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PROPERTIES OF BUFFALO MOZZARELLA CHEESE AS AFFECTED BY TYPE OF COAGULANTE

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Background. Mozzarella is one of several pasta filata or stretched curd cheeses that originated in Italy. The name pasta filata refers to a unique plasticizing and texturing treatments of the fresh curd in hot water that imparts to the finished cheese its characteristic fibrous structure and melting properties. Mozzarella cheese made from standardized buffalo milk with 3 and 1.5% fat. The effect of coagulant types (calf rennet, chymosin and *Mucor miehei* rennet) on the cheese properties was carried out.
Material and methods. Fresh raw buffalo milk and starter cultures of *Streptococcus salvarius* ssp. *thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* were used. The coagulants were calf rennet powder (HA-LA), microbial rennet powder (formase ISOTL from *Mucor miehei*) and chymosin derived by fermentation (CDF). Milk, curd, whey, kneading water and cheese were analysed. The slab gel electrophoresis patterns of Mozzarella cheese were also applied. Statistical analyses were also applied on the obtained data.
Results. Recovery of DM of both curd and cheese decreased in case of using *Mucor miehei* rennet while the recovery of TP and fat content in both curd and cheese and their loss to whey and kneading water were nearly the same. Soluble nitrogen and soluble tyrosine and tryptophan contents elevated with increasing the storage period. Increasing rate of the soluble nitrogen in case of using *Mucor miehei* rennet was higher compared to that made with the other types of coagulant. TVFA content increased with advancing the storage period, also increased with increasing the fat content of the original milk fat used. No effect can be seen due to the coagulant types. The meltability increased with storage period progress. While the effect of the type of coagulant enzyme hade neglect effect on meltability fat leakage and oiling off. Mozzarella cheese made with *Mucor miehei* rennet obtained the highest firmness compared with those made using calf rennet, or chymosin. Both scores of the flavour and body and texture improved with progressing of storage period, and were higher in case of using *Mucor miehei* rennet, while the appearance score of the cheeses decreased with increasing the storage period at 4°C.

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Conclusion. Using *Mucor miehei* rennet increased the rate of proteolysis during storage period more than the other 2 types of coagulant. They also, showed the higher sensory evaluation.

Key words: Mozzarella cheese, *Mucor miehei* rennet, calf rennet, chymosin, buffalo milk

INTRODUCTION

Mozzarella is one of several pasta filata or stretched curd cheeses that originated in Italy. The name pasta filata refers to a unique plasticizing and texturing treatments of the fresh curd in hot water that imparts to the finished cheese its characteristic fibrous structure and melting properties.

Mozzarella cheese is classified by standards of identity into four separate categories based on moisture content and fat in dry matter.

Several recent investigations have shown a significant correlation between milk fat intake and coronary heart diseases. Such studies have caused consumers to become more concerned about their consumption of milk fat in cheese and other dairy products. Studies by Dave et al. [2003] and Tunkick et al. [1993] showed that, low fat and low moisture Mozzarella cheese is too hard and non-meltable enough compared with full fat Mozzarella. However, refrigerated storage of the low fat cheese seemed to have textural properties similar to those of fresh high fat, low moisture Mozzarella. The proteolysis presumably taking place at 4°C causes some breakdown of its dense casein structure, resulting in a product with more desirable textural and melting properties. They concluded that, it is possible to produce low-fat Mozzarella which is comparable texturally and in meltability to unripened Mozzarella containing twice as much FDM.

Calcium plays an important role in the functional properties of cheese. Recent studies have shown that reduced calcium Mozzarella cheeses had improved softening, melting flow characteristics and higher proteolysis. Mozzarella cheeses were made by direct acidification using glucono-δ-lactone or using starter culture. Milk was preacidified using citric acid and acetic acid to a pH of 5.9 to lower level of calcium. Reduced calcium cheeses made with this preacidified milk were compared with control cheeses made with milk without preacidification. The level of reduction of calcium in final cheeses was approximately 45% [Thakur 2007].

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. This residual coagulant remains proteolytically active in most aged cheeses and plays an important role in the development of texture and flavour. Proteolytic activity by the coagulant in cheeses depends on the amount and-proteolytic characteristics of a specific coagulant and the amount of inactivation by temperature and pH that occurs during cheese making. In Mozzarella cheese, coagulant were widely reported to be fully inactivated by high temperature during stretching [Ahmed et al. 2008].

Coagulant type significantly affected the level of primary proteolysis, as measured by levels of pH 4.6 SN, with the *Rhizomucor pusillus* proteinase (RPP) giving significantly higher mean levels than the other coagulants at both 4 and 12°C. However, coagulant type did not significantly affect the firmness of the unheated cheese or the flowability of the cheese on heating at 180 or 280°C. Increasing storage temperature from 4°C to 12°C significantly increased the mean levels of proteolysis and non-expressible serum in the raw cheese and the mean flowability of the heated cheese [Sheehan et al. 2004].

However, recent reports [Farkye et al. 1991, Tunkick et al. 1993, Yun et al. 1993 a] 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, calf rennet and *Mucor miehei* rennet) used in cheese making. Several investigations [Oberg et al. 1992, Yun et al. 1993 b] have reported that the type of coagulant used in cheese making significantly influenced changes in cheese functionality during aging. Adding of glucono-δ-lactone, (GDL) alone to Mozzarella cheese milk decreases both actual and moisture and salt adjusted cheese yields more than using GDL with yoghurt starter as acidulate. The progressive reduction in moisture and salt adjusted cheese yield with increasing preacidification was caused by a substantial reduction in calcium recovery in the cheese and a tendency for decreased protein recovery in the cheese. This would decrease moisture and salt adjusted yield [Ismail et al. 2007, Metzger et al. 2000].

Since buffalo milk has a major share in the total milk produced and processed in Egypt and as this milk is quite suitable for Mozzarella cheese it also has a potential market in industry, such as pizza.

Therefore the aim of this study was to investigate the effect of some milk coagulating enzymes on the compositional, rheological and sensory qualities of Mozzarella cheese made from buffalo milk.

