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IMPACT OF HEAT TREATMENT OF MILK ON RHEOLOGICAL PROPERTIES AND MICROSTRUCTURE OF SET-YOGURT GEL

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ABSTRACT

Yogurt gels were made from milk heated at 72°C/15 s, 85°C/15 min and 100°C/5 min by acidification with *S. thermophilus* (TH4) and *L. delbrueckii subsp bulgaricus* (LB12). The pH, viscosity, shear stress and torque were measured during the yogurt gelation process. Images of sections of yogurt samples were obtained using scanning electron microscope in order to evaluate yogurt microstructure. Gel structure was examined for surface roughness, compactness, matrix area and pores area. With 100°C/5 min heat treatment, highest viscosity, shear stress, torque, surface roughness, compactness, and matrix area were exhibited, while, the lowest values were obtained with milk subjected to 72°C/15 s. The acid-induced gelation properties of heated milk are consistently different in some aspects as shifting in gelation pH, rougher gels and a different microstructure of the gels. Heat treatment of milk had significant effect ($p<0.001$) on all the rheological parameters investigated. The matrix area of the protein aggregates was a useful parameter to describe the differences between casein networks obtained by high and low heat treatments. The parameter of the average pores area in the yogurt gel gave an indication of its permeability. Significant positive correlations were observed between heat treatments and gel rheological properties measured. It was concluded that rearrangements of casein particles in the gel network occurred.

INTRODUCTION

Dairy industry is the area in which rheological tests are most extensively employed. Since milk can be converted into many products with different physical properties, rheological studies are of special importance for characterization and development of dairy products aiming consumer acceptability, elucidation of the structure and its relationship with textural properties (Kokini, 1992).

Quality of yoghurt and other fermented milks is largely dependent on its texture (Lankes *et al.*, 1998; Savello and Dargan, 1997 & 1995). Similarly to flavour, texture is a multi-parameter property of food (Domagala and Kupiec, 2003).

The rheology and syneresis of yogurt are markedly influenced by milk composition and processing treatments. Syneresis is undesirable in yogurt. It occurs when the protein network is unable to firmly held water. There are several reasons for development of yogurt syneresis. One of them is insufficient heating of used milk which means that casein micelle clusters are mainly formed with little branched chains resulting in a porous structure (Lucey, 2002).

Heat treatment of milk is one of the most important processing parameters affecting the texture, viscosity and consistency of yogurt (Walsh - O'Grady *et al*, 2001 and Mulvihill and Grufferty, 1995). The properties of milk are considerably changed during heating due to heat-induced denaturation of the whey proteins. Upon denaturation, the whey proteins either form soluble whey protein aggregates or associate with the casein micelles, resulting in whey protein-coated casein micelles (Vasbinder *et al.*, 2003a; Oldfield *et al.*, 2000; Singh *et al.*, 1996).

Understanding the gelation process during fermentation is critical for manipulating the physical properties of yogurt. Electron microscopy can reveal interesting features in the development of yogurt structure. Therefore, this research was undertaken to develop more knowledge of the rheological behavior of yogurt and its changes during the fermentation process as well as gel structure and acidity. The influence of heat treatment of the milk on the rheological properties and microstructure of the resultant yoghurt were also examined.

MATERIALS AND METHODS

Raw buffaloes' milk was obtained from the herd of the Faculty of Agriculture, Cairo University, Giza, Egypt, having total solids of 16.77%, fat content of 6.5%, titratable acidity from 0.16 - 0.18% and pH value from 6.6 to 6.8. Milk was divided into three parts which were heated at 72°C/ 15 s, 85°C / 15 min and 100°C /5min, respectively, then cooled to 42°C.

The heated milk was inoculated with 4% (wt/wt) of 1:1 mixed culture of *Streptococcus thermophilus* (TH4) and *Lactobacillus delbrueckii subsp. bulgaricus* (LB12); obtained from Chr. Hansen's, Laboratorium, Denmark. Fermentation was allowed to continue up to pH 4.6 or less during which the yogurt milk & gel samples were subjected to measurements.

The pH values were measured using pH-meter equipped with combined electrode (Jenway 3305, England). Triplicate measurements of pH were taken every 30 min intervals during fermentation.

