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EFFECT OF HEAT DENATURED WHEY PROTEINS ON THE QUALITY OF YOGHURT

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ABSTRACT

The effect of the addition of heat denatured whey proteins (frozen; nonfrozen and enzymatically hydrolyzed) to cow milk on the quality of yoghurt was investigated. Results revealed that addition of whey proteins extended duration of incubation time from 3 hours for control to 4 hours in the treated samples. The control samples showed the highest degree of curd syneresis and curd firmness in comparison with the treated samples. The water holding capacity of yoghurt made with whey proteins increased slightly compared with the control. However, total volatile organic acids; diacetyl and non protein nitrogen (NPN) were markedly increased in yoghurt fortified with whey proteins. These values increased with increasing whey protein concentration. Great differences in the sensory properties were observed with the addition of denatured whey proteins. Control samples had the highest score followed by the ones with added hydrolyzed whey proteins; nonfrozen whey proteins and frozen whey proteins. Cold storage at 7°C for 7 days resulted in a decrease in the pH of all samples after 3 days and then remained constant up to 7 days. Curd firmness and volatile organic acids showed an increase during the storage period while, diacetyl increased after 3 days, then decreased after 7 days.

INTRODUCTION

Whey is a byproduct of cheese and casein manufacture and it contains about 20% of the original milk proteins. The volume of whey produced in the world was reported to approach 200 million Tons / year (McIntosh, et al., 1998). About half of this amount is used for animal feeding; one-third for human nutrition and the rest is wasted (Korbonen, 1995).

The inclusion of whey protein in the diet has been linked with a number of physiological benefits which may involve specific bioactive factors. It was reported that consumption of whey proteins may retard the chemically induced cancers in the experimental animals (Bounous, et al., 1991 and melntosh et al., 1995); stimulate the immune system (Bounous, et al., 1988) and may increase the longevity (Bounous, *et al.*, 1989). Bounous and Gold, 1991 reported that heat-treated whey protein contains high concentration of specific dipeptides (glutamylcysteine), which can promote the synthesis of glutathion, an important factor for the protection and repair of cells. Moreover, addition of whey proteins in the diet has been linked with lowering of low-density lipoprotein (LDL) cholesterol (Zhang and Beynen, 1993). Peptides derived from whey proteins display opioid activity. B- lactotensin derived from B- lactoglobulin; lactoferroxin from lactoferrin and albutensin A from serum albumin, these and other bioactive peptides are used as infant food additive or as antimicrobial ingredient. (Tomita, *et al.*, 1991 and Regester, *et al.*, 1997). Incorporation of whey proteins in different dairy products are well established, i.e in cheese (Mead and Roupas, 2001; Mleko and Foegeding, 2001), in frozen yoghurt (Jayprakasha, *et al.*, 2000), in yoghurt, (Zedan, *et al.*, 2001; Bhullar, *et al.*, 2002 and Puvanenthiran, *et al.*, 2002).

The objective of this present investigation was to compare the effect of addition of heat denatured whey proteins (directly after heating or after freezing for one week) and trypsin hydrolyzate whey proteins on the physico-chemical and sensory properties of yoghurt.

MATERIALS AND METHODS

Milk samples.

Fresh cows milk was obtained from the herd of the farm of the El-minia University.

Preparation of whey proteins.

Denatured whey proteins were prepared from unsalted cheese whey as follow. The cheese whey was filtered through cheesecloth to separate the casein particles then centrifuged to remove the fat. The obtained whey was heated in boiling water bath for 30 min, then cooled under running tap water and left standing overnight. The denatured whey proteins were collect by centrifugation at 10000 g. The obtained whey proteins were divided into three parts, the first part was directly used; the second part was frozen for one week and the third part was air dried and enzymatically hydrolyzed using trypsin enzyme as follow: Trypsin enzyme was added to whey protein solution in concentration 1.2 anson unit / kg solution at pH 8 and 40°C for 16 h. The activity of enzyme was terminated by heating the solution in boiling water bath for 5 min. and then added to milk in required concentration.

The concentration of denatured whey protein was adjusted to be 10% in all treatments then added in concentration 1; 1.5 and 2%. This resulted in an increase in the whey protein / casein ratios to increase from 0.26 in control to 0.6; 0.8 and 1 respectively.

Manufacture of yoghurt:

Control yoghurt was made from 3% fat cow's milk heated at 90°C for 20 min. The milk was cooled to 42°C and inoculated with 0.5% (vol/vol) each of *lactobacillus delbruckii ssp. Bulgaricus* and *streptococcus thermophilus* cultures. The inoculated milk was incubated at 40°C until the coagulum was formed. After fermentation the samples were cooled in the refrigerator and kept overnight.