MATERIAL AND METHODS

Materials

Fresh raw buffalo milk was obtained from the herd of the Faculty of Agriculture, Cairo University. Starter cultures of *Streptococcus salvarius* ssp. *thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* were obtained from Microbiological Resources Centre (MIRCEN), Faculty of Agriculture, Ain Shams Univ. Calf rennet powder (HA-LA) from CHR-Hansen's Denmark; microbial rennet powder (formase ISOTL from *Mucor miehei*) Gist-brocades, France and chymosin derived by fermentation (CDF) obtained from Dairy Ingredients Division, Pfizer Inc. Dry Coarse table salt was obtained from El-Nasr Company at Alexandria, Egypt.

Cheese manufacture. Buffalo milk was separated to cream and skim milk using a mechanical separator. The obtained skim milk was divided into 2 equal portions. One portion was standardized to 3% fat and 1.5% fat, using the separated cream. Each standardized milk was further divided into 3 equal portions and manufactured to Mozzarella cheese as described by [Kosikowski 1982 a]. Using different types of coagulants. Three replicates were made from the control and treatments and analysed for chemical, physical and rheological properties when (fresh, 1, 2, 3 and 4) weeks of cold storage (4°C).

Methods of analysis

A. Milk, whey and kneading water (stretching water). Dry matter (DM), fat and total protein (TP) contents were determined according to the methods [AOAC 2007] while pH values were measured using a digital pH meter (M41150 USA) equipped with glass electrode, Sodium chloride content of the kneading water was determined by the method of [Case et al. 1985]. Total phosphorus (P) was determined in the ash according to [Snell and Snell 1949] and total calcium (Ca) content as described by [Francesco and Raffaello 1980].

B. Curd and cheese. Dry matter, fat, total protein contents and titratable acidity were determined according to the methods [AOAC 2007], soluble nitrogen (SN) content in cheese was determined by the micro-Kjeldahl method [Kosikowski and Mistry 1997], sodium chloride content according to [Richardson 1985], and pH using pH meter. The cheese was also analysed for total volatile fatty acids (TVFA) as described by [Kosikowski 1982 b] soluble tyrosine and tryptophan contents of cheese samples were determined according to Vakaleris and Price [1959], total phosphorus content as described by IDF [1966] and Calcium content as described by Raadsveld and Klomp [1971]. Separation of casein by slab gel electrophoresis was carried out by the method of Farkey et al. [1991]. The cheese recovery was calculated according to Vandeweghe and Maubois [1987].

The cheese samples were also analysed for meltability (tube) test as described by Muthukumarappan et al. [1999] meltability (disc) and fat leakage test were determined according to Guinee et al. [1999]. The method described by Kindsted and Fox [1991] was adapted for estimation of free oil in method Mozzarella cheese using Gerber fat testing equipment Bytrometer.

Cheese firmness. The firmness of cheese was measured at 15°C using Koehlerik K19500 penetrometer (Sycamore, AVE USA). The penetrometer was equipped with 2 different weights, the standardized rod weight of 47.5 gm and an additional 35 gm. Weights (total 82.5 gm) were used; the type of cone and the additional weight was selected to allow a better differentiation of firmness values. The whole scale was calibrated into (35) units; each unit was further divided into 10 parts, 0.1 mm each. In making the measurement, a cheese sample was placed on the base and the rod moved down until the tip of the cone release button was depressed for 5 s for recording the distance in units of 0.1 mm. The measurement was repeated three times using three different locations on the surface of a cheese sample and the average of three values was taken.

Sensory evaluation of cheese. The cheese samples were sensory evaluated according to the score card suggested by Land and Shepherd [1988]. Samples were judged by 20 persons of the staff at the Dairy Department, NRC.

Statistical analysis. Statistical analysis was preformed using the GLM procedure with SAS [2004] software. Duncan's multiple comparison procedure was used to compare the means. A probability to $P < 0.05$ was used to establish the statistical significance.

RESULTS AND DISCUSSION

Chemical composition of milk

Table 1 shows the chemical composition of standardized buffalo milk to 3 and 1.5% fat. It can be seen from the obtained results that the percentages of most components of the milk are nearly the same.

Table 1. Chemical composition of standardized fresh buffalo milk

Fat	Components, %									
	DM	fat/DM	TP	TP/DM	Ca	Ca/DM	P	P/DM	acidity	pH
3%	12.32	24.35	3.93	31.90	0.170	1.40	0.162	1.32	0.16	6.75
1.5%	10.85	13.83	3.94	36. 31	0.171	1.58	0.163	1.50	0.16	6.74

Chemical composition of Mozzarella cheese curd

The chemical composition of buffalo Mozzarella cheese curd produced from standardized milk with 3 and 1.5% fat by using different types of coagulant is presented in Table 2. The data revealed that, the moisture content of Mozzarella cheese curd made by using chymosin was highest compared with calf rennet and *Mucor miehei* rennet.

Table 2. Effect of different coagulant on the chemical composition of Mozzarella cheese curd

Components %	Calf rennet		*Mucor miehei* rennet		Chymosin	
	3% F	1.5% F	3% F	1.5% F	3% F	1.5% F
DM	38.95	37.31	42.71	39.76	38.72	37.09
Fat/DM	39.28	25.20	39.30	25.15	39.25	25.07
TP/DM	51.22	65.02	51.99	65.52	50.96	65.25
Ca/DM	1.87	2.28	1.91	2.16	1.85	2.33
P/DM	1.86	2.23	1.89	2.15	1.82	2.31
Yield	17.94	14.98	16.20	14.04	18.34	15.12

These results are in agreement with those reported by Barabano and Rasmussen [1992] and Dave et al. [2003] who found that, cheese made with fermented produced chymosin contained higher moisture than cheese made from other coagulants. Table 2 revealed that fat/DM and TP/DM of curd made using the 3 different coagulants seemed the same.

Furthermore, Table 2 shows the same trend for Ca and P/DM. It was nearly the same in all types of cheese curd. The same table indicates that, the yield percentage of Mozzarella cheese curd increased in case of using chymosin compared with curd produced by using calf rennet and *Mucor miehei* rennet.

These results are in line with the findings of Broom and Hickey [1990] who reported higher cheese yields with fermented produced chymosin than other kinds of coagulants. While Barabano and Rasmussen [1992] mentioned that the differences in cheese yield are related to cheese moisture content and not to the coagulant.

