Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Impact of the Supplementation with Partially Chymosin-Proteolyzed Buffalo's Total Milk Proteinate on the Properties of Cow's Set Yoghurt

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Abstract

The aim of this study was to experiment the supplementation of cow's yoghurt milk with partially chymosin-proteolyzed buffalo's TMP the relation to compositional, bacterial, biochemical, rheological and organoleptic attributes of the resultant product. Four treatments including the control were designed, where cow's full cream milk powder (FCMP) was reconstituted whether at the level of 14% FCMP with tap water (the control) or at the level of 13% FCMP with solution containing 1% TMP in the native form (i.e. non enzymatic hydrolyzed) or solution of TMP proteolyzed by chymosin for 4 or 8 h. Yoghurt milks were heat treated at 85°C for 5 min. followed by temperature adjustment to 42°C at which they were inoculated with 2% of activated yoghurt bacterial starter culture, filled into 100 ml polyvinyl chloride containers, covered, and incubated until complete coagulation (through about 3 h.). Thereafter, the container were transferred to the refrigerator (5±1°C), where they were kept for the periodical analyses. The results indicated that, the viscosity value as well as the capacity and stability of the emulsion property of solution containing 5% TMP proteolyzed by 1% chymosin enzyme decreased as the proteolysis time prolonged. On the other hand both of the water soluble nitrogen (WSN) and non-protein nitrogen (NPN) of TMP solution increased as the proteolysis time increased. No significant differences in both of moisture, fat, titratable acidity contents as well as the Log count of lactic acid bacteria between yoghurt treatments but the supplementation with 1% TMP, whether in the native or rather in the proteolyzed form, was associated with significantly gradual increases in the protein, WSN/TN, NPN/TN contents as well as pH value and decrease in the ash content of yoghurt. The prolonging of cold storage period (CSP) was associated with significant increase in yoghurt acidity. Neither yeasts & molds nor total coliform exhibited any considerable count in yoghurt along CSP. Although the cohesiveness criterion was not influenced, the hardness of yoghurt strengthened either due the presence of TMP or/and by the progressing of the cold storage period. Moreover, the degree of TMP proteolysis was associated with increase of yoghurt hardness. The adhesiveness appeared opposite trending to those of the hardness in all cases. The control sample possessed a value higher than that of non proteolyzed TMP. The TMP proteolysis whether for 4 h or rather for 8 h heightened the springiness values of yoghurt supplemented therewith. The gumminess value of yoghurt heightened when TMP added and as TMP was proteolyzed or cold stored. Chewiness behaved similar trending to gumminess in yoghurt when fresh. At the end of CSP, chewiness value of the control and yoghurt containing native TMP decreased, while those of proeolyzed TMP whether for 4 or 8 h increased. All TMP containing yoghurt gained the highest judging score and the sample of native or 4h-proteolyzed TMP kept the best sensory preference until the end of CSP. It can be concluded that: Although the TMP derivative is a protein compound, its proteolysis with chymosin, which κ-casein is its preferred substrate, resulting in the liberation of peptide, para κ-casein that helped more in strengthening the weak curd, from which cow's yoghurt is suffered and homogenizing its consistency, provided that the period of proteolysis does not exceed 4 h by 1% enzyme.

Keywords: Specifications of TMP and Chymosin; Viscosity as well as Emulsion property of TMP; Chemical, Microbiological and Texture profile parameters of Yoghurt

1. Introduction

Yoghurt or Zabadi is the most famous fermented dairy product in world and well-being has existed in many civilizations for a long time. The most important characteristics of yoghurt for the consumer

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EJCHEM use only: Received date 27 November 2022; revised date 01 October 2023; accepted date 07 December 2022
DOI: 10.21608/EJCHEM.2022.177257.7238

are its flavor, eye appeal, and mouthfeel of these only the last is in any way connected with rheology. The mouthfeel is largely influenced by smoothness of creaminess, which are in some way depended upon the viscosity and perception of solid-matter content (1).

A major concern facing the yoghurt industry is the production and keeping of a product with optimum quality, especially consistency and stability. Where a main criteria of quality assessment of yoghurt are the physical properties of the obtained gel (2), (3). Therefore, from the manufacture's point of view, the physical properties of yoghurt. e.g. viscosity / consistency of the end product (4).

Physical characteristics of yoghurt depend upon the type of yoghurt, with "set style" yoghurt exhibiting a firm gel, i.e. the strength of the coagulum and its ability to immobilize water (2),(3),(5),(6) and (7). The texture of set-style yoghurt, gel firmness and gel structure are important features which are mainly affected by protein. The smoothness, which is characterized by the particle size, is mainly related to the structure (permeability) of the yoghurt gel. Adequate firmness without synersis is essential for a superior quality set-yoghurt (8),(9). Physical properties of yoghurt are influenced by milk composition and manufacturing conditions. Variables affecting physical properties include heat treatment applied to milk, protein content, homogenization, developed acidity, mechanical handling of coagulum and the presence of stabilizers (2), (3),(5), and (10).