Chemical analysis.

pH was determined with a Beckman model 3550 digital pH meter (USA).

Curd firmness was expressed as a weight in grams required to penetrate the cured for 3 cm below the surface of coagulum.

Curd syneresis was determined using a drainage method described by Harwalkar and Kalab (1983). The test was performed in refrigerator for 2h and the volume of the whey was collected in graduated cylinder.

Water holding capacity was measured in 15 ml plastic tubes containing 10 ml of yoghurt. The tubes were centrifuged at 3000 g for 10 min and the supernatant fluid drained for 10 min. The water holding capacity was expressed as the weight of pellet after centrifugation (Verset, *et al.*, 2002).

Diacetyl and acetoin were determined colorimetrically as described by Westerfeild (1945)

Total volatile organic acids were determined according to Veip Scientific U.D.K. 130 Manual. The results were expressed as % of acetic acid.

Polyacrylamide gel electrophoresis was determined as described by Andrews, (1983).

Protein contents were determined as described by Gabor, (1989).

RESULTS AND DISCUSSION

Changes In pH.

Changes in pH of yoghurt during incubation represent the activity of starter culture and production of acidity. The changes in pH values were measured every 1 hour during incubation. Results in Table (1) showed that pH of control samples decreased from 6.6 to 4.3 within 3h incubation. However, addition of whey proteins to milk decreased the rate of pH decrease during incubation. Therefore, duration of incubation time was increased to 4h. It thus seems that addition of whey proteins resulted in a decreased acidity. Moreover, the rate of pH decrease was less with increasing the level of whey protein addition. It can be seen that with addition of hydrolyzed whey protein, the pH values during incubation were somewhat higher than that of frozen and nonfrozen whey proteins. These presented results are in agreements with those obtained by Tratnik and Bozanic, (1996), who found that addition of whey protein concentrate to milk extended the duration of culturing process than control by about 30 min to reach pH 4.6. However, Zedan, *et al.*, (2001) found that addition of acetylated whey proteins concentrate had no effect on the development of acidity during incubation.

Table (1): Changes in pH of yoghurt with and without whey protein during incubation.

Treatments	At zero time	2h	3h	4h
Control	Initial pH 6.6	6.13	4.30	
1% frozen		6.07	4.88	4.49
1.5% frozen		6.09	4.85	4.53
2% frozen		6.13	4.94	4.67
1% nonfroz.		6.11	4.85	4.47
1.5%nonfroz.		6.01	4.95	4.55
2% nonfroz.		5.97	5.01	4.59
1% hydrolyz.		6.28	4.91	4.57
1.5%hydrolyz		6.3	4.97	4.69
2%hydrolyz.		6.32	5.04	4.65

Curd firmness.

Results in Fig (1) showed that control samples had the higheST curd firmness. The curd firmness decreased with addition of whey proteins at 1% concentration in all treated samples. The decrease of curd firmness was more pronounced with increasing of whey proteins to 1.5 and 2%. Also Olivera, *et al.*, (2001), who found that yoghurt supplemented with whey powder exhibited low firmness and viscolelasticity. Curd firmness represents the formation of more linkages between micelles and micelles fusion (Green, 1980). However, Tratnik and Bozanic (1996); Zedan, *et al.*, (2001) and Bhullar, *et al.*, (2002) found that addition of whey protein concentrate;

acetylated whey protein and whey protein powder increased the strength of yoghurt curd. Mottar and Bassier (1987) reported that, heat denatured β-lactoglobulin exhibited superficial filaments and appendages on casein micelle surface inhibiting micelle fusion when milk was fermented. It is noteworthy to mention that, aggregates like gel were formed during heating with 1.5 and 2% whey protein addition. On the other hand, Molder and Kalab, (1983) observed that, when yoghurt was fortified with whey proteins, the casein micelles appeared in the form of individual entities linked to each other at relatively long distance with finely flocculated whey proteins. Thus, the strength of the yoghurt gel decreased.

Water holding capacity.

Water holding capacity represents the ability of yoghurt to bind sufficient water. Results in Fig (2) show the water holding capacity (W.H.C) expressed in gm /100 gm of treated and untreated samples. It appeared that yoghurt made with hydrolyzed whey proteins exhibited the highest WHC and yoghurt made with frozen whey protein showed more ability to bind water than control but less than that made with non frozen whey protein. It was observed that the solubility of hydrolyzed whey protein was more than the of frozen whey proteins which formed undissolved aggregates. This, perhaps the reason for the increase of the WHC of hydrolyzed whey proteins than the frozen ones.

