability and stretchability to the cheese by proteolytic enzymes to the original brine that may specifically act on cross linking bonds of casein. In this study, six commercial proteases were used. **Results:** It was found that Nabulsi cheese treated with papain developed an outstanding fibrous structure, this gives superiority in the application in kunafa, pizza and pastries. The meltability and stretchability of Nabulsi cheese treated with papain were still excellent after 4 weeks of storage; this indicated the restricted enzyme action, probably due to high salt concentrations (18%) in storage brine. **Conclusion:** Use of proteolytic enzymes to induce meltability and stretchability of Nabulsi cheese was proved to be an efficient method.

Key words: Nabulsi cheese, white brined cheese, meltability, stretchability, proteases, papain

INTRODUCTION

One of the most widely produced cheese type in the Mediterranean, South-East European countries and Middle East is white brined cheese[2]. In Jordan, white brined cheese of the Nabulsi type is the main traditional cheese produced by farmers, seasonal producers and dairy industry. The name is related to Nabulus, a town in the West Bank of Jordan. It is produced by renneting raw sheep milk or a blend of sheep and goat milk to which no starter culture is added[4]. Since Nabulsi cheese has a semi-hard texture and salty flavor it is often consumed after removing a part of its salt by soaking in water. Moreover; it is used after desalting in production of some bakery products and Arabic sweets particularly "Kunafa".

It is desirable that Nabulsi cheese shows melt and stretch abilities in order to fit in the production of a high quality "Kunafa". However, these characteristics are not always available, especially in cheese made from high quality milk where spontaneous fermentation is minimal in the milk as well as in the curd before boiling[3,4].

Different ways are being practiced to improve these characteristics, Firstly, using a mixture of cow, sheep or goat milk rather than ewe's milk alone, but this method is not reliable. Secondly, keeping the curd which is produced from raw milk at room temperature for a period of time till it becomes some what spongy before it is boiled. A third method is, storing boiled cheese in its cans for along period (i.e.) aging. This long period may allow substantial microbial growth

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namely halotolerant and halophilic microorganisms or the cheese may be altered by certain proteolytic enzymes that affect milk proteins. Also this method is neither practical nor reliable[1,2,5].

The idea of making cheese stretchable and meltable is to hydrolyze some of the peptide bonds of the more or less intact proteins (casein) or to destruct some covalent cross-linking bonds of the protein matrix, this can be accomplished through pH changes (either by direct acidification or through acid production by selected bacterial starter cultures or both), using protease enzymes; particularly coagulation enzymes and emulsifier salts (melting salts)[4,5].

Using protease enzymes and emulsifiers salts (melting salts) on the stretchability and meltability of local white brined Nabulsi cheese have not been investigated and there are no studies carried on the stretchability of local cheese.

The coagulant used in cheese making has a dual role. The primary function is to coagulate milk to produce cheese curd. In addition, a small proportion of the coagulant is carried over into the cheese. The residual coagulant remains proteolytically active in most aged cheeses and plays an important role in the development of texture and flavor[6]. Proteolytic activity by the coagulant in cheese depends on the amount and proteolytic characteristics of the specific coagulant and the amount of inactivation by temperature and pH that occurs during cheese making. For Swiss-type cheeses, high pH and temperature during cooking were generally thought to have completely inactivated residual coagulant[1,8].

Using a sensitive ELISA method for chymosin detection, showed that active chymosin was present in both experimental and commercial Swiss-type cheeses. In Mozzarella cheese, coagulant had been widely reported[5] to be fully inactivated by high temperature during stretching. However, recent reports have shown that, during aging of Mozzarella, extensive proteolysis occurs that can be largely attributed to residual coagulant. Moreover, the rate and specificity of proteolysis in Mozzarella are strongly influenced by the type of coagulant (e.g., chymosin and microbial rennet) used in cheese making. Several researches have reported that the type of coagulant used in cheese making significantly determine the functionality changes during aging[1,8].

The amount of coagulant retained in cheese varies with manufacturing conditions. Retention of chymosin is influenced by cooking temperature during cheese making. Residual chymosin is highly dependent on the rate of acidification during manufacture, particularly the pH of the whey at draining. In contrast, retention of the coagulants that were derived from the microbial sources was not influenced by acidification rate during cheese making[5]. And there is a direct relationship between the amount of chymosin added to cheese milk and the concentration of active chymosin in the cheese. Moreover, proteolysis rate and textural changes[6] in cheese during aging were directly related to residual chymosin concentration.