Chemical composition of Mozzarella cheese whey and kneading water

The chemical composition of whey and kneading water of Mozzarella cheese produced by using different types of coagulants from buffalo milk standardized to milk

3 and 1.5% fat are shown in Tables 3, 4. It was noticed that using different types of coagulants had no effect on DM content of both whey and kneading water of Mozzarella cheese made using the same level of fat percentage. Fat content of whey and kneading water of all Mozzarella cheese produced using different types of coagulants was nearly the same Tables 3, 4. Nearly the same values for TP, Ca and P of whey and stretch water were recorded when 3 and 1.5% fat pre-cheese milk used with the three coagulants types. These results are in agreement with those reported by Barabano and Rasmussen [1992] who found that fat and protein loss in whey was not significantly different for fermented produced chymosin and calf rennet.

Table 3. Effect of different coagulant on the chemical composition of Mozzarella cheese whey

Components %	Calf rennet		*Mucor miehei* rennet		Chymosin	
	3% F	1.5% F	3% F	1.5% F	3% F	1.5% F
DM	6.82	5.51	6.93	5.53	6.81	5.51
Fat	0.30	0.10	0.30	0.20	0.30	0.10
TP	0.43	0.41	0.44	0.42	0.43	0.35
Ca	0.05	0.04	0.05	0.05	0.04	0.04
P	0.04	0.04	0.05	0.04	0.04	0.03

Table 4. Effect of different coagulant on the chemical composition of Mozzarella cheese kneading water

Components %	Calf rennet		*Mucor miehei* rennet		Chymosin	
	3% F	1.5% F	3% F	1.5% F	3% F	1.5% F
DM	4.17	3.90	4.27	3.95	4.13	3.90
Fat	0.20	0.1	0.20	0.10	0.20	0.10
TP	0.06	0.04	0.07	0.05	0.06	0.04
Ca	0.04	0.03	0.05	0.04	0.04	0.03
P	0.03	0.02	0.04	0.04	0.04	0.03
Salt	3.82	3.74	3.85	3.78	3.79	3.75

Chemical composition of Mozzarella cheese

From Table 5 it is clear that Mozzarella cheese produced by using different types of coagulants showed that fat/DM%, TP/DM%, Ca/DM% and P/DM% nearly to be the same for all cheese treatments which made from standardized buffalo milk to 3 or 1.5% fat. Similar to the present results Sheehan et al. [2004], Sheehan and Guinee [2004], Rudan et al. [1999], Dave et al. [2003], Oberg et al. [1992] indicated that the coagulant type did not significantly affect cheese composition. This is contrary to the findings of Tunkick et al. [1993] who reported a significantly lower moisture in non-fat-substance content in low fat Mozzarella cheese manufactured with fermentation produced chymosin (FPC) than in the cheese made with *Rhizomucor pusillus* proteinase (RPP), *Cryphonectria parasitica* or calf rennet.

Table 5. Effect of different coagulant on the chemical composition of Mozzarella cheese

Components %	Calf rennet		*Mucor miehei* rennet		Chymosin	
	3% F	1.5% F	3% F	1.5% F	3% F	1.5% F
DM	44.50	43.38	46.80	43.84	44.05	43.30
Fat/DM	38.20	24.90	38.68	25.09	38.14	25.17
TP/DM	50.90	65.74	51.71	65.81	50.74	65.94
Ca/DM	1.65	1.96	1.61	1.92	1.66	2.13
P/DM	1.63	1.91	1.60	1.90	1.65	2.12
Salt	1.65	1.68	1.52	1.66	1.59	1.68
Yield	15.68	12.78	14.34	12.36	15.59	12.38

Recovery and loss of DM, fat and protein of Mozzarella cheese

Recovery of milk DM, fat and TP to both curd and cheese are presented in Table 6, while their loss to whey and kneading water is shown in Table 7. The obtained data showed that, the recovery of DM of both curd and cheese slightly decreases in case of using *Mucor miehei* rennet, while their loss in whey and kneading water also slightly increase, this may be due to the differences in moisture content of the cheese. These results are in agreement with those reported by Barabano and Rasmussen [1992] they mentioned that, there were significant differences in moisture content of the cheese made with different coagulants. This might cause a difference in retention of whey solids in cheese made with different coagulants and create yield differences that are related to cheese moisture content not to the coagulant.

Quality evaluated Mozzarella cheese from different milk sources Ayesha et al. [2008], Ahmed et al. [2008] they indicated that buffalo milk is richer in fat and protein especially casein and casein is the major constituent that influences the cheese yield and chemical composition.

The recovery of fat, TP to both curd and cheese and their loss to whey and kneading water is nearly the same when using the same standardized milk. Protein losses during cheese making can occur by two mechanisms, loss of soluble casein proteolysis products and loss of curd fines. In the present study, curd fines were collected during whey

Table 6. Recovery of dry matter, fat and protein in Mozzarella cheese as affected by application of different types of coagulant

Compo-nents %	Calf rennet				*Mucor miehei* rennet				Chymosin			
	3%		1.5%		3%		1.5%		3%		1.5%	
	curd	cheese	curd	cheese	curd	cheese	curd	cheese	curd	cheese	curd	cheese
DM	56.72	55.01	51.51	50.10	56.10	54.24	56.40	49.14	55.16	56.69	51.68	49.94
Fat	91.49	89.85	93.87	90.02	91.72	90.14	93.60	90.14	89.94	91.40	93.74	89.96
TP	91.37	90.17	92.17	90.51	91.51	89.99	92.82	90.50	90.12	91.50	92.87	89.71

Table 7. Losses of dry matter, fat and protein in Mozzarella cheese as affected by application of different types of coagulant

Components %	Calf rennet				*Mucor miehei* rennet				Chymosin			
	3%		1.5%		3%		1.5%		3%		1.5%	
	whey	kneading water	whey	kneading water	whey	kneading water	whey	kneading water	whey	kneading water	whey	kneading water
DM	33.46	11.53	36.19	13.40	33.90	11.86	37.26	13.60	11.41	11.41	36.66	13.40
Fat	7.50	2.70	8.65	1.33	7.06	2.80	8.53	1.33	7.36	2.70	8.71	1.33
TP	9.21	0.62	8.93	0.56	9.34	0.67	8.85	0.65	9.27	0.61	9.73	0.56

and were added back to the curd. Therefore, difference in protein losses reported in the present study should reflect loss of soluble casein proteolysis products. Barabano and Rasmussen [1992] found that, when fermentation produced chymosin, calf rennet and adult bovine rennet were compared; there were no significant differences between coagulants for protein recovery in the whey and cheese.