Curd syneresis

Water in yoghurt is distributed in three different forms bound, capillary and free water. The free water is the major source of wheying off (Rasic and Kurmann, 1978). Results in Fig (3) showed that control samples had the highest degree of syneresis compared to whey protein containing samples. The susceptibility to syneresis decreased with increasing whey protein concentration and the hydrolyzed whey protein samples exhibited the lower syneresis. These results are in accordance with the findings of Bhullar, *et al.*, (2002) and Punanenthivanr, *et al.*, (2002) who found that the rate of wheying off was reduced with increasing the whey protein / casein ratio.

Total volatile organic acid

Volatile organic acids arise from lactic acid bacteria action on lactose and citrate during fermentation or/and during lipolysis of milk fat. Many volatile compounds were detected in fermented dairy products (formic, pyruvic, uric, propionic, butyric, acetaldehyde, diacetyl, ethanol, aceton, acetoin, 2- butanon and hexanal (Hassanein, 1998). In the present study TVOA were expressed as mg acetic acid /100g samples.

Data in Fig (4) show that the control sample exhibited the lowest value (14.4 mg/ 100g), while the treated samples showed higher values being in the range of 19.2-31.2 mg /100g for hydrolyzed whey proteins and 19.2-20.4 mg/100g for nonfrozen whey proteins. While the moderate values were with frozen whey proteins. Kamaly, *et al.*, (1998) reported that the value of volatile acids in cultured milk was equivalents to 13.2 mg acetic acid /100gm sample and this was increased by about 10 –15 times with storage for 48h. The increase of volatile organic acids during storage may be attributed to the lipolytic activity of yoghurt culture (Alm,1982a and Hassan, 2004).

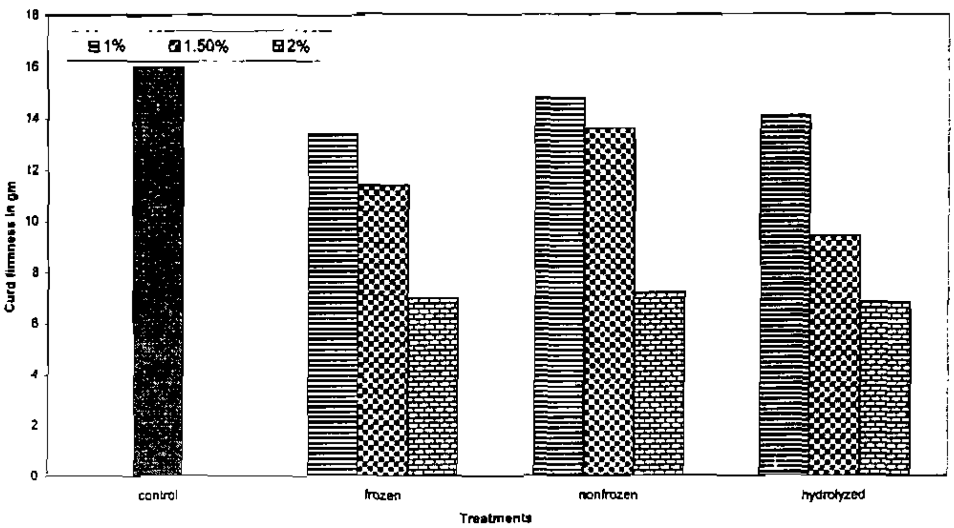


Fig. (1): Effect of whey protein addition on curd firmness of yoghurt.

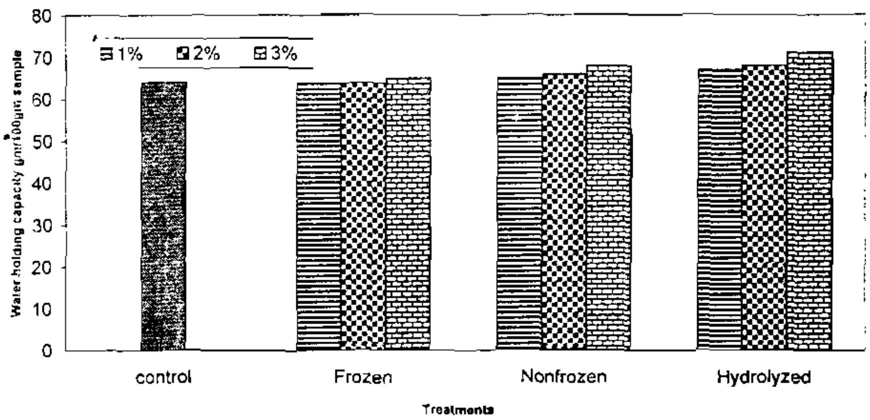


Fig. 2: Effect of whey proteins addition on the water holding capacity of yoghurt.