Proteolysis is usually slowed down by low-temperature storage and heat treatment of cheese milk, cheese from cow's milk shows more rapid proteolysis than that from buffalo milk which reflects differences in the rate of proteolysis of casein fraction of these two species, increasing the salt content in cheese slightly decreases proteolysis in Domiati cheese and the use of milk clotting enzymes other than calf rennet modifies proteolysis of Domiati Cheese[1,8].

With the exception of Emmental, Mozzarella and similar highly-cooked cheeses, the initial proteolysis in cheese is catalyzed by residual coagulant, αs1-casein undergoes considerable proteolysis during ripening but β-casein remains unchanged until an advanced stage of ripening[6].

The proteolytic activity of chymosin and pepsin on αs1-casein is stimulated by NaCl concentration up to an optimum of~5% and although activity is inhibited at higher NaCl levels, proteolysis of αs1-casein occurs up to 20% NaCl. In contrast, proteolysis of β-casein is strongly inhibited by 5% NaCl and completely inhibited by 10% NaCl. The inhibitory effect of NaCl on proteolysis is pH-dependent and at low pH NaCl alters the proteolytic specificity of chymosin and pepsin: NaCl (2.5%) inhibits the formation of β-III and promotes the formatting of β-IV and β-V[1].

The objective of this study was to study the effect of using different proteolytic enzymes particularly, those used as coagulants for cheese production on stretchability and meltability of local white brined Nabulsi cheese.

MATERIALS AND METHODS

Experimental cheese samples: Freshly drawn, cow and sheep milk was obtained from one local farm in Al-Dhulail (Abu-Hamdan Farm). This milk was used as follows to produce white brined Nabulsi cheese: Raw cow milk, raw sheep milk, a mixture of raw cow milk (34%) and raw sheep milk (66%) to produce the so called Mashmouleh cheese. The traditional method for the production of this kind of cheese as described by[4] was followed (Fig. 1) except the salting step which was carried out by in brine salting instead of dry salting.

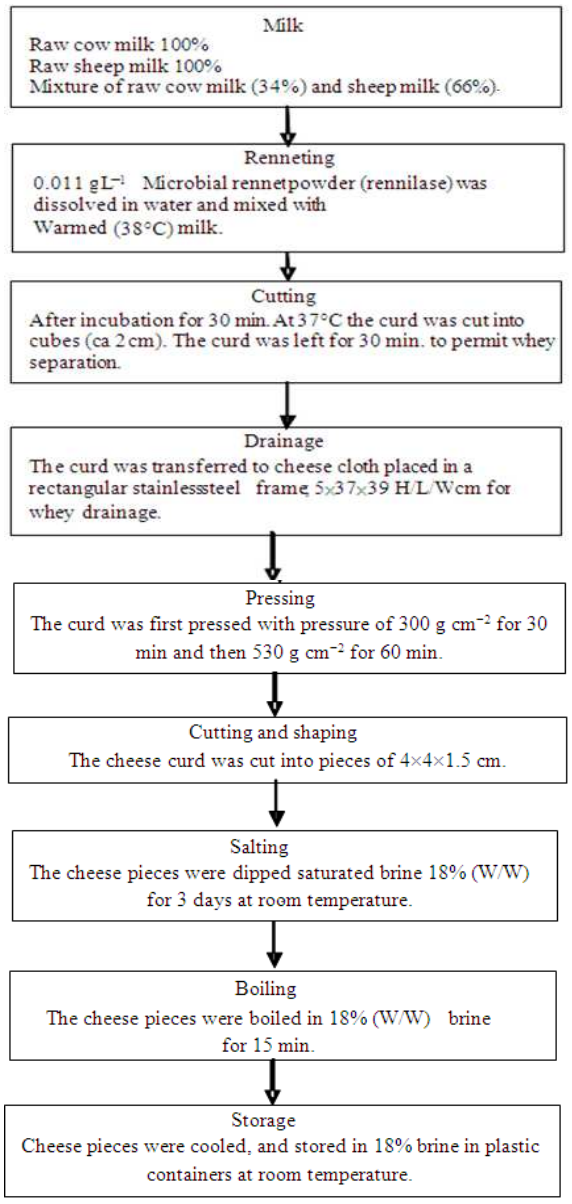

Fig. 1: Processing steps for the production of experimental white brined Nabulsi cheese

Local market samples: Four commercial white brined Nabulsi cheese samples and three desalted Mashmouleh cheese type specially used for Kunafa preparation were purchased from local market from different locations.