Viscosity, shear stress (SS) and torque (T) were measured in triplicate by means of rotational rheometer type Brookfield DVTII fitted with UL unit (Brookfield Engineering Lab., Inc., Stoughton, MA) connected to IBM compatible computer, equipped with Brookfield DV Gather+ 1.0 software, used to record every 30 min through 5 hrs.

Yogurt microstructure

Cubes (3x3x3mm) of the yogurt gel samples at pH 4.6 for the heat treatments of 72°C/ 15 s and 100°C /5 min and at pH 5.8, 5.0 and 4.6 for 85°C /15 min were prepared for SEM examination. The cubes were dipped in agar at 40°C, and then fixed in 4% glutaraldehyde in 0.33 M cacodylate buffer at pH 6.0 for overnight at 4°C. The cubes were rinsed three times with the buffer for 10 min intervals, and then post fixed with 1% osmium tetroxide (OsO_4) in 0.33 M cacodylate buffer (pH 6.0) for 2 hrs. The cubes were rerinsed three times with the buffer for 10 min intervals, and then dehydrated in series of acetone solution (25, 50, 75, 95% and three changes in 100%) for 10 min each. The specimens were dried to critical point using CO_2 in a Critical Point Dryer (Polaron, Waterford, England). Agar was removed by a razer blade before mounting the specimens on aluminum SEM stubs. The specimens were sputter coated with gold (EM Scope SC500 sputter coater, Ashford, Kent, England). Samples were viewed at 5 KV through Scanning Electron Microscope (JEOL- JSM – 35.Tokyo, Japan) equipped with an IBM-compatible computer to record the images. Three photographs were taken for each sample and the images of at least three fields for each micrograph were analyzed by Climax Vision computer program (Climax Technologies Inc., Longueil, Qc., J4GITS, Canada). The images were digitally processed to produce binary images which were measured through the system to obtain several rheological and geometrical properties. These include: compactness (describes how dense the surface is depending on the distance between the pexels; the smaller the distance between the pexels, the higher the compactness), surface roughness (measures the irregularity of the surfaces), the area of the matrix (area of protein and entrapped fat globules, 2-dimension) and the area of the pores in the protein matrix of the yogurt.

Statistical analysis

A randomized block design was used to evaluate the effect of the treatments (milk heating and incubation time) on the dependent variables measured in triplicates using sub-program MSTAT (v4c, 1989, MSU, USA). Linear regression analysis was applied to quantity the relationships between the rheological properties of yoghurt and the independent variables studied, while the "T" test and LSD were used to analyze the differences between means at $p < 0.01$.

RESULTS AND DISCUSSION

pH development of yogurt during fermentation

The changes in pH of milk as a function of fermentation time are shown in Fig. (1). During fermentation, the pH of milk decreased from 6.5 to ≤ 4.5, being slowly for the first 90 min and then decreased rapidly thereafter. Increasing the milk heating temperature and the incubation time, significantly ($p<0.001$) increased the acidity of yogurts (Table 1). Within means, there were significant differences at $\alpha = 0.01$. Milk gels were developed from milk heated at 72°C/15 s, 85°C/15 min and 100°C/5 min, at pH values of 4.8, 5.2 and 5.4, respectively. Therefore, gelation time was reduced from 270 min for milk heated at 72°C/15 s to 167 and 107 min for milk heated at 85°C/15 min and 100°C/5 min, respectively. This clear shift in gelation pH and time could be due to the heat denaturation of whey proteins. Consequently, when milk was heated, the denatured whey proteins, associate with the casein micelles, especially κ-caseins which have a high isoelectric pH (e.g. the main whey protein, β-lactoglobulin has an isoelectric pH of 5.3). Therefore, whey protein-coated casein micelles will flocculate at higher pH, resulting in a clear shift in gelation pH. These results were in agreement with those of Vasbinder and de Kruif, (2003); Vasbinder *et al.*, (2003b & 2001;

Lucey, (2004) Lucey *et al.* (1998) and Lucey *et al.*, (1997).stated that heating milk at temperatures> 80°C decreased gelation time.