Whereas, cow's milk is the main obtainable raw milk supply for dairy industries in many countries of world. With respect to yoghurt manufacture, due to the cow's milk is relatively characterized with thin body because of its solids deficiency, the obtained yoghurt is usually suffering from a pronounced weak consistency and wheying off defects. In some countries, the use of non dairy stabilizers in yoghurt making for consistency improvement is governed by legislative regulation (11). The use of such non dairy ingredients in yoghurt is not legally permitted in Egypt (12) as well as in many other countries (4) (13), therefore, solids enrichment became necessary when yoghurt was designed to be made from cow's milk for overcoming such disadvantages. Thermal treatment was the old traditional produce applied to prevent such defects by heat solids concentration. That has been also achieved by the direct addition either of skimmed milk powder (14), (15), total milk proteinate (13), (16), whey protein concentrate (17)

(18). Moreover, the technique of membrane filtration, especially the ultrafiltration, has successfully contributed with an efficient role to avoid such faults in yoghurt quality (19),(20) ,(21) and (22).

Total milk proteinate (TMP) are a form of milk protein isolate similar in composition to co-precipitate but retaining their proteins in a highly soluble and functional form (23).(13) supplemented yoghurt milk with 0.5, 1.0 and 1.5% (w/v) TMP powder instead of 3% SMP. They found that, although the acidity development was not affected by the TMP levels, yoghurt formulated with 1-1.5% TMP and higher protein and lower lactose and ash content as compared with the control samples. Apparent viscosity, yield stress value and consistency coefficient of the fresh and stored yoghurt were significantly increased while the curd syneresis was significantly decreased with addition of increasing amounts of TMP. Flow behavior index of the yoghurt samples was not markedly affected either by the type and concentration of the dry matter used or by cold storage for 7 days. Supplementation of milk with 1.5% TMP produced yoghurt of referable organoleptic properties, higher viscosity and reduced susceptibility to syneresis compared with yoghurt made from milk fortified with 3% SMP. In another study, yoghurt properties were considerably improved when whole milk protein was added at a level ranged from 1-3% (16).

For that in view, the aim of this study was to experiment the supplementation of cow's yoghurt milk with partially chymosin-proteolyzed buffalo's TMP the relation to compositional, bacterial, biochemical, rheological and organoleptic attributes of the resultant product.

MATERIALS AND METHODS

1. Materials

Fresh buffalo's skimmed milk was obtained from the herd of the dairy cattle at Faculty of Agriculture, Ain Shams University. Cow's full cream milk powder (FCMP) as well as skimmed milk powder (SMP) made by SFK DATABLAD, Hvidovre and Viborg, Denemark was obtained from the local market at Cairo. Calf chymosin (EC 3.4.23.4) and lyophilized mixed yoghurt starter culture (YSC) containing Streptococcus thermphilus and Lactobacillus delbrueckii subsp. bulgaricus were obtained from Chr. Hansen's Laboratory, Copenhagen, Denmark.

2. Experimental procedures

2.1. Preparation of buffalo's total milk proteinate

Buffalo's total milk proteinate (TMP) was prepared according to the method described by (23) but from buffalo skimmed milk as follow: Skimmed milk was alkalized by 1N NaOH to pH 10 and heated to 70°C for 10 min to solubilize casein micelles .The pH was then adjusted to pH 3.5 at the room temperature to complex the whey proteins and casein, using 1N HCl. Again the pH was raised to pH 4.6 by 1N NaOH to precipitate the complexed proteins. Proteins precipitate was drained using a cheese cloth. The precipitate was washed and dried using an air furnace under decreased pressure at 60 °C for 6-8 h as shown in Figure (1).

2.2. **Enzymatic proteolysis of bufallo's total milk proteinate**

Enzymatic proteolysis of bufallo's TMP by chymosin was carried out according to the method of (24) by dissolving dried TMP in distilled water at pH 8 using NaOH 2N and agitated by an magnetic stirrer to the complete dissolution. Mixing ceased and stored it overnight at 4 °C in a sealed vessel. The pH of the solution was adjusted 6.6 using lactic acid. The temperature was equilibrated to 42 °C thereafter, chymosin was added at the rate of 1 g enzyme per 100 g dried TMP at two proteolysis times 4.0 and 8.0 h. Then, the reactions were stopped by placing the flasks in a 90 °C water bath for 2 min followed by an ice bath. flasks in a 90 °C water bath for 2 min followed by an ice bath.