Soluble nitrogen (SN) content

Soluble nitrogen content of Mozzarella cheese made from standardized milk containing 3 or 1.5% fat is presented in Table 8. It is clear that, the soluble nitrogen of all cheeses increased with increasing the storage period.

Table 8. Effect of coagulants types on soluble nitrogen and total volatile free fatty acids of Mozzarella cheese during storage for 4 weeks at 4°C

Properties	Storage period weeks	Calf rennet		*Mucor miehei* rennet		Chymosin	
		3% F	1.5% F	3% F	1.5% F	3% F	1.5% F
Soluble nitrogen, %	fresh	0.135^{Aa}	0.214^{Ba}	0.144^{Aa}	0.245^{Ba}	0.140^{Aa}	0.230^{Ba}
	1	0.162^{a}	0.225^{a}	0.226^{b}	0.250^{a}	0.216^{b}	0.242^{a}
	2	0.210^{b}	0.248^{b}	0.252^{b}	0.284^{b}	0.238^{b}	0.254^{b}
	3	0.245^{c}	0.262^{b}	0.286^{c}	0.322^{c}	0.254^{c}	0.278^{c}
	4	0.268^{c}	0.284^{c}	0.318^{c}	0.348^{c}	0.288^{c}	0.311^{c}
T.V.F.A. ml 0.1 N NaOH/100 gm cheese	fresh	3.0^{Aa}	2.0^{Ba}	3.10^{Aa}	2.02^{Ba}	3.40^{Aa}	3.00^{Ba}
	1	4.0^{a}	3.14^{a}	4.0^{a}	3.19^{b}	4.60^{a}	3.42^{a}
	2	6.30^{b}	3.69^{a}	6.46^{b}	3.70^{b}	8.0^{b}	4.36^{a}
	3	8.52^{b}	6.28^{b}	8.37^{b}	6.24^{c}	8.70^{b}	6.82^{b}
	4	12.54^{c}	10.92^{c}	12.55^{c}	10.88^{c}	12.98^{c}	10.52^{c}

Dissimilar superscripts in the same row (for treatments) and the same column (for storage periods) are significantly different ($p < 0.05$). Each value is a mean of 3 replicates.

It could be seen from the previous table that the increasing rate of the soluble nitrogen in case of using *Mucor miehei* rennet in Mozzarella cheese making was higher compared with that made from the other types of coagulants. The increasing of soluble nitrogen content in case of using *M. miehei* rennet was due to the inactivation of the other types of coagulants during the stretching, while *Mucor miehei* rennet stills active.

In accordance with our results Dave et al. [2003] found that, when low moisture part-skim (LMPS) Mozzarella cheeses were made with single culture (SC) of *Streptococcus thermophilus* or mixed culture (MC) of *Streptococcus thermophilus* and *Lactobacillus helveticus* using coagulants chymosin or *Cryphonectria parasitica* (CP). Cheeses were analysed for total solids, fat, protein, ash, salt, and calcium on day 1. Changes in melt characteristics and proteolysis during storage (4°C) were monitored on 1, 7, 15, and 30 days. After 30 days storage, melt area increased only by approximately 2 times in cheeses made with SC as against 3-4 times in the cheeses made with MC. Soluble nitrogen was also higher in MC cheeses as compared to those made using SC only.

The statistical analysis of obtained results showed that, the soluble nitrogen content was highly significant affected by the storage period ($F = 249$). While coagulants types had highly significant effect on soluble nitrogen content in original milk used on cheese soluble nitrogen content ($F = 181$). Moreover, the influence of storage period and fat content in the original milk interaction with using different types of coagulants on the soluble nitrogen had a highly significant effect ($F = 546$).

Electrophoresis patterns of cheese

The slab gel electrophoresis patterns of Mozzarella cheese during 4 weeks of refrigerated storage at 4°C are shown in Figure 1 A and B zone one contains αs1- and αs2--caseins and zone two contains ß-casein. Proteolytic breakdown products of αs- and ß--casein are shown as new bands which showed faster movement than the original bands. The amount of αs1- and αs2-caseins as a percentage of total casein fractions decreased for all cheeses during refrigerated storage which may be due to the photolytic enzymes came from the bacterial cultures used in cheese making. The effect of coagulants types on the breakdown of the protein was not clear in case of using calf rennet and chymosin, while was clear in case of using, *M. miehei* rennet and could be attributed to the high heat treatment used in stretching. These results are in line with the finding of Fox [1993] who mentioned that, the protease from *M. miehei* was the most heat stable followed in order by *M. pusillus* protease, calf rennet, bovine pepsin, *E. parasitica* protease and porcine pepsin. These results are in agreement with those reported by Dave et al. [2003]. The degradation of total αs-casein was higher in the chymosin cheeses and that of β-casein in the *Cryphonectria parasitica* (CP) cheeses. After 30 d storage, the highest percentage breakdown of αs-casein was approximately 75% in cheeses made with mixed culture (MC) using chymosin and that of β-casein was approximately 50% in cheese samples prepared with MC coagulant from *Cryphonectria parasitica*. Meltability of Mozzarella cheese was better correlated to hydrolysis of β-casein and was comparable to soluble nitrogen but least to αs-casein.