Effect of heat treatment of milk on rheological properties and microstructure of yogurt

1-Viscosity, shear stress and torque

The effect of heat treatment of milk on viscosity, shear stress and torque-incubation time curves are shown in Fig. (2, 3 and 4). Heating the milk prior to yogurt manufacturing increased significantly ($p<0.001$) its viscosity, shear stress and torque (Table 1). The highest values for these parameters were occurred by gels from milk heated at 100°C/ 5 min, followed by that heated at 85°C/ 15 min and 72°C/15 s. Also, results showed that the viscosity, shear stress and torque of the yogurts ranged from 350.8 cP, 42.9 D/cm2 and 54.9% for yogurt from milk heated at 72°C/ 15 s to 575.2 cP, 70.9 D/cm2 and 89.9% and 679.4 cP, 83.8 D/cm2 and 106.2% for the yogurt from milk heated at 85°C /15 min and 100°C/5 min, respectively. The increased of heat denaturation whey protein was probably the reason for the increase in these properties. Heating at elevated temperatures for long time would result in complete denaturation of whey proteins. Also, heating at high temperatures can produce intermolecular S-S cross-links which have a great effect on the strength of curd. The viscosity is influenced by covalent (SH/SS) interactions arising from denaturation of globular whey proteins. The viscosity-pH relationship (not shown) was nearly the same as for the viscosity-incubation time. Viscosity was highly correlated with shear stress and torque (R^2= 0.942 and 0.958, respectively). These results are consistent with the findings of Shaker *et al.*, (2000); Shaker & Tashtoush, (2000) and Ross-Murphy, (1995). Dave and Shah, (1998) reported that the apparent viscosity of yogurt was 75-120 pas.sec., while, Ozer, (2004) stated that the yogurt viscosity values were 225.0 poise and 189.0 poise, respectively, when yogurt were sheared for 15 and 30 s.

2-Surface roughness and compactness of yogurt gels

Surface roughness and compactness of yogurt from milk heated at 85°C/15 min. at pH 5.8, 5.0 and 4.6 were measured using the scanning electron micrographs (Fig. 6). Statistical analysis showed a significant increase in roughness and compactness ($p<0.001$) with lowering the pH during fermentation of yogurt. Also, negative correlations were found for roughness and compactness with pHs studied. The highest roughness and compactness were apparent at pH 4.6.

Fig. (5) shows the surface roughness and compactness of yogurts made from milk heated at 72°C/15 s, 85°C/15 min and 100°C/5 min. The electron micrographs showed increases in surface roughness and compactness of the protein network with increasing heat treatment ($p<0.001$). The gel formed at 100°C had the highest gel roughness and compactness with high correlations (Table 1). The surface roughness and compactness of the gels made from heated milk at 85°C and 100°C were higher than that made from milk heated at 72°C (α=0.01) due to the interaction between protein particles especially casein micelle surface (κ-casein) and the denatured whey proteins (β-lactoglobulin). Predominant amount of casein comparing to whey proteins affected the rough and coarse-grain structure of yogurt.

The surface roughness and compactness correlated well with temperature (Table 1). The high temperature caused probably an extensive rearrangement of the gel network that increased the yogurt compactness. The observed decrease in the yoghurt pH with increasing heat treatment was also considered. Cayot *et al.,* (2003); Vasbinder *et al.,* (2004) and Lucey *et al.*, (1998 &1997) showed close findings.

3-Casein matrix and pores areas of yogurt gels

Yogurt gels exhibited larger pores at higher pHs (Fig. 6), which indicated more permeability at pH 5.8 than at 5.0 than at 4.6. The pores area decreased significantly ($p<0.001$) with time and high acidity values (Table 2) from 51.7% for the gel at pH 5.8 to 18.8% and 12.42% for those at pH 5.0 and 4.6, respectively. After gel formation, the pores and the matrix areas in yogurts were reduced with increasing the acidity at the three pHs tested. Weak yogurt gels had a less stable network and large pores.