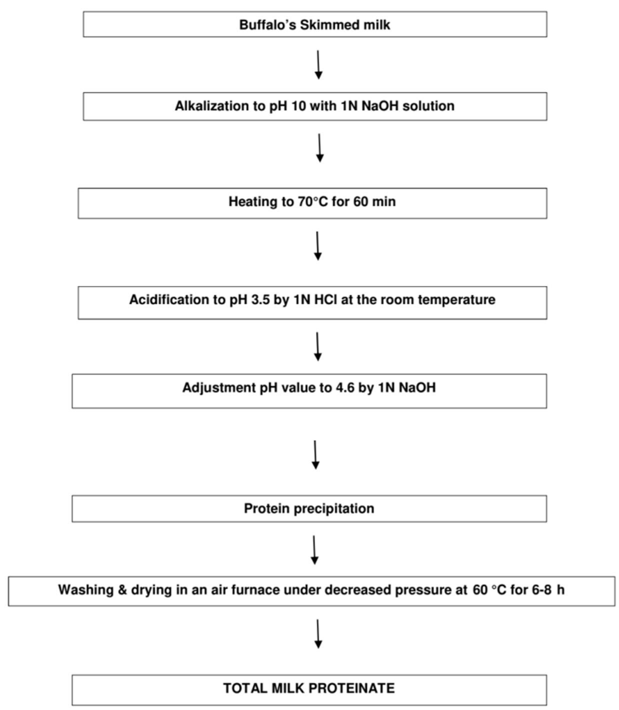

Figure (1): Schematic diagram of bufallo's total milk proteinate preparation

2.3. Activation of yoghurt starter culture

Yoghurt starter culture (YSC) was activated at 42°C using antibiotic free SMP reconstituted to 12% total solids (TS) and autoclaved at 120°C for 10 min. After incubation at 42°C for 4-5 h, the obtained culture was freshly used.

2.4. Preparation of cow's yoghurt supplemented with partially chymosin-hydrolyzed buffalo's total milk proteinate

Four treatments including the control were designed, where cow's FCMP was reconstituted at the temperature of 45°C for 15 min to achieve the full hydration as recommended by **(25)** whether at the level of 13% FCMP (the control) with tap water or at the level of 12% FCMP with solution containing 1% TMP in the native form (*i.e.* non enzymatic hydrolyzed) or solution of TMP hydrolyzed by chymosin for 4 or 8 h. The yoghurt bases were procedure as described by **(4)** with adopting the manufacture conditions enacted by (**12**), where they were heat treated at 85°C for 5 min. followed by temperature adjustment to 42°C at which yoghurt milks were inoculated with 2% of YSC activated as before mentioned, filled into 100 ml polyvinyl chloride containers, covered, and incubated until complete coagulation (through about 3 h.). Thereafter, the container were transferred to the refrigerator (5±1°C), where they were kept for the periodical analyses. Three replicates were done for every treatment. **Figure (2)** shows the flow scheme the manufacture procedures of yoghurt supplemented with chymosin-proteolyzed TMP.

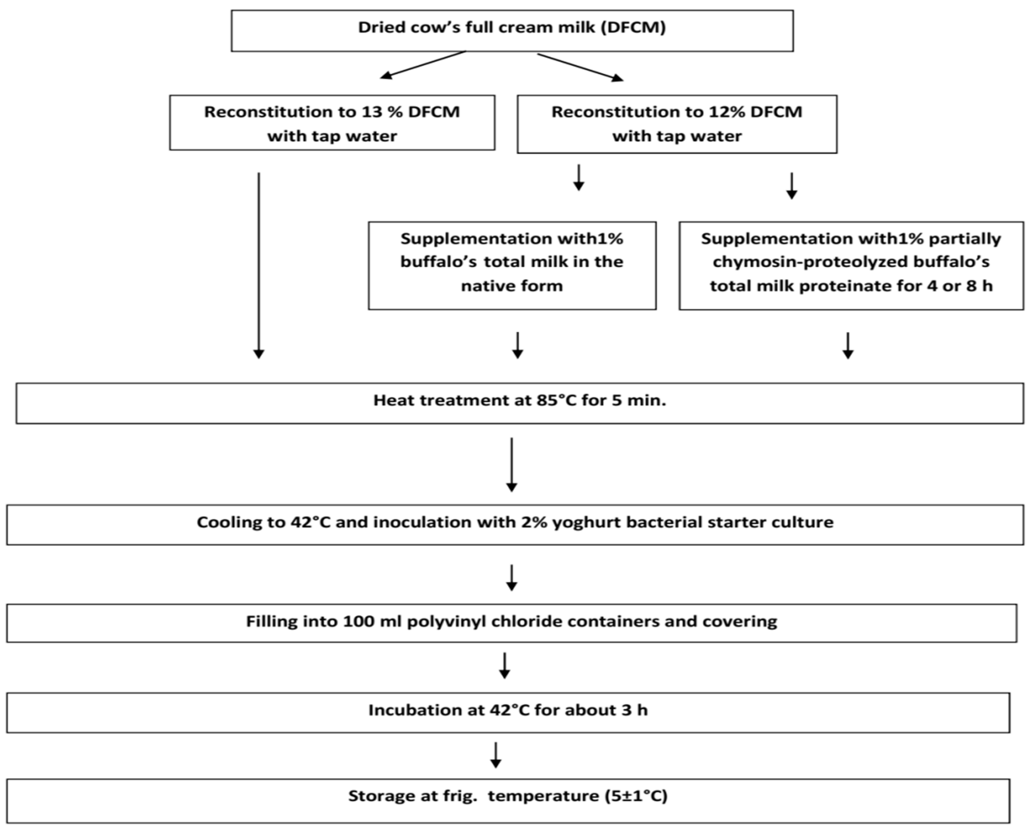

Figure (2): The flow scheme of the manufacture of yoghurt supplemented with 1% partially chymosin-proteolyzed buffalo's total milk proteinate