Di-Matteo et al. [1982], Farkye et al. [1991], Yun et al. [1993 b] who pointed that, the residual coagulant activity in Mozzarella cheese was demonstrated by either a decrease in intact casein content or an increase in soluble nitrogen content. Also,

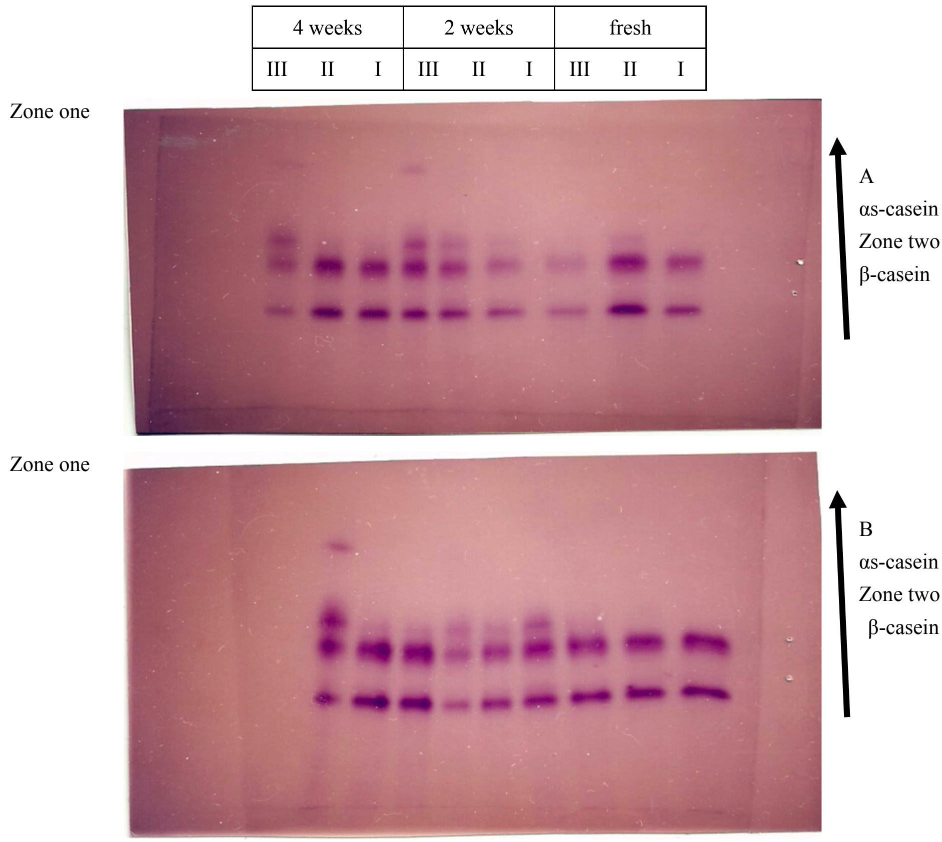

Fig. 1. The eletrophoresis gel of Mozzarella cheese made using (I) calf rennet, (II) chymosin and (III) *Mucor miehei* rennet. A – 3% fat and B – 1.5% fat. Zone one – αs-casein and zone two – β-casein

Creamer [1976] observed a slower rate of casein degradation in Mozzarella cheese than in Cheddar or Gouda and suggested that it may have been due to the high temperature used during stretching. Also, the effect of the coagulant types on the breakdown of ß-casein is not clear. Yun et al. [1993 a] mentioned that, ß-casein in cheese made with CDF and *M. miehei* protease remained relatively constant during refrigerated storage. However, ß-casein in cheese made with *E. parasitica* protease decreased significantly.

Total volatile free fatty acids (TVFA) content

Variations in total volatile free fatty acids of Mozzarella cheese made from standardized buffalo milk to 3 or 1.5% fat with using different types of coagulants are illustrated in Table 8.

The statistical analysis of the obtained results indicated that, the TVFA content of cheese was highly significantly affected by the storage period (F = 807.91) and fat content of cheese (F = 194.73). Moreover, no significant effect could be seen by the actions of coagulants types on TVFA (F = 0.72). Furthermore, a significant influence was observed on the TVFA of Mozzarella cheese by the interaction between the storage period, fat content and the coagulant types (F = 2.01).

Soluble tyrosine and tryptophan contents

Soluble tyrosine and tryptophan expressed as mg/100 gm cheese are mentioned in Table 9. Soluble tyrosine and tryptophan considered good indicators for cheese ripening during the storage period. The cheese content of tyrosine and tryptophan showed the same trend during the storage period it could be noticed that they elevated with the increasing of the storage period and decreased with increasing the fat content in the original milk used. The rate of elevation in case of using *M. miehei* rennet was higher compared with the other types of coagulants because *M. miehei* rennet remains active when exposing to high temperature, than the other types.

Table 9. Effect of coagulants types on soluble tyrosine and soluble tryptophan of Mozzarella cheese during storage for 4 weeks at 4°C

Properties	Storage period weeks	Calf rennet		*Mucor miehei* rennet		Chymosin	
		3% F	1.5% F	3% F	1.5% F	3% F	1.5% F
Soluble tyrosine mg/100 g cheese	fresh	3.62^{Aa}	4.24^{Ba}	3.68^{Aa}	5.95^{Ba}	3.65^{Aa}	5.56^{Ba}
	1	8.92^{a}	9.79^{a}	9.76^{a}	11.89^{a}	8.99^{a}	11.32^{a}
	2	18.75^{b}	20.26^{b}	20.41^{b}	23.21b	18.82^{b}	22.18^{b}
	3	40.87^{c}	44.30^{c}	54.32^{c}	56.45^{c}	44.84^{c}	45.74^{c}
	4	52.21^{c}	56.88^{c}	60.85^{c}	68.74^{c}	56.40^{c}	59.95^{c}
Soluble tryptophan mg/100 g cheese	fresh	1.64^{Aa}	1.89^{Ba}	1.92^{Aa}	2.20^{Ba}	1.72^{Aa}	2.04^{Ba}
	1	5.26^{a}	6.62^{a}	6.87^{a}	8.21^{a}	5.64^{a}	7.86^{a}
	2	12.64^{b}	15.28^{b}	14.47^{b}	17.18^{b}	12.82^{b}	16.74^{b}
	3	25.86^{c}	30.18^{c}	27.38^{c}	33.37^{c}	26.08^{c}	32.12^{c}
	4	37.87^{c}	43.11^{c}	39.74^{c}	46.76^{c}	37.97^{c}	44.78^{c}

Dissimilar superscripts in the same row (for treatments) and the same column (for storage periods) are significantly different ($p < 0.05$). Each value is a mean of 3 replicates.

The statistical analysis of results indicated a higher significant effect of storage period (F = 243.81), (F = 457.11), fat content of the original milk used (F = 180.2), (F = 860.12) and coagulant types (F = 156.2), (F = 46.25) on the cheese tyrosine and tryptophan contents respectively. Furthermore, the interaction between storage period, fat content and coagulant types was highly significant affected both soluble tyrosine and tryptophan contents (F = 103.64), (F = 24.4) respectively.

