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EFFECT OF IRON FORTIFICATION ON THE QUALITY OF YOGHURT

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ABSTRACT

Sheep milk was used in making of yoghurt by a traditional method in five treatments. Yoghurt was manufactured without ferrous salts (control), the other four treatments of yoghurt were fortified by adding 10 and 20 mg. Ferrous chloride (T_1,T_2) and 10,20 mg. Ferrous gluconate (T_3,T_4) / litre of sheep milk. The resultant yoghurt was kept in refrigerator for 7 days and analysed when fresh and after 3,5 and 7 days for T.S% fat%; T.P. %; pH; lactose content; Thiobarbituric acid (T.B.A).; Total volatile fatty acids(T.V.F.A).; acetaldehyde and diacetyl contents. All yoghurt samples were evaluated organoleptically for flavour; consistency and appearance. The obtained results showed that fortification of yoghurt with ferrous (chloride and gluconate) increased T.V.F.A; T.B.A; and diacetyl contents. While decreased pH; lactose % and acetaldehyde content during storage period, on the other hand, ferrous gluconate took the same trend but with more pronounced effect on these properties whether by increasing or decreasing except the T.B.A. content which increased more with adding ferrous chloride. Also, results revealed that, no prounced differences (not significant., $P>0.05$) were noticed for T.S%; fat % and T.P.% in control and fortified samples either fresh or during storage period. However, clear difference (highly significant $P<0.01$) for pH; lactose content; T.V.F.A; T.B.A; acetaldehyde and diacetyl contentswere noticed. Sensory evaluation indicated that fortification of yoghurt with iron salt (chloride or gluconate) and storage had no effect on the appearance (not significant $P > 0.05$) whereas a clear effect was founed in flavour and consisitency (highly significant $P<0.01$) of the resultant product. Sensory evaluation indicated that adding ferrous gluconate was considered as the most suitable source of iorn supplement with its two ratios. It produced gyoghurt having favourable flavour, acceptable consistency, good appearance and no wheying – off.

INTRODUCTION

The production of sheep and goat milk is important in several countries whose climatic conditions are suitable such as Egypt. Buffaloe and cow represent the major dairy animals but goat and sheep are also found in a large population that amount to about 4 millions, Mashaly *et al* (1984) The sheep population in Egypt in 1996,were 7346000 Capita (AST, 1997). Milk and dairy products contribute high quality protein, calcium, and some minerals such as magnesium, phosphorous, but it is poor in zinc and iron. Dairy products containing added iron are uncommon since, iron promotes lipid oxidation and reacts with milk components. (Zhang & Mahoney ,1991; and Jackson ,1992).

Yoghurt is considered as a good fermented dairy product for health, therefore, its production have become widely increased, (Tamime & Deeth ,1980). Moreover, the availability of yoghurt was also higher than that of raw milk. Yoghurt Contains all the elements of nutrition found in milk in a more digestable form, (Abou-Dawood *et al*.,1993). Dairy products are appropiate vehicles for iron fortification since they have high nutritional density, reach target populations and are widely distributed. Iron fortification could increase nutritive value and consumer appeal of dairy products. Fortifying dairy products with iron would enhance their nutritional value, Cheddar Cheese have been successfully fortified with iron,(Zhang & Mahoney, 1989). Hengenauer, *et al*., (1979), found that, chelated forms of iron that rapidly donated iron to casein prevented oxidation of milk lipids. Iron fortification of various foods stands as a reasonable solution to increase dietary iron levels. Iron deficiency anemia is still a most prevalent nutritional problem in the world as well as in Egypt, Degheidi *et al*. (1996). The purpose of the present study is to manufacture yoghurt from sheep milk fortified with iron and to investigate the chemical and organoleptic properties of the resultant yoghurt during storage as affected by the source and concentration of iron used.

MATERIALS AND METHODS

Material :

Fresh whole sheep milk was obtained from a private farm, Dakahliah Governorate, Egypt.

Source of iron:

Ferrous chloride; ferrous gluconate (food grade) were purchased from sigma chemical company, and used to prepare the experimental samples for the current work.

Yoghurt culture :

Mixed culture consisted of ***lactobacillus delbrueckii sub. sp. bulgaricus*** and ***streptococcus salivarus sub. sp. thermophilus*** (1:1) from Chr. Hansens Copenhagen, Denmark was obtained from Misr Dairy company, Egypt.