3. Analytical methods

Dry matter (DM), total nitrogen (TN) and titratable acidity (TA) contents were quantified as mentioned in **(26).** The pH value was measured using a pH meter (HANNA Instruments, USA). Fat, water soluble nitrogen (WSN) and non protein nitrogen (NPN) contents were determined as in **(27).** Lactose content was determined according to **(28).** The technique **(29)** was used to measure emulsifying capacity and stability. The specific activity of chymosin was determined as in **(30).** Yoghurt samples were subjected to texture profile analysis (TPA) using a Texture Analyzer (TMS-Pro, USA) according to **(31)**. The samples were subjected to two successive compressions (bites) at 50% deformation using a cylindrical probe of 20 mm diameter and 35 mm length at three different locations for each yoghurt sample. The speed of the crosshead was kept at 1mm/sec with a load cell of 25 N. Fracturability, Hardness 1 and Hardness 2, work carried out on the sample during the first bite (A1) and on the second bite (A2), cohesiveness (A2/A1), springiness (elasticity) and chewiness were obtained using soft were provided with the used of computerized texture analyzer as in **(32).**Apparent viscosity (ηa) was measured at 10°C using a rotary viscometer (RHEOTEST, type RV and Pruefgeraetewerk Medingn, Dresden) as described by **(33).**Lactic acid bacteria were counted according to **(34).**Yeasts & Molds and total count were enumerated according to American Public Health Association **(35)**. Coliform were determined according to **(36)** on violet red bile agar. The count is expressed as colony forming units (cfu) *per* g of product.Sensory evaluation of yoghurt samples for every criteria namely appearance, flavor and consistency was carried out using the five point scale (five= best quality, zero = worst quality) as illustrated by **(37).** The data obtained were exposed to proper statistical analysis according to statistical analyses system user's guide **(38).**

RESULTS AND DISCUSSION

1. Specifications of substrate and enzyme

The specifications given in Table (1) demonstrate some properties of substrate *ie* total milk potentate as well as chymosin enzyme used in this study. The specifications of TMP are in coincidence with those of (**13**).

2. Physiochemical properties of proteolysis resultant

As could be seen in Table (2), the viscosity value of solution containing 5% TMP proteolyzed by 1% chymosin enzyme decreased as the proteolysis time prolonged. Likewise, both of the capacity and stability of the emulsion property were proportionally declined due to TMP proteolysis. On the other hand both of the water soluble nitrogen (WSN) and non-protein nitrogen (NPN) of TMP solution increased as the proteolysis time increased. It is worthy to mention that the increasing rates of NPN always doubled of those of WSN. In this respect **(39) (40)**reported that, the native substrate of chymosin is usually the fraction of κ-casein which is specifically cleaved at the peptide bond between amino acid residues 105 and 106, phenylalanine and methionine. When the specific linkage between the hydrophobic (para-casein) and hydrophilic (acidic glycopeptide) groups of casein is broken, the hydrophilic group, named glycomacropeptide is water soluble(*i.e.,* WSN) as well as remained soluble in the presence of 12 % trichloroacetic acid and hence determined as NPN.

Table (1): Specifications of dried buffalo's total milk proteinate and chymosin enzyme

Chemical composition % of dried buffalo's total milk proteinate		**Specification of chymosin enzyme**	
Moisture	**4.34**	**Activity (U/g)**	**66.79**
Protein/dry matter	**95.41**	**Protein (mg / g)**	**0.0243**
Ash	**3.697**	**Specific Activity (U/mg)**	**2748.5**

Table (2): Physiochemical properties of solution containing 5 % of buffalo's total milk proteinate as affected by proteolysis time with 1% chymosin enzyme at 42°C

Proteolysis time (h)	**Viscosity (cP)**	**Emulsion**				**Water soluble nitrogen**		**Non protein nitrogen**	
		Capacity (m^2g^{-1})	**Stability (%) after (day)**			**(ppm)**	**Increase (%)**	**(ppm)**	**Increase (%)**
			1	**2**	**3**				
0	**4.20**	**135**	**100**	**100**	**88**	**0.0979**	**-----**	**0.070**	**-----**
4	**3.24**	**125**	**95**	**90**	**82**	**0.1024**	**89.0**	**0.210**	**200**
8	**3.00**	**120**	**92**	**86**	**79**	**0.4790**	**389.3**	**0.560**	**700**

3. Properties of yoghurt supplemented with the native or proteolyzed TMP

As mentioned in Table (3) the data reveal that no significant differences in both of moisture as well as fat contents between yoghurt treatments but the supplementation with 1% TMP, whether in the native or in the proteolyzed form, was associated with significant increase in the protein content of yoghurt. This is considered logically regarding the chemical composition of the TMP given in Table (1) which showed its protein / dry matter content being 95.41%.