Fig. (5) shows the effect of heating (72°C/15 s, 85°C/15 min and 100°C/5 min) on yogurt matrixes and pores areas. Increasing the heating temperature of the milk up to 100°C increased significantly ($p<0.001$) protein matrix areas and decreased the pores areas (Table 1). The matrix area increased by 44.9% when heating of milk increased from 72°C/15 s to 100°C/5 min. In contrast, the pores areas were reduced from 38.46% to 12.42% and 10.87% for the gel made from milk heated at 72°C/15 s to those heated at 85°C/15 min and 100°C/5 min, respectively. Results gave a clue to the increased compactness of the gels at the high heat treatment. On the other hand, decreasing the pore area as a result of heat treatment may reduce wheying off. Whey separation of yogurt gels increased with a decrease in heating temperature indicating an increased possibility for rearrangements, which was confirmed by presence of large pores in microstructures of gels made from milk heated at 72°C. Generally, increasing milk heat treatment temperature resulted in more compact protein network and decreased the pore sizes in the protein matrix. Consequently, water is more firmly bound in the matrix. The obtained results agree with those of Lee &Lucey, (2004); Harte *et al.*, (2003); Lucey, (2002); Lucey *et al.* (1998); Raynol & Remeuf (1998) and van Vliet *et al.*, (1991).

4- Microstructure of yogurt gels

The micrographs of yogurt gels are presented in Fig. (7). Yogurts made from milk heated at 72°C, exhibited a clustered protein network at gelation time, whereas a more interconnected network was observed in yogurts made from milk heated at 85 and 100°C .At the end of fermentation, protein clusters became denser and were closer to each other, and yogurt gels exhibited a more branched and interconnected protein matrix. Yogurts made from milk heated at 72°C was less interconnected and exhibited larger pores (and clusters) compared with yogurts made from milks heated at 85 and 100°C which exhibited more interconnected structure with smaller pores. This confirms that extensive rearrangements occurred in yogurt gel networks made at 100°C.

At pH 4.6, the formation of yogurt gels was completed with the casein particles aggregated into a three-dimensional network of chains and clusters. Heating milk at 100°C resulted in gels with a cross-linked microstructure. However, the heat denaturation of whey proteins and reaggregation of the casein micelles during yogurt fermentation may explain the observed results which were consistent with those of Harte *et al.*, (2003); Lucey (2002) and Raynol & Remeuf (1998).

Table (1): Statistical analysis of pH, rheological and microstructural parameters of yoghurt as a function of heating temperature or incubation time.

Factor	Source	Mean Square	F Value	Prob	LSD	R^2	Equations
pH	Factor A	2.569	913.0636	***	0.0567	0.966	Y=7.607863-2.5551e-001 X
	Factor B	6.281	2232.256	***	0.1182		Y=7.607863-2.0949e-001 X
	A x B	0.075	26.7875	***			
Viscosity	Factor A	233257.402	3718.7070	***	8.207	0.903	Y=-301.812096+7.7182e+001 X
	Factor B	398909.171	6359.6109	***	17.09		Y=-301.812096+5.2144e+001 X
	A x B	11550.937	184.1509	***			
Shear Stress	Factor A	3540.071	3200.4999	***	1.090	0.912	Y= -37.207376+9.5066e+000 X
	Factor B	6007.183	5430.9614	***	2.269		Y=-37.207376+6.3983e+000 X
	A x B	176.878	159.9114	***			
Torque	Factor A	5719.458	3337.3605	***	1.357	0.934	Y=-47.392052+1.2083e+001X
	Factor B	9777.103	5705.0369	***	2.824		Y=-47.392052+8.1646 X
	A x B	284.443	165.9755	***			
Roughness	Factor A	0.050	176.1174	***	0.1030	0.945	Y=0.476667+1.2500e-001 X
compactness	Factor A	0.050	176.1174	***	0.0651	0.959	Y=0.504444+1.3833e-001 X
matrix area	Factor A	14440520847.654	42108.42	***	3813	0.888	Y=231264.44+6.1615e+004 X
Pores area	Factor A	18382139419.179	3709192.317	***	458.4	0.883	Y=219741.013+-6.9126e+004 X

Factor A= heating condition (72°C/15 s, 85°C/15 min and 100°C/5 min) **Factor B**= incubation period (every 30 min for 5 hrs).

Table (2): Statistical analysis of rheological and microstructural parameters of yoghurt as a function of pH.