Proteases: Sex Commercial protease enzymes were selected to test their ability to modify meltability and stretchability of the brined cheese. Table 1 shows names, origin and some specifications of these enzymes.

Table 1: Names, origin and pH of proteases tested for their ability to modify meltability and stretchability of Nabulsi cheese

No.	Name of enzyme	Origin	pH	Description
1	Marschall (blue label)	France, Rhodia	5.4	Animal extract coagulant
2	Meito Rennet	Japan, Meito Sangyo	3.8	
3	Marzyme	France, Rhodia	5.6	Microbial Coagulants
4	Rennilase	Holland, Gist-brocades	3.8	Microbial Coagulants
5	Papain	France, Rhodia	6.2	From papaya Latex EC (3.4.23.2)
6	Pepsin	France, Rhodia	5.6	Bovin pepsin extract EC (3.4.23.1)

Testing methods:

Meltability test: The method used in measuring meltability of cheese was described by Arnott *et al*.[2]. It is based on heating a standardized cylindrical cheese specimen under specified conditions (oven temperature and time), followed by measuring the specimen's diameter. This was done as follows:

Cylindrical samples of 2 diameter and 1.5 cm height were prepared as follows:

A test tube of 2 cm diameter was used as a probe to cut a specimen from a desalted cheese sample. The specimen was wrapped again and stored at 4°C until testing.

The cheese specimen was placed on a glass Petri-dish and heated at 100°C in a laboratory oven for 15 min. At the end the diameter of the specimen was measured.

Stretchability test: A new apparatus was designed and constructed for measuring the stretchability of cheese. It is based on heating a specified amount of cheese under specified conditions on a thermostatically controlled hot plate, followed by measuring the distance between the hot plate and mobile stretching plate before the cheese strands are torn off.

Measuring procedure: A desalted 15 g cheese sample was manually disintegrated using a spatula and spread evenly over the filter paper on the hot plate surface and preheated to 80°C (40 sec). The stretching plate was lowered by the pulley handle until settling on the heated sample and held for 5 sec. The stretching plate was then consciously and steadily lifted by the pulley handle until the stretched cheese strands are torn off. The distance between the two plates in cm is considered as a measure of stretchability.

Treatments with selected proteases to modify meltability and stretchability of Nabulsi cheese: An experiment was conducted on experimental cheese samples prepared from cow milk.

Boiled brined cheese cubes (2×2×1.5 cm) samples of weighing 71.4 g, each were filled in 500ml glass jars with 425 mL of 18% brine solution. The selected protease enzymes were individually added to each jar at 0.01% (w/w) concentration. Two pH values (5.8, 5.4) were adjusting using (10%) citric acid solution along with two storage temperatures (room and 4°C temperature). This procedure was done in triplicate with control treatment (zero concentration). The meltability and stretchability of the stored cheese under two temperature were measured at one week interval for 4 weeks.

Statistical analysis of experiment data: The data obtained were analyzed for significance using the General Linear Model (GLM) procedure of the SAS institute Inc., Cary, NC and USA 1998 version seven software. LSD mean were applied to determine significance between different treatments[7].

RESULTS

A pre-experiment was conducted using 0.3% (w/w) concentration of rennilase, to principally test the effectiveness of proteases in inducing meltability and stretchability within few days. This relatively high concentration resulted in high meltability with little stretchability which demonstrates the hydrolytic action of the proteases. Hence, a screening systematic test was conducted at a lower concentration namely 0.01% (w/w) at two different pH values, for four weeks storage and two storage temperatures.

Table 2 shows meltability values of enzymes treated cheese samples at room temperature, while Table 3 shows stretchability of the same samples. Table 4 and 5 gives the results of the parallel experiment at 4°C storage temperature.

Out of the six enzymes used, four enzymes induced different levels of meltability and stretchability, the other two enzymes (Marchall and Marzymes) led to disintegration and lyses of cheese samples upon testing (neither meltability nor stretchability).

As can be seen from the Table 2-5, considering both meltability and stretchability, papain gave the best results already after one week of storage, regardless the variables tested and throughout the three next weeks.

Rennilase and pepsin followed papain in their effectiveness respectively, with significant differences between values ($p<0.05$). Papain treated cheese samples at room temperature were 71.5 and 55 cm at pH 5.8, 5.4 respectively, after three weeks of storage.