Moreover, both of WSN/TN and NPN/TN of yoghurt significantly increased as the yoghurt was supplemented with TMP whether, in the native or in the proteolyzed form. The ash content of yoghurt appeared a significant reduction as a result of TMP adding regardless its proteolysis period.

Table (3): Chemical composition of set yoghurt as affected by the supplementation with 1% of chymosin-proteolysed buffalo's total milk proteinate (TMP) for different times

Component %	Without added TMP (Control)	Proteolysis time (h) of 1% TMP solution used for yoghurt supplementation		
		0 (without enzyme)	4	8
Moisture	**87.07**	**87.62**	**87.20**	**87.14**
Fat	**3.50**	**3.51**	**3.52**	**3.51**
Protein (TN X 6.38)	**3.43[b]**	**4.77[a]**	**4.54[a]**	**4.88[a]**
WSN/TN	**0.262[d]**	**0.299[c]**	**0.373[b]**	**0.630[a]**
NPN/TN	**0.036[d]**	**0.044[c]**	**0.057[b]**	**0.074[a]**
Ash	**1.010[a]**	**0.967[b]**	**0.933[b]**	**0.967[b]**

Data in Table (4) confirm that, although titratable acidity % of fresh yoghurt did not exhibit any significant variations between treatments. The prolonging of cold storage period was associated with significant increase in yoghurt acidity.

The fresh TMP yoghurts appeared pH values relatively higher than that of the control that could be attributed to the relatively higher protein content possessed them and hence their relatively higher buffering capacity because of that (Table, 5). While all pH values of yoghurt lowered as the cold storage period extended.

Microbiologically, the Log count of lactic acid bacteria (LAB) did not appear any significant differences between the fresh yoghurt treatments. All Log counts of LAB reached to the highest value after seven days and then reduced again at the end of cold storage period (14 days) (Table, 6). These findings are in coincidence with those reviewed by**(22)**,**(41)** and **(42)**, who reported that, during cold storage period, both counts of *Str. thermophilus* and *Lb. acidophilus* increased significantly along the first week, then decreased gradually thereafter.

Table (4): Titratable acidity (as lactic acid %) of set yoghurt during cold storage period at 5 ± 1 °C as affected by the supplementation with 1% of chymosin-proteolysed buffalo's total milk proteinate (TMP) for different times

Cold storage period (day)	Without TMP (Control)	Proteolysis time (h) of 1% TMP solution used for yoghurt supplementation		
		0 (without enzyme)	4	8
Fresh	**0.75[a,c]**	**0.72[a,c]**	**0.72[a,c]**	**0.74[a,c]**
7	**0.79[a,b]**	**0.77[a,b]**	**0.79[a,b]**	**0.80[a,b]**
14	**0.81[a,a]**	**0.79[a,a]**	**0.82[a,a]**	**0.84[a,a]**

Table (5): The pH value of set yoghurt during cold storage period at 5 ± 1 °C as affected by the supplementation with 1% of chymosin-proteolysed buffalo's total milk proteinate (TMP) for different times

Cold storage period (day)	Without TMP (Control)	Proteolysis time (h) of 1% TMP solution used for yoghurt supplementation		
		0 (without enzyme)	4	8
Fresh	**4.29[b,a]**	**4.50[a,a]**	**4.44[a,a]**	**4.39[a,a]**
7	**4.27[b,b]**	**4.39[a,b]**	**4.40[a,b]**	**4.35[a,b]**
14	**4.25[b,c]**	**4.32[a,c]**	**4.34[a,c]**	**4.30[a,c]**

Neither yeasts & molds nor total coliform exhibited any considerable count (Table, 6). That could be ascribed to the thermal processing of milk as well as the relatively high sanitation conditions adapted during manufacturing, filling and handling of yoghurt treatments.

Hardness is the force required to compress a sample between the molars as explained by**(31)**,**(43)**. Data displaying in Table (7) declare that, the hardness of yoghurt strengthened either due the presence of TMP or/and by the progressing of the cold storage period. Moreover, the degree of TMP proteolysis was associated with increase of yoghurt hardness. This is probably due to the contribution of the fraction of para κ-casein to the yoghurt matrix, especially that this fraction was previously released during the chymosin proteolysis of TMP and encountered in the yoghurt milk of the availability of ionized calcium, which is necessary to achieve the chemical step of the network formation.**(39)**,**(40)** reported that, when the specific linkage between the hydrophobic (para κ-casein) and hydrophilic (acidic glycopeptide) groups of casein is broken by chymosin, the hydrophobic groups unite and form a 3D network that traps the aqueous phase of the milk. The latter happens due to the availability of calcium ions in the medium as is the case in milk.