Experimental procedure :

Fresh whole sheep milk was heated to 85^{O}C. for 20 min. and cooled to 42^{O}C. Preheated milk was divided into five parts of two kg. milk, the first one served as a control. Two parts were treated with ferrous chloride and the other two parts were treated with ferrous gluconate at levels of 10 and 20 mg/L. milk respectively. Both control and treated sheep milk samples were inoculated with 3% starter culture and incubated at 42^{O}C. until a firm curd was formed. The resultant yoghurt was kept in refrigerator at (5-8^{O}C.). The samples of yoghurt were analysed for chemical and organoleptic properties at fresh and after 3, 5 and 7 days of storage. Three replicates of each treatment were carried out.

Methods of analysis :

pH-value of inoculated sheep milk was test every ½ hr. during incubation time until it was curdled. A combined electrode (MV-870) digital – pH – meter was used for measuring the pH values. The resultant yoghurt samples were analysed for total solids (T.S.)%; fat%; total protein (T.P.)%; lactose % and pH – values according to the methods mentioned in the A.O.A.C. (1990). Acetaldehyde and diacetyl contents were estimated as mentioned by Lees & Jago (1969) and Wasterfeld (1945), respectively. Thiobarbituric acid (TBA) was determined by the method of Keeny (1971), while total volatile fatty acids (T.V.F.A) were estimated according to the method of Kosikowski (1978). Organoleptic properties of yoghurt were assessed using a panel test of 10 persons of the department staff, these properties were : flavour (50 point); consistency (40 point). appearance (10 points) and total acceptability (100 point). Statistical analysis was carried out according to Snedecor and Cochran method (1982).

RESULTS AND DISCUSSION

Development of pH – value during incubation time :

Results in table (1) revealed that pH – values gradually decreased along the incubation time in all treatments, due to the developed acidity by the yoghurt culture. Also, results indicated that. the pH value decreased with the incubation time to reach pH 4.6 ,and it varied depending on the level of ferrous chloride and ferrous gluconate (iron sources) added and also the milk type. Control yoghurt reached pH – value of 4.6 after 210 min., while it reached that level after 180 min, in all other treatments. These results indicated that, the addition of ferrous chloride and ferrous gluconate resulted in a further decrease in pH – value (increase in acidity) as a result of the growth of the yoghurt starter but it was higher in case of farrous gluconate than ferrous chloride. Results were in agreement with those of Koladkin *et al*. (1974) and Jarrstt (1979) , who reported that addition of trace elements Zn, Fe and Cu sulphate to milk increased acidity (decrease pH-value). This is due to more intensive growth of lactic streptococcus and total number of microflora during incubation in yoghurt during storage.

Table (1) : Changes in pH – values in inoculated milk fortified with iron during incubation time at 42^{o}C.

Treatments	Incubation time (min.)							
	Zero	30	60	90	120	150	180	210
T_0	6.62	6.45	6.25	5.88	5.70	5.35	4.80	4.60
T_1	6.64	6.34	6.18	5.85	5.60	5.20	4.68	-
T_2	6.60	6.30	6.10	5.80	5.40	5.02	4.65	-
T_3	6.62	6.28	5.90	5.45	4.90	4.65	4.60	-
T_4	6.61	6.32	5.80	5.40	4.80	4.62	4.60	-

T_0 = Sheep milk without additives (control)
T_1 = Sheep milk with 10mg. ferrous chloride / Litre milk.
T_2 = Sheep milk with 20mg. ferrous chloride / Litre milk.
T_3 = Sheep milk with 10mg. ferrous gluconate / Litre milk.
T_4 = Sheep milk with 20mg. ferrous gluconate / Litre milk.

Degheidi and Abd-Rabou (1998) confirmed that the decrease in pH-values might be due to the reduction of lactose in milk products. Also, results are in accordance with Degheidi, *et al*. (1996), who found that titratable acidity of Edam Cheese fortified with iron (ferrous chloride and with ferrous gluconate) was higher in all treatments than in control (decreasing pH values) along the ripening periods. This might be attributed to the proteolysis and increase in nitrogen fraction during ripening.