Factor	Source	F Value	Prob	LSD value	R^2	Equations
Roughness	Factor A	387.8183	0.001	0.103	0.972	Y=0.403333+1.5000e-001x
compactness	Factor A	1608.11	0.001	0.103	0.984	Y=0.178889+2.2500e-001x
matrix area	Factor A	5087046.49	0.001	474.1	0.926	Y=148729.2+8.7791e+004x
Pore area	Factor A	382581.005	0.001	1838	0.951	Y=308683.6-9.5882e+004x

Factor A= pH (5.8, 5.0 and 4.6)

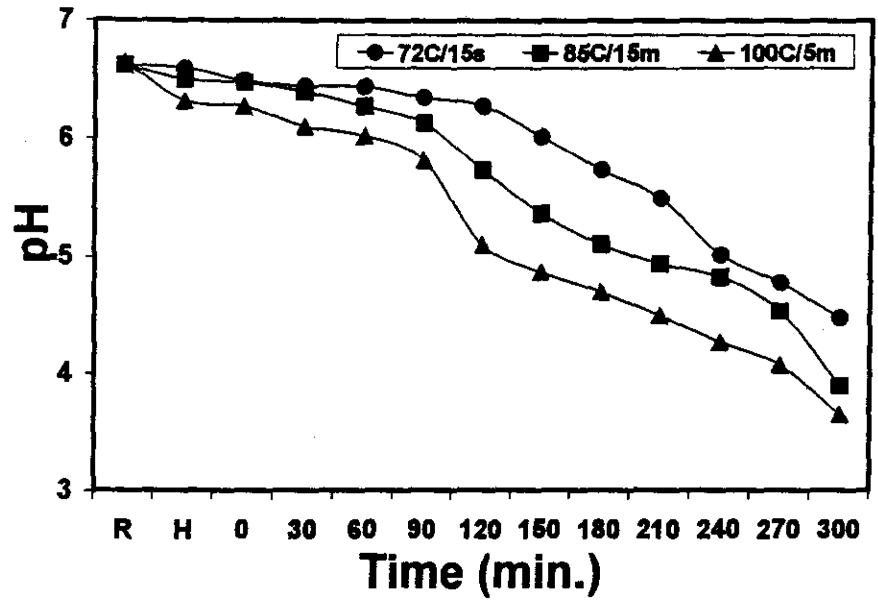

Fig. (1): pH profiles as a function of fermentation time for yogurt gels made from heated milk. R= raw milk, H= heated milk and 0= inoculated heated milk.

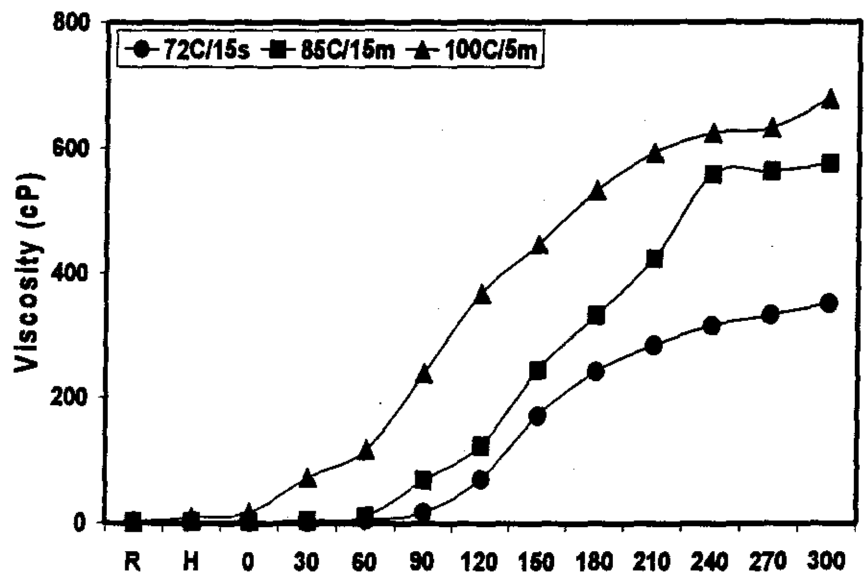

Fig. (2): Viscosity profiles as a function of fermentation time for yogurt gels made from heated milk. R= raw milk, H= heated milk and 0= inoculated heated milk