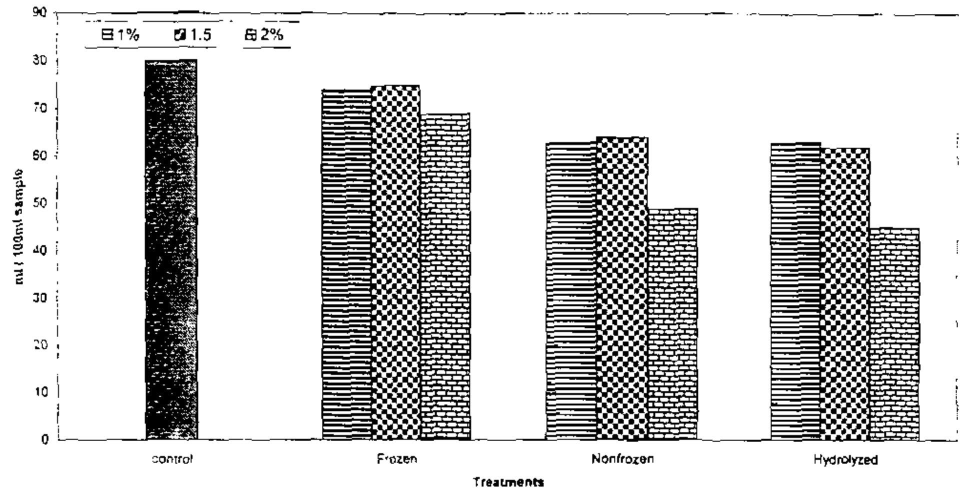


Fig. 3: Effect of whey protein addition on curd syneresis of yoghurt

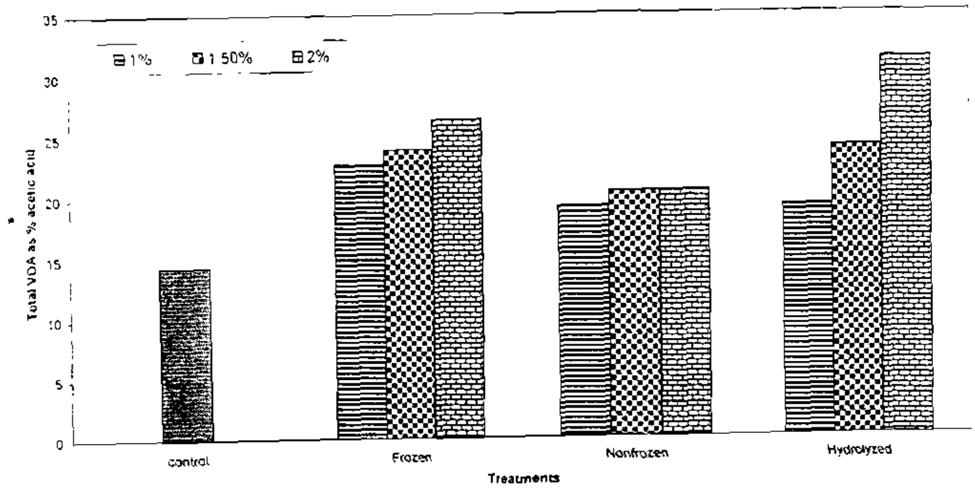


Fig. 4: Effect of whey protein addition on total volatile organic acids of yoghurt

Diacetyl and acetoin

Volatile compounds produced by culture bacteria such as diacetyl, acetoin and acetaldehyde contribute to the distinctive flavor and aroma of yoghurt. Diacetyl is produced mainly by *Strepococcus thermophilus* and is responsible for full flavor and aroma in particular for products that contain low acetaldehyde concentration (Hassanein,1998). Data in Fig (5) show the diacetyl content in control and treated samples. It can be seen that the concentration of diacetyl were lower in the control (1.19 mg/L) than all treatments. Samples with whey protein hydrolyzate had the highest quantities of diacetyl (1.83 – 2.5mg/L). Samples containing nonfrozen whey proteins had diacetyl value between 1.5-1.63 mg/L and the ones with frozen whey proteins ranged 1.29- 1.46mg/l. Kogan and Accolas, (1996) and Hassanein, (1998) suggested that diacetyl is derived from acetyldehyde and /or α – acetolactate during fermentation by specific enzymes that are more active at pHoptimum pH 4.6- 5.0. In this study, addition of whey proteins into milk increased the incubation time from 3h for control to 4h for treated samples and this may allow better condition for the production of diacetyl. In addition, the pH of control samples was lower (4.3) while those of treated samples were about 4.6 and this is may be another reason for the lower value in control samples.

Acetoin was also determined in the same samples as optical absorption at 540 nm after the colors were developed for 1h. Results in Fig (6) showed that optical absorption values were less in the control samples than the treated samples. Increasing the level of added whey protein frozen and nonfrozen resulted in a reduction in optical absorption, but the values were still more than the control, However, with the hydrolyzed whey proteins increasing the level up to 2% reduced optical absorption to the extent that become less than the control. (Fig. 6).