Acid and pH values

Table 10 shows the acidity and pH values of Mozzarella cheese made from standardized buffalo milk to 3 or 1.5% fat using different types of coagulants.

Table 10. Effect of coagulants types on the acidity and pH value of Mozzarella cheese during storage for 4 weeks at 4°C

Properties	Storage period weeks	Calf rennet		*Mucor miehei* rennet		Chymosin	
		3% F	1.5% F	3% F	1.5% F	3% F	1.5% F
Acidity, %	fresh	0.64	0.70	0.68	0.74	0.66	0.72
	1	0.66	0.72	0.74	0.78	0.70	0.74
	2	0.68	0.74	0.78	0.82	0.72	0.76
	3	0.70	0.76	0.84	0.80	0.74	0.78
	4	0.74	0.78	0.90	0.84	0.78	0.80
pH	fresh	5.3	5.3	5.3	5.3	5.3	5.3
	1	5.2	5.2	5.10	5.0	5.2	5.1
	2	5.1	5.0	4.80	4.65	5.0	4.8
	3	4.8	4.8	4.6	4.40	4.7	4.6
	4	4.4	4.60	4.00	4.00	4.25	4.4

It is clear from the obtained data that, the acidity increasing and pH value decreased with the progressing of the storage period of all treatments. The types of coagulants had no effect on the development of the acidity and decreased pH value due to the high temperature used during stretching, and the changes of the obtained data caused by the action of the bacterial cultures used. The fore-mentioned data showed that, high fat cheese had lower acidity and higher pH value compared with that made low fat milk (1.5%). These results are in line with the finding reported by Patel et al. [1986] who mentioned that, high fat Mozzarella cheese was found to have lower acidity and higher pH than those from low fat cheese.

Fat leakage and oiling off

The fat leakage and oiling off of Mozzarella cheese prepared from standardized buffalo milk 3 or 1.5% fat using different types of coagulants are presented in Table 11. It was clear that, a direct relation was found between both fat leakage and oiling off of cheese on the one hand and fat content of the original milk used in cheese making on the other hand. The effect of coagulants types on fat leakage and oiling off was neglected because of the high temperature used during stretching. Matheson [1981] found that, residual rennet is not active in Mozzarella cheese owing to the high cooking temperature.

The data revealed that fat leakage and oiling off of all the treatments increase during the storage period at 4°C with sharp increases in the first 2 weeks. These results are

Table 11. Effect of coagulants types on fat leakage and oiling off of Mozzarella cheese during storage for 4 weeks at 4°C

Properties	Storage period weeks	Calf rennet		*Mucor miehei* rennet		Chymosin	
		3% F	1.5% F	3% F	1.5% F	3% F	1.5% F
Fat leakage, cm²	fresh	66.24^{Aa}	45.68^{Ba}	67.42^{Aa}	45.32^{Ba}	67.86^{Aa}	46.90^{Ba}
	1	89.10^{a}	52.62^{a}	90.82^{b}	52.80^{a}	89.64^{b}	69.72^{b}
	2	93.88^{b}	68.10^{a}	94.41^{b}	69.64^{b}	94.62^{c}	70.98^{c}
	3	96.46^{b}	71.82^{b}	97.48^{b}	72.43^{c}	97.80^{c}	74.88^{c}
	4	102.12^{c}	74.74^{b}	103.63^{c}	76.45^{c}	106.34^{c}	79.70^{c}
Oiling off, %	fresh	3.5^{Aa}	0.5^{Ba}	3.4^{Aa}	0.6^{Aa}	3.6^{Aa}	0.5^{Ba}
	1	5.5^{a}	1.0^{a}	5.5^{a}	1.1^{a}	5.6^{a}	1.1^{a}
	2	7.7^{b}	1.5^{b}	7.8^{b}	1.5^{b}	7.7^{b}	1.4^{b}
	3	8.0^{b}	1.6^{b}	8.7^{b}	1.6^{b}	8.4^{c}	1.6^{b}
	4	9.5^{c}	1.8^{b}	9.6^{c}	1.9^{c}	10.0^{c}	1.8^{c}

Dissimilar superscripts in the same row (for treatments) and the same column (for storage periods) are significantly different ($p < 0.05$). Each value is a mean of 3 replicates.

in agreement with those reported by Fox [1993] who mentioned that, free oil formation showed characteristic pattern of change during aging, with a sharp increase in the first 1-2 weeks followed by a gradual increase thereafter. Also, the data revealed that, fat content of the original milk used had a high effect on the fat leakage and oiling off of the cheese. Fox [1993] maintained it was obvious that, fat content is a strong determinant of oiling off, as indicated from surveys of commercial Mozzarella cheese. He mentioned also that, cheese with high F/DM tended to yield high free oil levels as measured by the disk fat leakage test or the centrifugal free oil test.

The statistical analysis of the results showed that, both fat leakage and oiling off of Mozzarella cheese was highly significantly affected by the fat percentage in the initial milk used ($F = 80.64$) ($F = 90.41$). Also, the storage period showed a highly significant effect on the fat leakage and oiling off of Mozzarella cheese ($F = 33.43$) ($F = 27.08$). These influences of interaction of fat percentage of the original milk used and the storage period had a significant effect ($F = 2.29$) ($F = 2.54$).