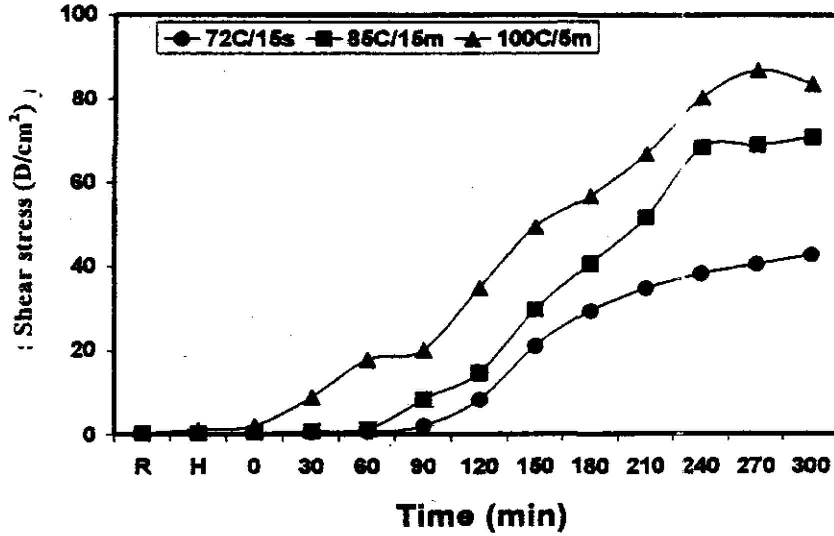

Fig. (3): Shear stress profiles as a function of fermentation time for yogurt gels made from heated milk. R= raw milk, H= heated milk and 0= inoculated heated milk.

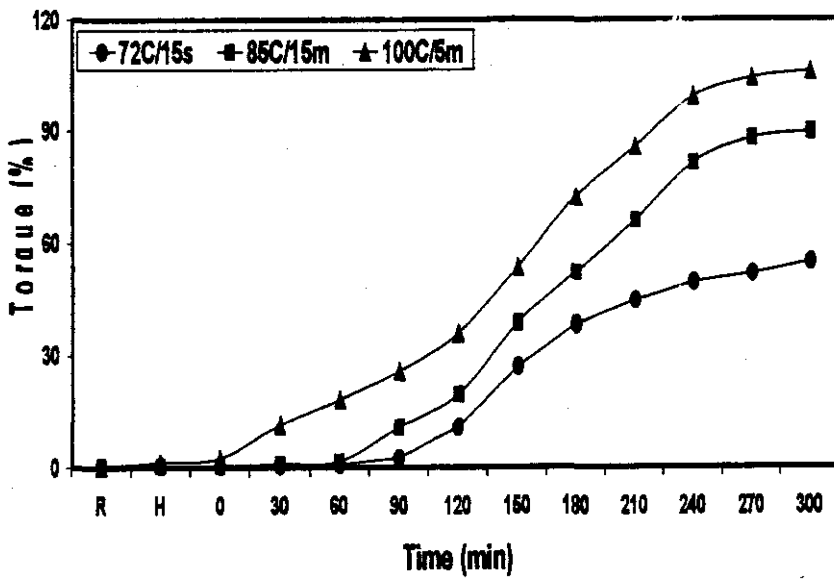

Fig. (4): Torque profiles as a function of fermentation time for yogurt gels made from heated milk. R= raw milk, H= heated milk and 0= inoculated heated milk.

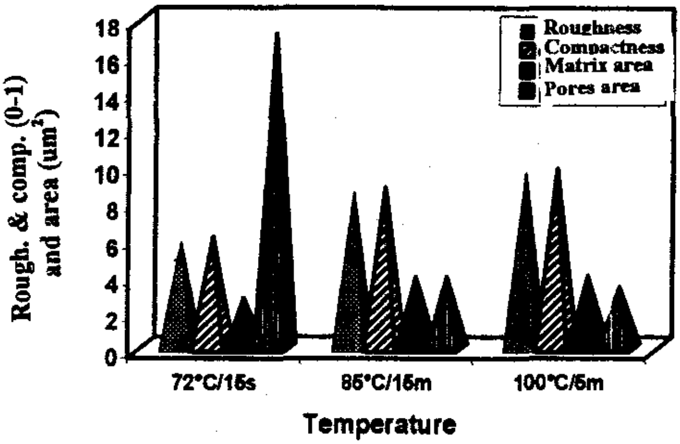

Fig. (5): Effect of milk heating at 72°C/15 s, 85°C/15 min and 100°C/5 min on gel surface roughness ($x10^{-1}$), compactness ($x10^{-1}$), matrix area ($x10^{5}$) and pores area ($x10^{4}$).