Non-Protein Nitrogen (NPN)

JNPN was determined in 12% TCA soluble nitrogen spectrophotometrically and the results were expressed as optical absorption at 280nm. Results in Fig (7) demonstrated that incorporation of whey proteins into milk enhanced the formation of NPN and such increase was found to be proportional to the amount of whey protein added. Moreover, the highest rate of NPN was found with hydrolyzed whey proteins and the lowest was with frozen whey proteins. The increase in NPN as a result of whey proteins addition could be attributed to the stimulating effect of whey proteins on yoghurt culture, which enhanced the proteolysis. Alm, (1982b) reported that casein degraded to a lesser extent during fermentation, while whey protein tended to be more hydrolyzed. Gassem and Frank, (1991) also found that yoghurt culture produces proteases during fermentation causing an increase in NPN. The high levels of NPN in yoghurt made with hydrolyzed whey protein may be attributed not only to proteases produced by yoghurt starter but also to trypsin added during the hydrolysation process. Candloti, *et al.*,(2001) reported that addition of microbial proteases to native whey proteins produced high nitrogen in 12% TCA. Moreover, Bertrand- Harp *et al*, (2002) demonstrated that denaturated whey proteins were more susceptible to tryptic hydrolysis (trypsin or pepsin) than native form, probably because of the exposure of their hidden tryptic cleavage sites (Lys- and Arg- x bonds).

Electrophoretic patterns.

The electrophoretgrams of the experimental yoghurt after 24h of refrigerated storage are shown in Fig. (8). All samples gave essentially the same electrophoretic patterns One faint band was resolved in the γ-casein region, which is assumed to be derived from β-casein (Andrews, 1983). Several fast moving bands were resolved a head of αs-casein region. It appeared that β and αs-casein were more hydrolyzed in samples containing hydrolyzed whey proteins. These bands were absent or present at low relative concentration in the yoghurt made with nonfrozen whey proteins. These findings are in line with NPN analysis (Fig. 7). Also Kananen, *et al.*, (2000) reported that, whey proteins hydrolyzed more readily with formation of high and low (<2000 D) molecular fractions.

Sensory evaluation

Organoleptic properties of yoghurt samples with and without whey proteins are presented in Table (2). The results showed that, the control samples had the highest score (94 point), followed by those from hydrolyzed whey proteins (90, 90 and 81 point for 1; 1.5 and 2% respectively). The lowest score was obtained samples containing frozen whey proteins (82; 69 and 57 in the same order. While, the score with nonfrozen whey proteins were 87; 87 and 70 respectively. It is noteworthy to mention that, samples with frozen whey proteins had undesirable tough aggregates in the mouth feel and some of them were precipitated during incubation particularly at high concentrations.

Table (2): Sensory evaluation of yoghurt fortified with whey protein.

Sample	control	1% F	1.5% F	2% F	1% NF	1.5% NF	2% NF	1% HD	1.5% HD	2% HD
Flavor	52	48	37	35	50	50	45	51	52	48
Body ; Texture	30	22	20	15	25	25	15	27	26	22
Appearance	12	12	11	7	12	12	10	12	12	11
Total	94	82	69	57	87	87	70	90	90	81

F = Frozen NF= Nonfrofen HD= Hudrolyzed

Changes during cold storage.

Yoghurt samples were stored under refrigeration (7°C) for 7 days. As expected, pH values exhibited further decrease on storage for 3 days and then remained almost constant until 7 days (Table 3). These results are in accordance with those of Laye, *et al.*, (1993) who found that lactic acid concentration of yoghurt was either increased slightly or remained constant during 12 days of refrigerated storage.