Table (6): Microbiological quality (log cfu/ g) of set set yoghurt during cold storage period at 5± 1 °C as affected by the supplementation with 1% of chymosin-proteolysed buffalo's total milk proteinate (TMP) for different times

Microbe	Cold storage Period (day)	Without added TMP Control)	Proteolysis time (h) of 1% TMP solution used for yoghurt supplementation		
			0 (without enzyme)	4	8
Lactic Acid bacteria	Fresh	7.36	7.44	7.38	7.28
	7	7.35	7.51	7.41	7.30
	14	7.33	7.40	7.30	7.24
Yeasts and Molds	Fresh	<10	<10	<10	<10
	7	<10	<10	<10	<10
	14	<10	<10	<10	<10
Total Coliform	Fresh	<10	<10	<10	<10
	7	<10	<10	<10	<10
	14	<10	<10	<10	<10

On the contrary the adhesiveness appeared opposite trending to those of the hardness in all cases, namely the yoghurt supplementation with TMP as well as the proteolysis time besides, the cold storage period. Adhesiveness is an adverse quality parameter in texture profile analysis of yoghurt samples. Storage of yoghurt samples for 7 or 14 days led, in general, to decrease the adhesiveness energy, may be due to the resulting higher hardness values.

Cohesiveness is a measure for the structure stability of a food specimen and if it withstands repeated compression or not. Cohesiveness is the strength of internal bonds making up the body of the product as explained by **(31),(43).** It is a parameter for measuring the ability of yoghurt to adhere with each other. Nevertheless, the cohesiveness criterion of yoghurt did not evidence any significant variation between treatments towards all factors studied.

With regards to the springiness values of yoghurt, the control sample possessed a value higher than that of non proteolyzed TMP. The TMP proteolysis whether for 4 h or rather for 8 h heightened the springiness values of yoghurt supplemented therewith. At the end of cold storage period (14 days) significant reductions were recorded in the springiness values of all treatments.**(31),(43)** explained that, springiness is the rate at which a deformed material returns to its original shape on removal of the deforming force, *i.e.* springiness is a textural parameter expressing the degree of ability of a product to springs back after it had been deformed during the first compression.**(44)** found that, incorporating the rennet within the coagulation led to increase the springiness value of the resultant Kariesh cheese when compared with the application of the acidic coagulation alone. That could be due to the higher water holding ability of the acidic-enzymatic coagulum than crumbly one of the acidic coagulation.

The gumminess value of yoghurt heightened when TMP added and as TMP was proteolyzed. The gumminess value of control sample was not changed by cold stage period whereas the value of this criterion of TMP containing yoghurt raised as the cold storage period extended. **(31), (43)** reported that, gumminess is the force needed to disintegrate a semisolid food to a state ready for swallowing. It could be observed that the relationship between hardness values and cutting force (gumminess) is positive. Moreover,(**44)** found that incorporating the rennet with the acidic coagulation led to increase the gumminess also as a result of increasing the hardness of Kariesh cheese.

Likewise, chewiness criterion of fresh yoghurt behaved responses towards the supplementation with TMP similar to those exhibited by gumminess. Whereas, the chewiness value of yoghurt heightened when TMP added and rather as TMP was proteolyzed. The prolonging of cold storage period of yoghurt was significantly met with different reactions, the chewiness value of the control and yoghurt containing non proteolyzed TMP decreased, while those of proeolyzed TMP whether for 4 or 8 h increased. Gumminess and Chewiness are the force and the energy required to cut and move the food specimen in the mouth.**(31),(43)** explained that, chewiness is the work needed to masticate a solid food to a state ready for swallowing.

Table (7): Texture profile parameters of set yoghurt when fresh as well as at the end of cold storage period at 5°± 1°C as affected by the supplementation with 1% of chymosin-proteolysed buffalo's total milk proteinate (TMP) for different times

Parameter	Cold storage period (day)	Without added TMP (Control)	Proteolysis time (h) of 1% TMP solution used for yoghurt supplementation		
			0 (without enzyme)	4	8
Hardness (N)	Fresh	$1.225^{c,b}$	$1.611^{b,b}$	$2.110^{a,b}$	$2.128^{a,b}$
	14	$1.296^{d,a}$	$1.885^{c,a}$	$3.024^{b,a}$	$3.177^{a,a}$
Adhesiveness (mJ)	Fresh	$3.181^{a,a}$	$2.889^{b,a}$	$2.821^{b,a}$	$2.167^{c,a}$
	14	$2.240^{b,b}$	$1.754^{d,b}$	$2.560^{a,b}$	$1.797^{c,b}$
Cohesiveness (Ratio)	Fresh	0.44	0.36	0.36	0.38
	14	0.39	0.34	0.43	0.39
Springiness (mm)	Fresh	$9.95^{c,a}$	$8.23^{d,a}$	$10.57^{b,a}$	$13.89^{a,a}$
	14	$5.84^{d,b}$	$6.90^{c,b}$	$8.43^{b,b}$	$9.37^{a,b}$
Gumminess (N)	Fresh	$0.533^{d,-}$	$0.597^{c,b}$	$0.753^{b,b}$	$0.807^{a,b}$
	14	$0.507^{d,-}$	$0.634^{c,a}$	$1.241^{b,a}$	$1.298^{a,a}$
Chewiness (mJ)	Fresh	$5.74^{d,a}$	$6.20^{c,b}$	$7.41^{b,a}$	$8.54^{a,b}$
	14	$3.70^{d,b}$	$4.75^{c,b}$	$8.95^{b,a}$	$10.46^{a,a}$

Organleptically, all TMP containing fresh yoghurts gained the full score in the criterion of appearance, consistency and total score. Likewise the flavor of all fresh treatments including the control obtained the full score.