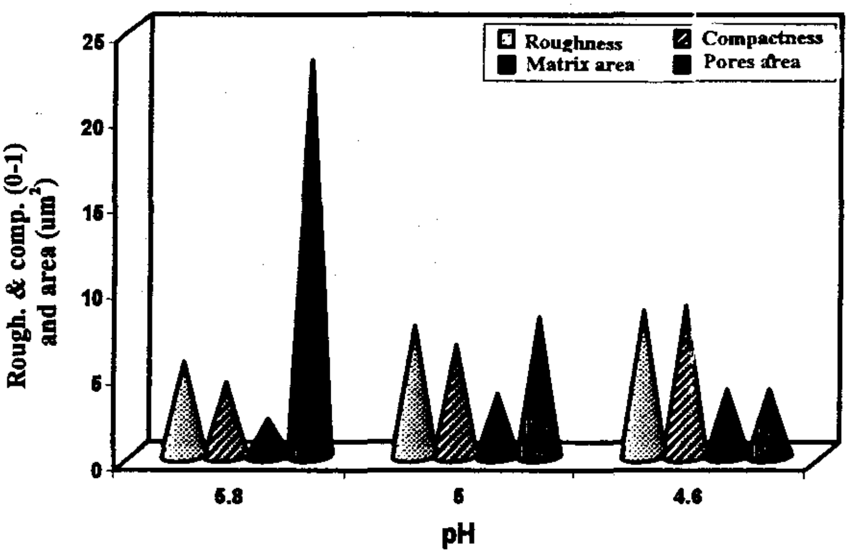

Fig. (6). Effect of yoghurt pH on gel surface roughness ($x10^{-1}$), compactness ($x10^{-1}$), matrix area ($x10^{5}$) and pores area ($x10^{4}$).