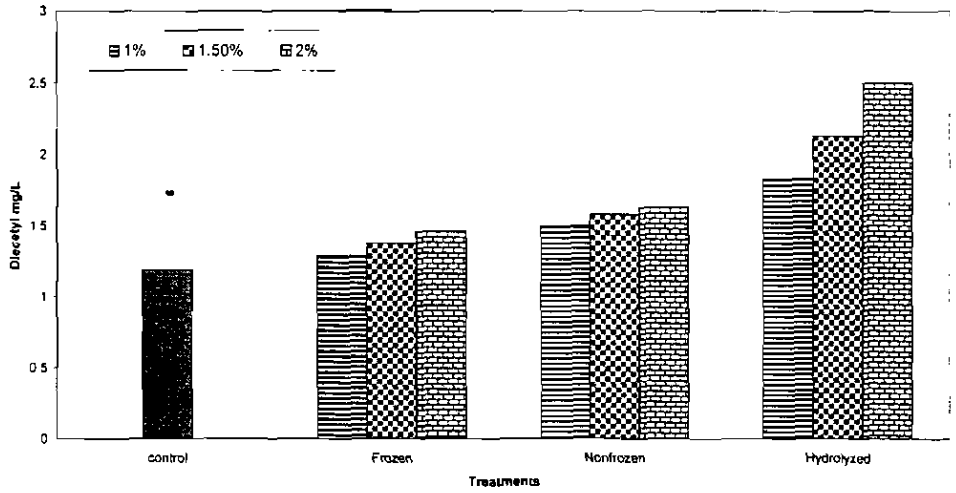


Fig. 5: Effect of whey protein addition on diacetyl formation in yoghurt

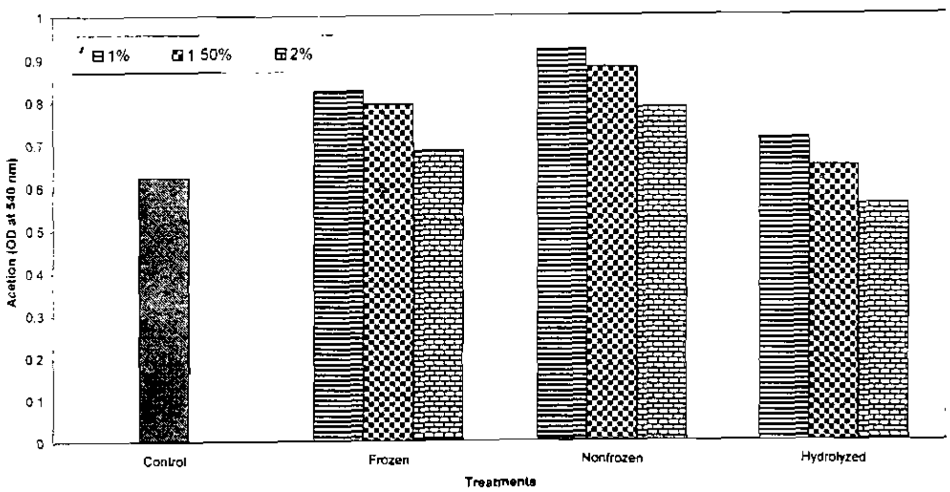


Fig. 6: Effect of whey protein addition on acetoin of yoghurt

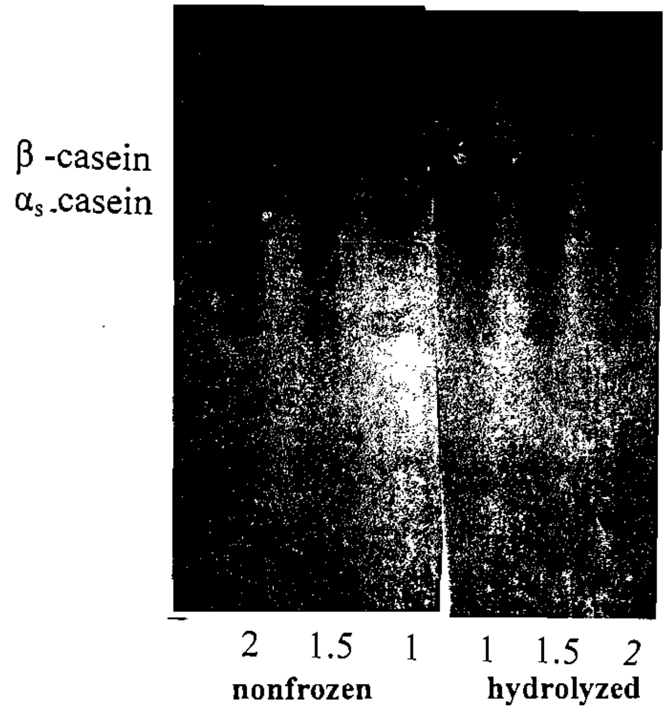


Fig. (8): Poly acrylamide gel electrophotograms of nonfrozen and proteins hydrolyzed whey