Table 2: Effect of adding selected proteases*, two pH values and storage time on meltability of Nabulsi cheese made from cow milk, stored at room temperature

		Meltability** (diameter in cm)								
		pH	5.8				5.4			
No.	Type of enzyme	Storage time (week)	1w	2w	3w	4w	1w	2w	3w	4w
1	Marchall (blue label)		None$_b$	None$_c$	None$_e$	None$_e$	None$_d$	None$_c$	None$_d$	None$_f$
2	Meito Rennet		2.45$_a$	2.65$_b$	3.45$_b$	4.10$_a$	2.4$_c$	2.75$_b$	3.45$_a$	4.0$_a$
3	Marzyme		None$_b$	None$_c$	None$_e$	None$_e$	None$_d$	None$_c$	None$_d$	None$_f$
4	Rennilase		2.40$_a$	2.65$_b$	3.15$_c$	3.65$_c$	2.45$_{b,c}$	2.65$_b$	2.10$_b$	3.70$_c$
5	Papain (EC 3.4.22.2)		2.45$_a$	3.25$_a$	3.45$_b$	3.80$_b$	2.75$_a$	3.00$_a$	3.20$_{a,b}$	3.55$_d$
6	Pepsin (EC 3.4.33.1)		2.80$_a$	3.10$_a$	3.65$_a$	4.00$_a$	2.70$_a$	3.00$_a$	3.20$_{a,b}$	3.85$_b$
7	Control (only pH treated)		2.45$_a$	2.50$_b$	2.75$_d$	3.00$_d$	2.50$_b$	2.65$_b$	2.80$_c$	2.85$_e$

*: Protease enzyme added in a concentration of 0.01% (w/w); **: Meltability measurement was performed on cheese after desalting, using Arnott method[2]. Means with the same letter within the same column are not significantly different

Table 3: Effect of adding selected proteases*, two pH values and storage time on meltability of Nabulsi cheese made from cow milk, stored at 4°C

		Meltability** (diameter in cm)								
		pH	5.8				5.4			
No.	Type of enzyme	Storage time (week)	1w	2w	3w	4w	1w	2w	3w	4w
1	Marchall (blue label)		None$_d$	None$_e$	None$_f$	None$_e$	None$_c$	None$_d$	None$_e$	None$_f$
2	Meito Rennet		2.65$_{a,b,c}$	2.85$_c$	2.95$_d$	3.35$_c$	2.65$_{a,b}$	2.90$_b$	3.0$_c$	3.30$_d$
3	Marzyme		None$_d$	None$_e$	None$_f$	None$_c$	None$_c$	None$_d$	None$_e$	None$_f$
4	Rennilase		2.60$_{b,c}$	3.05$_b$	3.15$_c$	3.35$_c$	2.70$_a$	3.10$_a$	3.20$_b$	3.45$_c$
5	Papain (EC 3.4.22.2)		2.90$_a$	3.25$_a$	3.60$_a$	3.85$_b$	2.80$_a$	3.0$_{a,b}$	3.45$_a$	3.80$_b$
6	Pepsin (EC 3.4.33.1)		2.75$_{a,b}$	3.00$_b$	3.50$_b$	4.10$_a$	2.6$_{a,b}$	2.90$_b$	3.35$_a$	3.90$_a$
7	Control (only pH treated)		2.45$_c$	2.45$_d$	2.80$_e$	3.10$_d$	2.45$_b$	2.55$_c$	2.65$_d$	2.85$_e$

*: Protease enzyme added in a concentration of 0.01% (w/w); **: Meltability measurement was performed on cheese after desalting, using Arnott method[2]. Means with the same letter within the same column are not significantly different

Table 4: Effect of adding selected proteases*, two pH values and storage time on stretchability of Nabulsi cheese made from cow milk, stored at room temperature

		Stretch ability **(length in cm)								
		pH	5.8				5.4			
No.	Type of enzyme	Storage time (week)	1w	2w	3w	4w	1w	2w	3w	4w
1	Marchall (blue label)		None$_e$	None$_d$	None$_f$	None$_f$	None$_f$	None$_f$	None$_e$	None$_e$
2	Meito Rennet		11.5$_c$	23.5$_c$	27.5$_d$	17.5$_c$	10.5$_d$	22.0$_d$	26.0$_c$	16.5$_c$
3	Marzyme		None$_e$	None$_d$	None$_f$	None$_d$	None$_f$	None$_f$	None$_e$	None$_e$
4	Rennilase		13.5$_c$	31.5$_b$	35.5$_b$	31.5$_b$	13$_c$	30.0$_b$	34.0$_b$	26.0$_b$
5	Papain (EC 3.4.22.2)		42.5$_a$	61$_a$	71.5$_a$	70$_a$	38$_a$	57$_a$	60$_a$	55$_a$
6	Pepsin (EC 3.4.33.1)		19$_b$	29$_b$	32$_c$	28.5$_b$	16.5$_b$	24.5$_c$	27$_c$	25.5$_b$
7	Control (only pH treated)		6.5$_d$	0.5$_d$	15$_e$	17.5$_c$	5.5$_e$	7.0$_e$	10$_d$	12$_d$