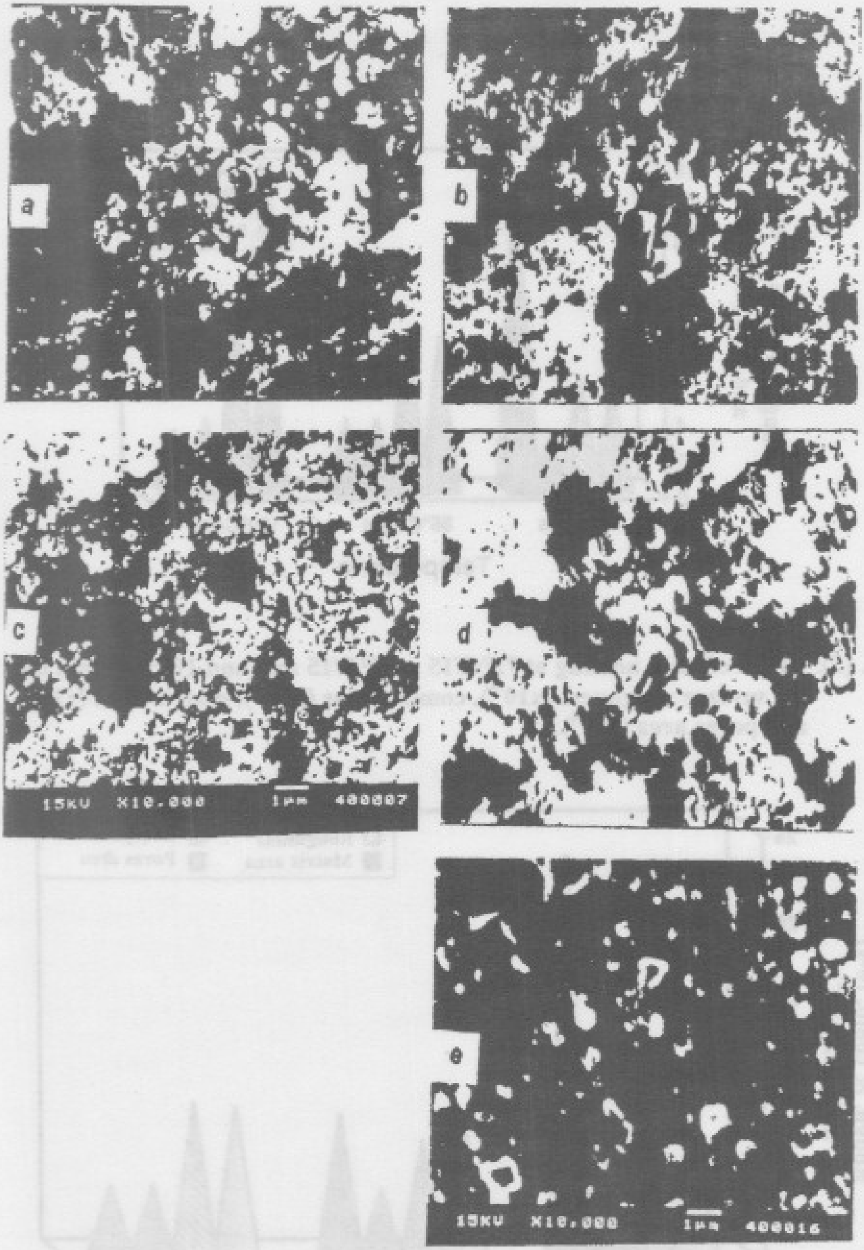

Fig. (7): Microstructure of yogurt gels made from milk heated at 72°C/15 s (a), 85°C/15 min (b) and 100°C/5 min (c) at pH 4.6 and at 85°C/15 min at pH 5.0 (d) and 5.8 (e).

CONCLUSIONS

Heating milk at 100°C for yoghurt production changes the rheological properties of yogurt. With manipulating heat treatment, yogurt of variable textures can be maintained to meet consumers' demands. A high heat treatment may reduce the syneresis as a result of reducing the pores areas in the yogurt matrix. Measuring the matrix pore area seems to be a good technique as an indirect way of predicting the gel syneresis.

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تأثير المعاملة الحرارية للبن على الخواص الريولوجية والتركيب البنائي الدقيق لخثرة الزبادى الجالس Set-Yoghurt

هدى محمود الزينى
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تم تصنيع الزبادى الجالس Set-Yoghurt من اللبن الجاموسى المعامل بدرجات حرارة مختلفة من التسخين المبدئى (٧٢°م/١٥ث ، ٨٥°م/١٥ق ، ١٠٠°م/٥ ق) والملقح ببادئ خليط من (TH4) *Streptococcus. thermophilus* و *L.delbrueckii subsp. bulgaricus* (LB12) وتم تتبع ال pH ، اللزوجة، معامل القص shear stress، العزم torque، أثناء تكوين خثرة الزبادى، كما تم فحص عينات من خثرة الزبادى بالميكروسكوب الألكترونى وأستخدمت طريقة الimage analysis فى تحليل صور الميكروسكوب المتحصل عليها وكذلك لتقييم التركيب البنائى الدقيق لعينات الزبادى المختبرة وكذ لك الخواص الريولوجية الخاصة بهذا التركيب وهى:
surface roughness, compactness, matrix area and pores area .

وقد أشارت النتائج الى أن المعاملة الحرارية للبن على ١٠٠°م/٥ ق أعطت أعلى قيم لكل من: surface roughness, compactness, matrix area, shear stress، torque، viscosity بينما انخفضت هذه الخواص مع المعاملة الحرارية ٧٢°م/١٥ث. وكانت خواص الجيل الحمضى المتكون من اللبن المسخن مختلفة فى بعض الأتجاهات مثل التغير فى الـ pH ، الخشونة ، التركيب البنائى الدقيق. وكانت للمعاملة الحرارية تأثير معنوى عى جميع الخواص الريولوجية المختبرة. وكان قياس المساحات التى تشغلها تجمعات البروتين المتجبن matrix area مفيداً فى تمييز الفروق بين شبكات البروتين المترسب Casein network والمتكونة من اللبن المعامل بمعاملات حرارية مختلفة. كما أعطى قياس مساحة الفراغات pores area فى الجيل دليلاً واضحاً على قدرة الجيل على النفاذية Permeability وكان الأرتباط بين الخواص الريولوجية المختبرة والمعاملات الحرارية سالباً بدرجة معنوية.