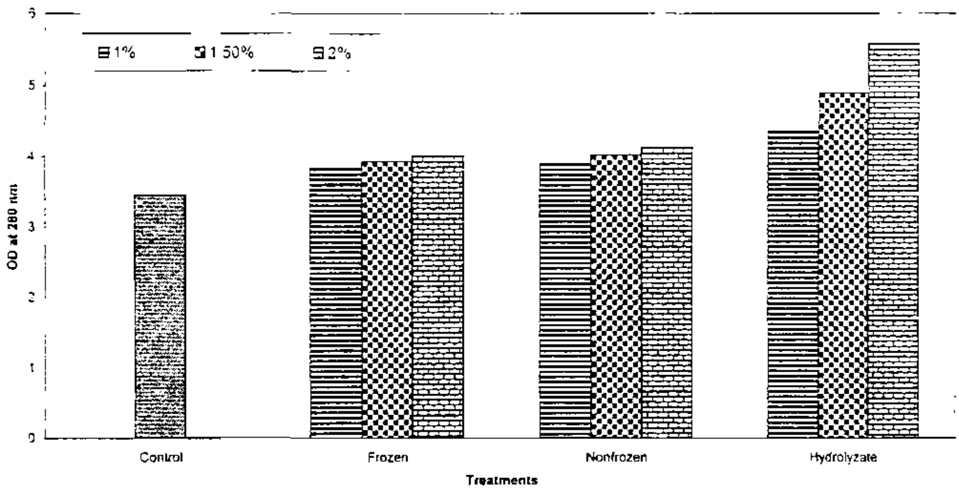


Fig. 7: Effect of whey protein addition on formation of NPN in yoghurt

Table(3):Changes in pH of yoghurt samples on storage for7days at 7°C

Sample	One day	three days	Seven days
Control	4.3	4.1	4.07
1% nonfrozen	4.47	4.13	4.11
1.5%nonfrozen	4.55	4.39	4.28
2%nonfrozen	4.59	4.32	4.31
1% hydrolyzate	4.57	4.07	4.02
1.5% hydrolyz.	4.69	4.05	4.04
2% hydrolyzate	4.65	4.11	4.09

The changes in curd firmness throughout the storage period are shown in Fig. (9). It can be seen that, curd firmness was greatly increased with increasing storage period up to 7 days. Hassan, (1997) claimed that low storage temperature favors better firmness, while high temperature encourages formation of weak body.

The concentrations of volatile organic acids were markedly increased during the storage. With high concentration of whey protein addition the values of total volatile organic acids increased to 57.6 and 43.2 mg / 100 gm in nonfrozen and hydrolyzed whey protein containing samples respectively (Fig. 10). Tammime and Robinson, (1985) reported that acetic acid as well as formic, propionic and butyric acids are among the volatile compounds that generally produced during fermentation of yoghurt by lactic acid bacteria.

Fig. (11) shows the effect of storage on the diacetyl contents of yoghurt. Diacetyl contents increased on storage for 3 days followed by a decrease thereafter up to 7 days. Laye, *et al.*, (1993) suggested that the changes in the concentration of flavor components during storage could be due to the reactions that resulted in the formation or conversion to other compounds or/and their losses due to volatilization. These also showed that, diacetyl concentration decreased on storage for 10 days.

It could be concluded that, nonfrozen or hydrolyzed denatured whey proteins could be used as suitable ingredient additives for production of yoghurt. Frozen denatured whey proteins at 1.5 and 2% concentration had lower acceptability because of the undesirable tough aggregates in mouth feel.