Table (2) shows that no clear differences in the total solids (T.S.)% of all iron fortified sheep yoghurt when compared with untreated control samples. All values ranged between 15.94 to 16.38 against 15.86% for control, it means that addition of ferrous chloride and ferrous gluconate slightly increased the T.S. % in the resultant yoghurt. The same trend was recorded for total protein (T.P.)%, values ranged between 4.28 to 4.54 against 4.30% in control. On the other hand, fat % slightly decreased and ranged between 6.42 to 6.51 against 6.51% in control yoghurt samples during storage periods. Results are in agreement with those found by Abou-Dawood *et al*., 1993 ; Alm, 1982 found that the silght increase of total protein content after fermentation and storage could be explained by the production of volatile substances that evaporate in lyophlization of samples and caused slight decreased in fat percent . this alters the dry matter content so that the nitrogen moiety in the fermented milk samples shows an increase. Abd – Rabou *et al*., 1992, those found that T.P % and T.S.% increased in yoghurt during storage and fat% decreased , also Abd-Rabou *et al*., 1999, found the same trend in yoghurt manufacture from buffalo milk fortified with zinc during stoage. Also, results are in agreement with Zhang & Mahoney (1991), those found that in processed Cheddar Cheese fortified with iron. On the other hand, El-Sayed, *et al*. (1997) indicated that T.S.% and T.P. % slightly increased and pH – value; fat % slightly decreased in all samples of processed Ras Cheese fortified with ferrous chloride and ferrous gluconate, these results are in accordance with our results. Jackson (1992) found that T.S.% slightly increased and fat % decreased by iron fortification (ferrous chloride) of Cheese. Results also are in agreement with that reported by Degheidi, *et al*. (1996) for Edam Cheese fortified with ferrous (chloride, and gluconate).

Fig (1) shows the changes in pH-values of sheep yoghurt fortified with ferrous chloried and ferrous gluconate during storage periods. It is clear that, pH-values of the resultant sheep yoghurt decreased during storage periods in all samples fortified with iron salts. This might be due to the development in acidity by the yoghurt culture. Addition of ferous chloride and ferrous gluconate increased acidity (decreased pH-values) depending on the iron and its concentration. Koladkin, *et al*. (1974) found that, the addition of trace elements of Zn; Fe and Cu sulphate to milk increased the acidity. Results are in accordance with those found by Degheidi et. al. (1996), who revealed that, the fortification of Edam Cheese with ferrous salts (chloride and gluconate) increased acidity in Edam Cheese and decreased pH-values.

Table (2): Chemical composition of sheep yoghurt fortified with iron.

Storage period (day)	Treatments	Total solids (T.S.%)	Fat %	Total protein (T.P.%)

Fresh	Control	15.86	6.51	4.30
	T_1	16.01	6.51	4.28
	T_2	16.12	6.50	4.29
	T_3	15.94	6.51	4.30
	T_4	15.96	6.50	4.28
3	Control	15.89	6.50	4.35
	T_1	16.13	6.49	4.36
	T_2	16.20	6.49	4.37
	T_3	16.02	6.48	4.38
	T_4	16.03	6.48	4.38
5	Control	15.92	6.47	4.40
	T_1	16.16	6.46	4.42
	T_2	16.31	6.46	4.43
	T_3	16.08	6.45	4.44
	T_4	16.11	6.45	4.45
7	Control	15.96	6.45	4.46
	T_1	16.22	6.44	4.48
	T_2	16.38	6.43	4.49
	T_3	16.11	6.42	4.52
	T_4	16.15	6.42	4.54

*** means of 3 replicates**

The changes in lactose content in sheep yoghart treated with ferrous salts during storage periods are shown in Fig (2). It could be observed that lactose % decreased with different ratios in all treatments. However, the highest decrease in lactose content was noticed in T_3 and T_4 (ferrous gluconate) while the lowest decrease was recorded in control samples during storage periods of yoghurt. Tamime and Robinson (1985) reported that the decrease in lactose % during storage periods of yoghurt was an indicative to the action of enzyme galactosidase permease and B-D galactosidase produced by the organisms of ***S.thermophilus*** and ***L.bulgaricus***. Results of lactose content are in agreement with thosenoticed by El-Sayed, *et al*. (1997) who found that, lactose content decreased in processed Ras Cheese fortified with ferrous chloride and ferrous gluconate , however the effect of gluconate was higher. Degheidi, et. al. (1996), found that, Edam cheese forified with iron (ferrous chloride and ferrous gluconate) contained lower lactose content than control. Zhang & Mahoney (1989) showed these observations in the study of the chemical composition of Cheddar cheese , yoghurt and white soft cheese.