Meltability

Meltability of Mozzarella cheese made from standardized buffalo milk 3 or 1.5% fat using different types of coagulants are shown in Table 12. The meltability of Mozzarella cheese either by disc or by tube methods grew with increasing of the storage period. Although, many investigations showed that, the increase in meltability of the cheese made using chymosin increased with faster rate during storage compared with the other types of coagulants. In our study, the meltability increased with storage time in case of using *M. miehei* rennet than calf rennet and chymosin because, the residual coagulant

Table 12. Effect of coagulants types on the meltability by of Mozzarella cheese during storage for 4 weeks at 4°C

Properties	Storage period weeks	Calf rennet		*Mucor miehei* rennet		Chymosin	
		3% F	1.5% F	3% F	1.5% F	3% F	1.5% F
Meltability, mm (tube method)	fresh	0.0^{Aa}	0.0^{Ba}	10^{Aa}	4^{Ba}	0.0^{Aa}	0.0^{Ba}
	1	58^{b}	50^{b}	72^{b}	64^{b}	60^{b}	58^{b}
	2	76^{b}	60^{b}	92^{b}	77^{b}	78^{cb}	68^{cb}
	3	82^{c}	70^{bc}	106^{c}	89^{c}	85^{c}	78^{c}
	4	112^{c}	78^{c}	134^{c}	100^{c}	116^{c}	86^{c}
Meltability, cm^2 (disc method)	fresh	15.11^{Aa}	8.78^{Ba}	20.43^{Aa}	9.88^{Ba}	17.60^{Aa}	9.12^{Ba}
	1	20.24^{b}	8.89^{a}	36.62^{b}	11.24^{b}	25.81^{b}	10.42^{ab}
	2	25.30^{b}	12.23^{b}	42.81^{cb}	14.87^{b}	31.64^{bc}	13.42^{b}
	3	30.85^{c}	19.48^{c}	48.67^{c}	23.25^{c}	34.62^{c}	21.86^{c}
	4	41.68^{c}	24.85^{c}	54.88^{c}	26.81^{c}	45.73^{c}	25.97^{c}

Dissimilar superscripts in the same row (for treatments) and the same column (for storage periods) are significantly different ($p < 0.05$). Each value is a mean of 3 replicates.

retained in the cheese inactivated by temperature and pH that occurred during cheese making in case of using calf rennet and chymosin. These results are in line with the findings of Dave et al. [2003] who showed that Melt characteristics of 7 days cheeses prepared with mixed culture (MC) almost corresponded to those of 30 days cheeses made with single culture (SC), suggesting faster ripening and increase in melt of cheeses made with MC. Oberg et al. [1992] mentioned that stretch and melt of direct acidification of Mozzarella cheese vary with choice of enzyme. Kindsted [1993] reported that, in Mozzarella cheese, coagulant had been widely reported to be fully inactivated high temperature during stretching. Fox [1993] found that, the protease from *M. miehei* was the most heat stable followed in order by *M. Pusillus* protease, calf rennet, bovine pepsin and porcine pepsin. Also, he mentioned that the native protease from M. *miehei* and *M. Pusillus* are rather thermo stable at pH 5.9-6.6. Abu-Alruz et al. [2009] showed that use of proteolytic enzymes to induce meltability and stretchability of Nabulsi cheese was proved to be an efficient method. The meltability and stretchability of Nabulsi cheese treated with papain were still excellent after 4 weeks of storage than the after coagulant enzyme.

The meltability of Mozzarella cheese made from pre-cheese milk 3% fat was higher than that made from 1.5% fat. Patel et al. [1986], Tunkick et al. [1991], Sundar and Upadhyay [1992] found that, the high fat cheeses had significantly higher meltability which attributed to initially higher fat content in cheese milk resulting in cheese with low viscosity or with soft texture, thus rendering it more meltable both horizontally and vertically.

The statistical analysis of the obtained results showed a highly significant effect on the cheese meltability either when disc or tube methods was applied (F = 2468.6 and

457.1 for disc and tube test respectively). Also, the storage period was found to be highly significant and affected the meltability of Mozzarella cheese (F = 1684.2 and 46.2 for disc and tube test respectively). Moreover, the inter action of fat content of the original milk used with storage period and coagulants type significant was (F = 2.19 and 2.44 for disc and tube test respectively).

Firmness

Mozzarella cheese firmness values made from standardized buffalo milk 3 or 1.5% fat using different types of coagulants are shown in Table 13. The data obtained revealed that, the firmness of the cheese when fresh and after storage period at 4°C decreased with increasing the fat content of the original pre-cheese milk used. Also, the data showed that, Mozzarella cheese made using *M. miehei* rennet, obtained the highest firmness compared with those made using calf rennet or chymosin. These results are in agreement with those reported by Walstra and Jenness [1984] who stated that, increasing the fat content gives a weaker gel because of the reduced level of gel forming protein and a physical interference of the fat globules with protein net work.

Table 13. Effect of coagulants types on the firmness* of Mozzarella cheese (as penetration distance in mm) during storage for 4 weeks at 4°C

Storage period weeks	Calf rennet		*Mucor miehei* rennet		Chymosin	
	3% F	1.5% F	3% F	1.5% F	3% F	1.5% F
Fresh	68^{Aa}	56^{Ba}	64^{Aa}	52^{Ba}	65^{Aa}	55^{Ba}
1	65^{a}	52^{a}	61^{a}	48^{a}	62^{a}	50^{a}
2	60^{b}	48^{b}	55^{b}	44^{b}	57^{b}	46^{b}
3	52^{c}	44^{c}	48^{c}	40^{c}	50^{c}	42^{c}
4	45^{c}	40^{c}	40^{c}	34^{c}	44^{c}	38^{c}

*The higher record the less firmness.

Dissimilar superscripts in the same row (for treatments) and the same column (for storage periods) are significantly different ($p < 0.05$). Each value is a mean of 3 replicates.

The statistical analysis of Mozzarella cheese firmness showed that, the firmness of Mozzarella cheese was highly significant affected by the fat content of the original milk used (F = 457.1). Furthermore, the storage period of Mozzarella cheese had a highly significant influence on the firmness (F = 46.25). Moreover, the interaction between pre-cheese milk fat, storage period and coagulants types had a significant influence on the firmness of Mozzarella cheese (F = 2.44).