Fig. 9: Effect of storage on the curd firmness og yoghurt

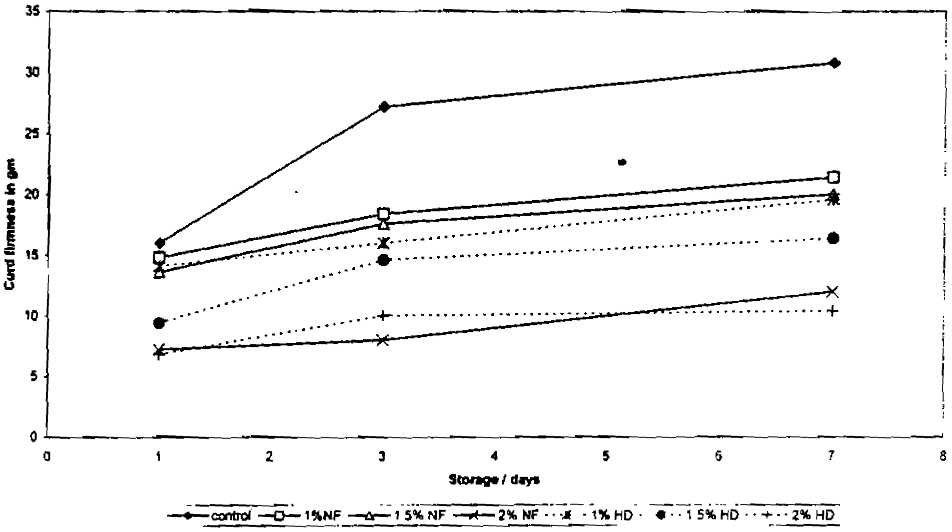


Fig. 10: Effect of storage on the total volatile organic acid in yoghurt

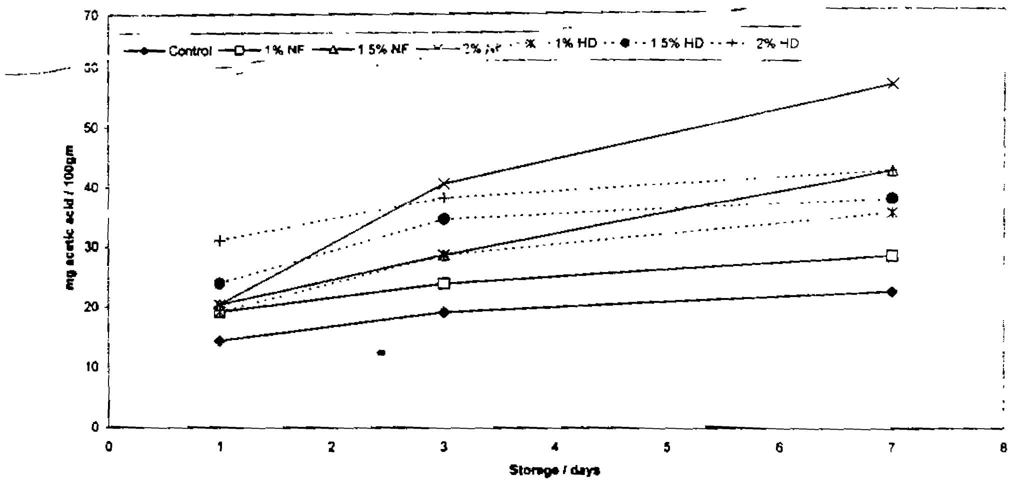
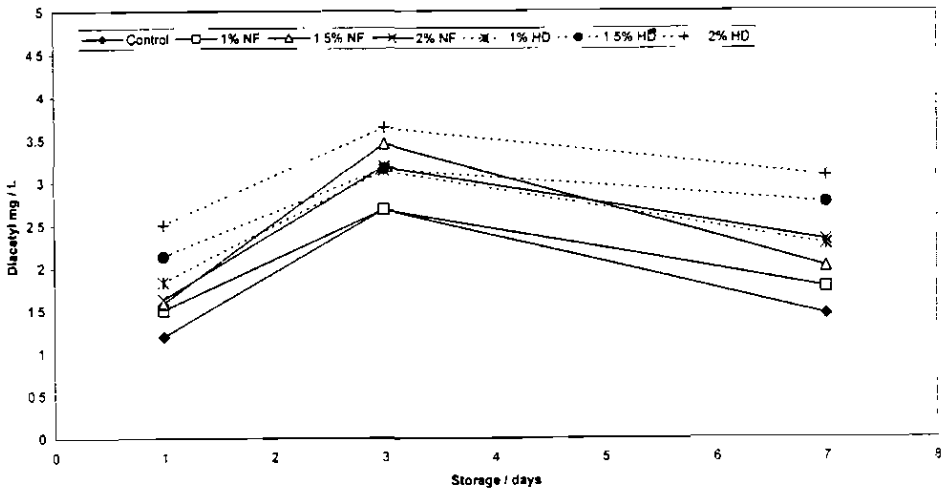


Fig. 11: Effect of storage on the formation of diacetyl in yoghurt



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تأثير إضافة بروتينات الشرش المدنترة حراريا على درجة جودة الزبادى

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تم دراسة تأثير إضافة بروتينات الشرش المدنترة حراريا (المجمدة و الغير مجمدة والمحللة إنزيميا) على درجة جودة الزبادى المصنع من اللبن البقرى. أدت إضافة بروتينات الشرش المدنترة إلي اللبن المعد للصناعة إلي زيادة فترة التحضين من ٣ إلى ٤ ساعات.واظهر الكنترول أعلى درجة فى قوة الخثرة كما زاد انفصال الشرش بالمقارنة بالعينات المعاملة بينما ازداد الماء المحتجز داخل الخثرة فى العينات المعاملة قليلا مقارنة بالكنترول.إزدادت كلا من الأحماض العضوية الكلية و الداى استيل والنتروجين الغير بروتينى زيادة واضحة مع زيادة بروتينات الشرش المضافة و ظهر تباين واضح بين العينات فى الخواص الحسية , أظهر الكنترول أعلى درجات فى الخواص الحسية ثم بروتين الشرش المتحلل انزيميا ثم الغير مجمد وأخيرا" المجمد.

خلال التخزين على ٥٧م لمدة ٧ أيام انخفض ال pH لكل العينات بعد ٣ أيام ثم ثبت بعد ذلك حتى اليوم السابع. كما زادت قوة الخثرة و الأحماض العضوية المتطايرة خلال فترة التخزين بينما الداى أستيل زاد بعد ٣ أيام ثم انخفض بعد ذلك.