Data in Fig (3) illustrated that T.V.F.A. content in the resultant sheep yoghurt gradually increased over the storage periods for all treatments. However, the increase in total volatile fatty acids (T.V.F.A.) was more pronounced in experimental yoghurt treated with ferrous gluconate (T_3 , T_4) than with ferrous chloride (T_1 , T_2) during storage periods. Shalaby, et. al. (1992) reported that, the increase in fatty acids of yoghurt during storage might be due to the lipolytic activity of lactic culture resulting in the liberation of fatty acids during the hydrolysis of milk fat. Results are in agreement with those reported by El-Shibiny, *et al*. (1979); Hofi, *et al*. (1978) El-Senaity (1999), who revealed that T.V.F.A. content increased gradually during storage for 7 days of yoghurt. On the other hand results also are in accordance with Hermansen, *et al.* (1995) and El-Etriby, *et al.* (1997).

Thiobarbituric acid (TBA) content as shown in Fig (4) increased by prolonging the storage periods and these increase was more pronounced by fortification of sheep yoghurt with ferrous chloride while ferrous gluconate had the lowest effect on T.B.A. Results illustrated that the average of TBA values as optical density (O.D.) gradually increased during storage periods of sheep yoghurt fortified with ferrous (chloride and gluconate). Results are in accordance with that noticed by Abd-Rabou *et al* ,(1999) they noticed that TBA content inceased during storage of buffalo yoghurt . Degheidi, *et al.* (1996) who reported that TBA values gradually increased during the ripening periods of Edam Cheese fortified with ferrous chloride more than with ferrous gluconate and took the same trend of TBA increasing. El-Sayed, *et al.* (1997) found that TBA increased in processed Ras Cheese fortified with ferrous chloride more than with ferrous gluconate Jackson, (1992) reported that TBA values increased in a higher ratio when Cheese fortified with ferrous chloride than with ferrous gluconate.

Acetaldehyde Content :

The average of acetaldehyde content of sheep yoghurt fortified with ferrous chloride and ferrous gluconate was shown in Fig (5). Acetaldehyde values decreased during storage periods of sheep yoghurt, the highest values of acetaldehyde were noticed in yoghurt samples fortified with ferrous gluconate followed with ferrous chloride. Results are in agreement with those reported by Abd-Rabou, *et al.* (1999) and El-Senaity, (1999) who revealed that acetaldehyde content decreased during storage period of yoghurt made from Buffalo milk and goats milk.

Diacetyl Content :

Fig (6) shows diacetyl content during storage periods of sheep yoghurt fortified with ferrous (chloride and gluconate), these values took the opposite trend of acetaldehyde as the highest level of increase was observed in samples of yoghurt fortified with ferrous gluconate. Results are in accordance with those of Abd-Rabou, *et al*. (1999) and El-Senaity, (1999) who reported that diacetyl content increased in yoghurt during storage.

Organoleptic properties :

Table (3) gives the averages scores for the organoleptic properties of sheep yoghurt as affected by the salt and level of ferrous chloride and ferrous gluconate. However, these different levels. of ferrous (chloride and gluconate) had variable effects in both flavour and consistency of sheep yoghurt. The highest total scores was obtained in yoghurt fortified with ferrous ghuconate (T_3 , T_4), however, its highly preferable than all other treatments (T_1 , T_2) along storage periods.

Table (3) : The organoleptic properties of sheep yoghurt treated with iron.

Storage period (day)	Treatments	Flavour	Consistency	Appearance	Total
		(50)	(40)	(10)	100

Fresh	T_0	45	39	9	93
	T_1	45	36	9	90
	T_2	46	37	9	92
	T_3	48	38	9	95
	T_4	49	37	9	95
3	T_0	45	38	9	92
	T_1	44	37	8	89
	T_2	45	36	9	90
	T_3	47	38	9	94
	T_4	45	38	9	92
5	T_0	45	36	9	90
	T_1	43	35	8	86
	T_2	42	36	9	87
	T_3	44	38	8	90
	T_4	43	38	8	89
7	T_0	43	34	9	86
	T_1	40	35	8	83
	T_2	40	36	9	85
	T_3	43	37	8	88
	T_4	43	36	8	87

T_0 = Sheep milk without additives (control)
T_1 = Sheep milk with 10mg. ferrous chloride / Litre milk.
T_2 = Sheep milk with 20mg. ferrous chloride / Litre milk.
T_3 = Sheep milk with 10mg. ferrous gluconate / Litre milk.
T_4 = Sheep milk with 20mg. ferrous gluconate / Litre milk.