*: Protease enzyme added in a concentration of 0.01% (w/w); **: Stretchability measurement was performed on cheese after desalting, using the new apparatus designed for the purpose of the study. Means with the same letter within the same column are not significantly different

Table 5: Effect of adding selected proteases*, two pH values and storage time on stretchability of Nabulsi cheese made from cow milk, stored at 4°C

		Stretchability** (length in cm)								
		pH	5.8				5.4			
No.	Type of enzyme	Storage time (week)	1	2	3	4	1	2	3	4
1	Marchall (blue label)		None$_f$	None$_c$	None$_f$	None$_f$	None$_f$	None$_f$	None$_e$	None$_f$
2	Meito Rennet		15.0$_d$	29.0$_c$	30.0$_d$	26.0$_d$	15.0$_d$	26.5$_d$	28.0$_c$	25.0$_d$
3	Marzyme		None$_f$	None$_e$	None$_f$	None$_f$	None$_f$	None$_f$	None$_e$	None$_f$
4	Rennilase		17.5$_c$	37.5$_b$	40.0$_b$	40.0$_b$	17$_c$	39.5$_b$	37.5$_b$	37.0$_b$
5	Papain (EC 3.4.22.2)		45$_a$	66$_a$	73$_a$	72$_a$	41$_a$	61$_a$	68$_a$	65$_a$
6	Pepsin (EC 3.4.33.1)		21.5$_b$	30.5$_c$	33$_c$	29.5$_c$	26$_b$	34$_c$	37.5$_b$	33$_c$
7	Control (only pH treated)		6.2$_e$	8.0$_d$	14.5$_e$	18$_e$	5.5$_e$	6.5$_e$	9.0$_d$	12.5$_e$

*: Protease enzyme added in a concentration of 0.01% (w/w); ** Stretchability measurement was performed on cheese after desalting, using the new apparatus designed for the purpose of the study. Means with the same letter within the same column are not significantly different.

DISCUSSION

The Corresponding stretchability values of rennilase treatment were 31.5 and 34.0 cm, whereas values of pepsin treatment were 32 and 27 cm respectively after 3 weeks of storage at room temperature.

It is worth noting that the two pH values were selected since they gave the best meltability and stretchability (Table 5).

It can be clearly seen from the Table 2-5 that the optimum duration of the enzymatic treatments with the best three enzymes was three weeks. It is interesting that papain showed an excellent stretchability value just after one week of storage (42.5 cm). This suggests to start the treatments with papain one to two weeks before desalting for uses.

Further findings were that no significant (practical) differences are observed in stretchability due to the two pH treatments (5.8, 5.4.) tested; therefore, any pH in this range would be acceptable. The pH adjustment using citric acid should consider the buffering capacity of the cheese and as it was explained before, the amount could be experimentally determined by titration of a slurry of cheese with aliquot amount of the original brine.

The same observation regarding pH effect is valid for the two storage temperatures (room temperature and 4°C). Though refrigeration temperature gave somewhat higher stretchability values, selecting room temperature for the treatment is trivial, so long cheese is kept in its original brine.

According to the results obtained from the tables, papain and rennilase were selected for further investigations.

CONCLUSION

Nabulsi cheese treated with papain developed an outstanding fibrous structure, this gives superiority in the application in Kunafa, Pizza and pastries. The meltability and stretchability of Nabulsi cheese treated with papain were still excellent after 4 weeks of storage, this indicates the restricted enzyme action, probably due to high salt concentrations (18%) in storage brine.

Further studies are needed to investigate the long-term effect of proteolytic enzymes, on the physical and

sensory characteristics as well as the keeping ability of the cheese; and to investigate the suitability of every proleolytic enzyme to induce meltability and stretchability should be tested at a wide range of pH, in order to judge its effectiveness for this purpose.

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