Sensory evaluation

The Sensory evaluation of Mozzarella cheese made from standardized buffalo milk to 3 or 1.5% fat with different types of coagulants are presented in Table 14. The flavour of all cheese is nearly the same at the same level of the fat in the initial milk used when fresh, while the appearance records increased with decreasing the fat content of the

Table 14. Effect of coagulants types on the sensory evaluation of Mozzarella cheese during storage for 4 weeks at 4°C

Sensory evaluation	Storage period weeks	Calf rennet		*Mucor miehei* rennet		Chymosin	
		3% F	1.5% F	3% F	1.5% F	3% F	1.5% F
Flavour (50)	fresh	36	34	37	35	37	35
Body and texture (35)		23	20	22	19	23	19
Appearance (15)		13	14	13	15	13	15
Total (100)		72[Aa]	68[Ba]	72[Aa]	69[Ba]	73[Aa]	69[Ba]
Flavour (50)	1	37	35	38.5	35.5	38	36
Body and texture (35)		24	21	24	21	24	20
Appearance (15)		11	13	12	14	11	13.5
Total (100)		72[a]	69[a]	74.5[a]	70.5[a]	73[a]	69.5[a]
Flavour (50)	2	38	36	40	37.5	39	37
Body and texture (35)		26	22	27	24	25	22
Appearance (15)		10	12	11	13	10	12
Total (100)		74[b]	70[ab]	78[b]	74.5[b]	74[a]	71[b]
Flavour (50)	3	39	37	42	40	40	37
Body and texture (35)		28	24	29	27	26	23
Appearance (15)		9.5	11	10	12	9.5	11
Total (100)		76.5[b]	72[b]	81[c]	79[c]	75.5[b]	72[c]
Flavour (50)	4	41	38	45	42	41	39
Body and texture (35)		29	25	31	30	27	24
Appearance (15)		9	10	8	9	9	10
Total (100)		79[c]	73[b]	84[c]	81[c]	77[c]	73[c]

Samples were judged by 20 persons of the colleague staff members.

original milk used. The body and texture values of the cheese increased with the fat content of the initial milk used. Also, the flavour records of all cheese increased during the storage period, this increase can be attributed to the proteolysis of the cheese curd. The rate of increasing flavour score in case of Mozzarella cheese made by using *M. miehei* rennet was higher compared with that made by using calf rennet and chymosin.

The statistical analysis variance of Mozzarella cheese showed that, the flavour of the cheese was highly significant effected by the fat content of the original milk used ($F = 16.66$). While the storage period was found to be highly significant effected the flavour of Mozzarella cheese ($F = 3.65$). Furthermore, the cheese flavour was found be significant effected as a result of the interaction between the fat content of the original milk used, storage period and coagulants types ($F = 2.65$).

Body and texture of Mozzarella cheese tended to improve with increasing of the storage period. It was cleared from the obtained data, the rate of improving in body and texture was higher in Mozzarella cheese made by using *M. miehei* rennet compared with the other type's coagulant and this could be attributed to the heat resistance of *M. miehei* enzymes during stretching which remained active during the storage period at 4°C for 4 weeks.

The statistical analysis of the results showed that, body and texture of the cheese were highly significant affect by the fat content of the initial milk used (F = 19.2). While the storage period was found to be highly significant and affected the body and texture of Mozzarella cheese (F = 30.00). Moreover, the influence of fat percentage in the original milk used interacted with the storage period and coagulant types on the body and texture had a significant effect (F = 2.48). The same table shows the appearance score of the cheese with increasing the storage period at 4°C.

The statistical analysis showed that, the appearance score of Mozzarella cheese when fresh was highly significant and affected by the fat percentage of the initial milk used (F = 19.24.71). Also, the storage period was found to be highly significant affected the appearance records of Mozzarella cheese (F = 21.83). Furthermore, the influence of the interaction between the fat percentage of the initial milk used, storage period and type coagulants on the appearance had a significant effect (F = 2.38). It could be seen that the total scores of Mozzarella cheese made using *M. miehei* rennet either when fresh or after storage period for 4 weeks at 4°C gained the highest score. Also, the total scores increased during the storage period for all treatments.

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WŁAŚCIWOŚCI SERA MOZZARELLA Z MLEKA BAWOLEGO W ZALEŻNOŚCI OD RODZAJU UŻYTEGO KOAGULANTU

Wstęp. Ser mozzarella należy do najpopularniejszych serów podpuszczkowych miękkich bez skórki. Przyczynami jego rozpowszechnienia, a tym samym podejmowania badań, są nie tylko walory smakowo-zapachowe i wartość odżywcza, ale przede wszystkim wartość funkcjonalna i użytkowa. Celem pracy była ocena jakości świeżego i przechowywanego sera mozzarella z mleka bawolego oraz powstałej po jego wygniataniu serwatki i wody w zależności od użytej podpuszczki.
Materiał i metody. Mozzarellę zrobiono z wystandaryzowanego mleka bawolego zawierającego 3 i 1,5% tłuszczu. Efekt koagulacji mleka uzyskano z użyciem podpuszczki cielęcej, chymozyny otrzymanej na drodze fermentacji i podpuszczki mikrobiologicznej otrzymanej z *Mucor miehei*. Poddano analizie mleko, mleko ukwaszone, wodę po wygniataniu i gotowy ser. Do rozdziału białek kazeinowych zastosowano metodę elektroforezy płytowej. Uzyskane wyniki poddano analizie statystycznej.
Wyniki. Większy był odzysk suchej substancji w serze otrzymanym z wykorzystaniem podpuszczki mikrobiologicznej *Mucor miehei*, mimo zawartości ogólnej białka i tłuszczu takiej samej jak w pozostałych serach. Wraz z wydłużeniem czasu przechowywania doszło do zwiększenia ilości azotu rozpuszczalnego, rozpuszczonej tyrozyny i tryptofanu. Zaobserwowano wzrost zawartości azotu rozpuszczalnego w serze otrzymanym z użyciem podpuszczki *Mucor miehei* w porównaniu z pozostałymi serami, w których zawartość wolnych kwasów tłuszczowych rosła z zawartością tłuszczu w mleku przerobowym. Rozpływalność serów zwiększała się wraz z wydłużaniem czasu przechowywania. Rodzaj użytego enzymu koagulującego nie miał wpływu na rozpływalność serów i wyciek frakcji olejowej.
Wnioski. Ser mozzarella robiony z wykorzystaniem podpuszczki z *Mucor miehei* uzyskał większą zwięzłość w porównaniu z serami otrzymanymi z użyciem chymozyny z procesów fermentacyjnych i podpuszczki cielęcej. Wyniki oceny smaku, wyglądu oraz parametrów tekstury polepszyły się wraz z wydłużeniem czasu przechowywania i jednocześnie były lepsze dla sera uzyskanego z udziałem podpuszczki mikrobiologicznej *Mucor miehei*. Natomiast pożądalność ich wyglądu zmniejszyła się z wydłużeniem okresu przechowywania w 4°C.

Słowa kluczowe: ser mozzarella, mleko bawole, jakość, podpuszczka

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