After seven days of cold storage period the samples containing TMP proteolyzed for 4 or 8 h kept the high score of appearance while those of non proteolyzed TMP or TMP proteolyzed for 4 h obtained the high score of consistency and flavor attributes.

At the end of cold storage period, the appearance scores of both proteolyzed TMP yoghurts were lowered to be as those of the control and non proteolyzed TMP, while the consistency of all sample except of that of 8 h proteolyzed TMP gained the high score and the flavor of all sample except of the control gained also the high score at the end of cold storage period. The total judging score indicated that, the yoghurt treatment of TMP proteolyzed for 4 h kept its overall sensory quality followed by that of 8 h- proteolyzed TMP more than that of non proteolyzed TMP and even than the control treatment.

Table (8): Organoleptic scores of set yoghurt during cold storage period at 5± 1°C as affected by the supplementation with 1% of chymosin-proteolysed buffalo's total milk proteinate (TMP) for different times

Sensory attribute	Cold storage period (day)	Without added TMP (Control)	Proteolysis time (h) of 1% TMP solution used for yoghurt supplementation		
			0 (without enzyme)	4	8
Appeara nce (out of 5)	Fresh	4	5	5	5
	7	4	4	5	5
	14	4	4	4	4
Consisten cy (out of 5)	Fresh	4	5	5	5
	7	4	5	5	4
	14	5	5	5	4
Flavor (out of 5)	Fresh	5	5	5	5
	7	4	5	5	5

	14	4	5	5	5
Total score (out of 15)	Fresh	13	15	15	15
	7	12	14	15	14
	14	13	14	14	13

Conclusion:

It can be concluded that: Although the TMP derivative is a protein compound, its proteolysis with chymosin, which κ-casein is its preferred substrate, resulting in the liberation of peptide, para κ-casein that helped more in strengthening the weak curd, from which cow's yoghurt is suffered and homogenizing its consistency, provided that the period of proteolysis does not exceed 4 h by 1% enzyme.

Reference:

[1]Prentice, J.H. (Ed.) (1992). Dairy Rheology A Concise Guide. pp. 123-136.VCH Pub., Inc., New York, USA.

[2]Parnell-Clunies, E.M.; Y. Kakuda and J.M. deMan (1986a). Influence of heat treatment of milk on flow properties of yoghurt. J. Food Sci., 51: 1459-1462.

[3]Parnell-Clunies, E.M.; Y. Kakuda; K. Mullen; D.R. Arnott and J.M. deMan (1986b). physical properties of yoghurt. A comparison of vat *versus* continuous heating systems of milk. J. Dairy Sci., 69: 2593-2603.

[4]Tamime, A.Y. and R.K. Robinson, (Eds) (1999). Yoghurt: Science and Technology. Pergamon Press, Oxford. New York, USA. pp. 300-305.

[5]Rasic, J.L. and J.A. Kurmann (Eds) (1978). Yoghurt, Scientific Grounds, Technology, Manufacture and Preparations. Tech. Dairy Pub. House, Copenhagen, Denmark.

[6]Tamime, A.Y. and G.C. Deeth (1980). Yoghurt: Technology and biochemistry. J. Food Prot., 43: 939-977.

[7].Dannenberg, F and H.G. Kessler (1988). Effect of denaturation of β-Lactoglobulin on texture properties of set-style nonfat yoghurt. 1. Synersis. Milchwissenschaft, 43: 632-635.

[8]Kroger, M. (1973). Controlling the quality of yoghurt. Dairy Ice Cream Field, 156: 38-42.

[9]Zoon, P. (2003). Viscosity, smoothness and stability of yoghurt as affected by structure and EPS functionality. Proc. IDF Seminar on Aroma and Texture of Fermented Milk, Kolding, Denmark, June, 280-289.

[10]Nielsen, V.H. (1975). Factors which control the body and texture of commercial yoghurt. Amer. Dairy Rev., 37: 36-38.

[11]FAO/WHO (1976). In Joint Food Agricultural Organization / World Health Organization Committee of Government Experts on the Code of Principles Concerning Milk Product. Report of the 18th Session, No. C x 5 / 70, FAO, Rome, Italy.

[12]EOSQC (2005). Egyptian Organization for Standardization and Quality Control, Cairo, Egypt. Egyptian standard No. 1000 Yoghurt.

[13]Fayed, A.E.; I.M. Roushdy and A.I. Metwally (1996). Physical and organoleptic properties of yoghurt made from standardized buffalo's milk fortified with total milk proteinate. Proc. 6th Conf. Agric. Dev. Res. Ain Shams Univ., Cairo, Dec., Ann. Agric. Sci., Sp. Issue: 109-124.

[14]Lankes, H.; H.B. Ozer and R.K. Robinson (1998). The effect of elevated milk solids and incubation temperature on the physical properties of natural yoghurt. Milchwissenschaft, 53: 510-513.

[15]Folkenberg, D.M. and M. Martens (2003). Sensory properties of low fat yoghurt. Part A: Effect of fat content, fermentation culture and addition of non fat dry milk on the sensory properties of plain addition of non fat dry milk on the sensory properties of plain yoghurt. Milchwissenschaft, 58: 48-51.

[16]Omar, M.M. and A.M. Abou El-Nour (1998). Reological properties of yoghurt enriched with whole milk protein. Egypt. J. Dairy Sci., 26: 77-88.

[17]Abd El-Salam, M.H.; S. El-Shibiny; M.B. Mahfouz; H. Fakher El-Dein; H.M. El-Atriby and V. Antila (1991). Preparation of whey protein concentrate from salted whey and its use in yoghurt. J. Dairy Res., 58:503-510.

[18]Remeuf, F.; S. Mohammed; I. Sodini and J.P. Tissier (2003). Preliminary observation on the effect of milk fortification and heating on microstructure and physical properties of stirred yoghurt. Int. Dairy J., 13: 773-782.

[19]Renner, E. and U. Eiselt-Lomb (1985b). Investigations on the protein fortification of yoghurt. 2. Effect on the product composition Milchwissenchaft, 40: 462-463.

[20]Renner, E. and U. Eiselt-Lomb (1985a). Investigations on the protein fortification of yoghurt. 1. Effect on the ripening behavior. Milchwissenchaft, 40: 388-390.

[21]Haggag, H.F. and A.E. Fayed (1988). Production of Zabady from ultrafiltered bufflo's milk. Food Chem., 39; 29-36.

[22]Aita, O. A.; Y. A. Husein; A. E. Fayed and M. A. El-Nawawy (2015). Quality attributes of protein fortified yoghurt. J. Food and Dairy Sci., Mansoura Univ. 6: 227 – 241.

[23]Morr, C. V. (1985). Composition, physiochemical and functional properties of reference whey protein concentrates. J. Food Sci., 50: 1406-1411.

[24]Banach, J.; Lin, Z. and Lamsal, B. (2013). Enzymatic modification of milk protein concentrate and characterization of resulting

functional properties. LWT Food Sci. Technol., 54: 397–403.
[25]Fayed, A. E. (1986). Protein Fortification of some Dairy Products. Ph.D. Thesis, Fac. Agric., Ain Shams Univ., Cairo, Egypt.
[26]AOAC (2016). Association of Official Analytical Chemists. Official methods of analysis of AOAC International, 19th ed., Benjamin Franklin, Washington D.C., USA.
[27]Ling, E.R. (Ed.) (1963). A Text Book Dairy Chemistry. 2. practical 3rd ed. Chapman and Hall. Ltd London, UK, pp. 76-98.https://www.researchgate.net/publication/39879592.
[28]Barnett, A. J. G., and Tawab, G. A. (1957). A rapid method for the determination of lactose in milk and cheese. J. Sci. Food and Agric., 8: 437-441.
[29]Pearce, K.N. and Kinsella, J.E. (1978). Emulsifying properties of proteins: Evaluation of a turbidimetric technique. J. Agric. Food Chem., 26: 716-723.
[30]Chopra, A.K. and D. K. Mathur (1983). Factors affecting protease production by *Bacillus stearothermophilus* RM-67. J. Food Prot., 46:1020-1025.
[31]Bourne, M. C. (Ed.) (2002). Food Texture and Viscosity: Concept and Measurement. 2nd Ed., pub. Elsevier Sci. Technol. Books, UK.
[32]Kumar, P. and Mishra, H. N. (2004). Mango soy fortified set yoghurt: Effect of stabilizer addition on physicochemical, sensory and textural properties. Food Chem., 87: 501–507.
[33]Toledo, T.T. (Ed.) (1980). Fundamental of Food Process Engineering. AVI pub. Co. West port, Connecticut, USA.
[34]Elliker, P.; A. Anderson and G. Hannesson (1956). An agar culture medium for lactic acid streptococci and lactobacilli. J. Dairy Sci., 39:1611-1956.
[35]APHA (1992). American Public Health Association. Standard Methods for the Examination of water and Wastewater, 18th Ed., Washington, D.C.
[36]Nunez, M. (1976). Microbial flora of Manchego cheese. VI. Pediococci. Anim. Inst. Nac. Invest. Agrar., Gen. No. 4,75 (Dairy Sci. Abstr. 1978, 458).