Statistical analysis :

Statistical analysis of variance (table 4) for the above results confirmed by the analytical data where there were highly significant effect ($P<0.01$) of iron fortification on pH, lactose, T.V.F.A., TBA, acetaldehyde, and diacetyl values as well as sensory evaluation except appearance. While not significant effect ($P>0.05$) was observed for T.S., Fat, and T.P. contents. The effect of storage periods on chemical properties and organoleptic scores was the same as well as the effect of fortification.

Table (4) :Statistical analysis of properties of sheep yoghurt fortified with iron.

Source		DF	MS	Prob.
T.S. %	A	4	0.064	ns

	B	3	0.031	ns
Fat %	A	4	0.009	ns
	B	3	0.104	ns
T.P. %	A	4	0.018	ns
	B	3	0.023	ns
pH-value	A	4	0.038	**
	B	3	0.703	**
Lactose %	A	4	0.220	**
	B	3	0.920	**
T.V.F.A.	A	4	250.630	**
	B	3	348.850	**
TBA	A	4	0.00002	**
	B	3	0.00003	**
Acetaldehyde	A	4	920.300	**
	B	3	1350.980	**
Diacetyl	A	4	907.200	**
	B	3	2868.460	**
Flavour	A	4	4.170	**
	B	3	22.530	**
Consistency	A	4	3.170	**
	B	3	9.380	**
Appearance	A	4	0.050	ns
	B	3	0.070	ns

A = Treatments ; B = Storage periods (day)
ns= not significant (P > 0.05) ; ** = high significant (P < 0.01)

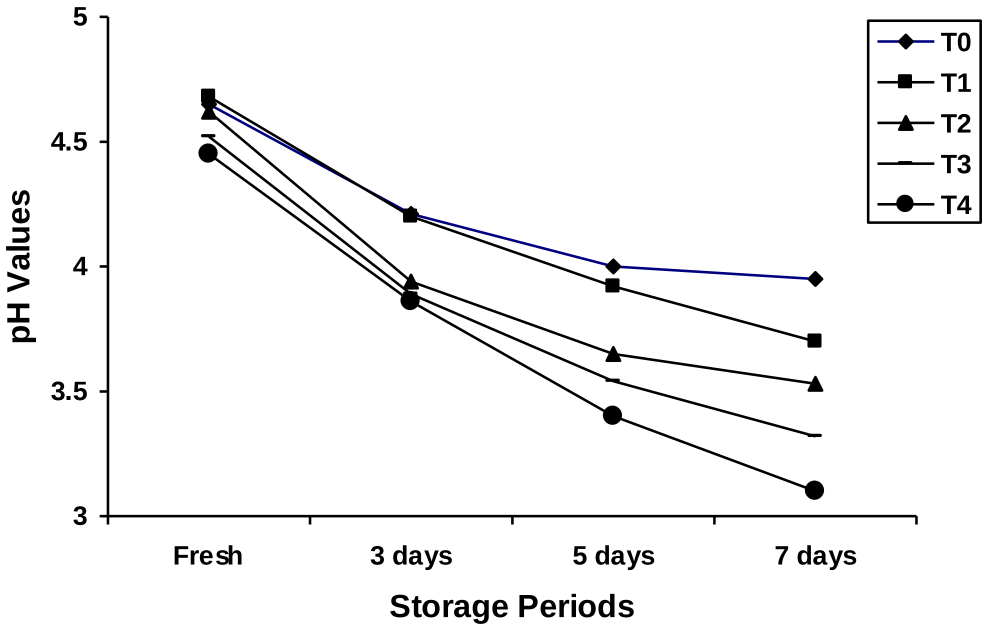

Fig. (1): Effect of adding iron salts on the pH values of sheep yoghurt during storage.

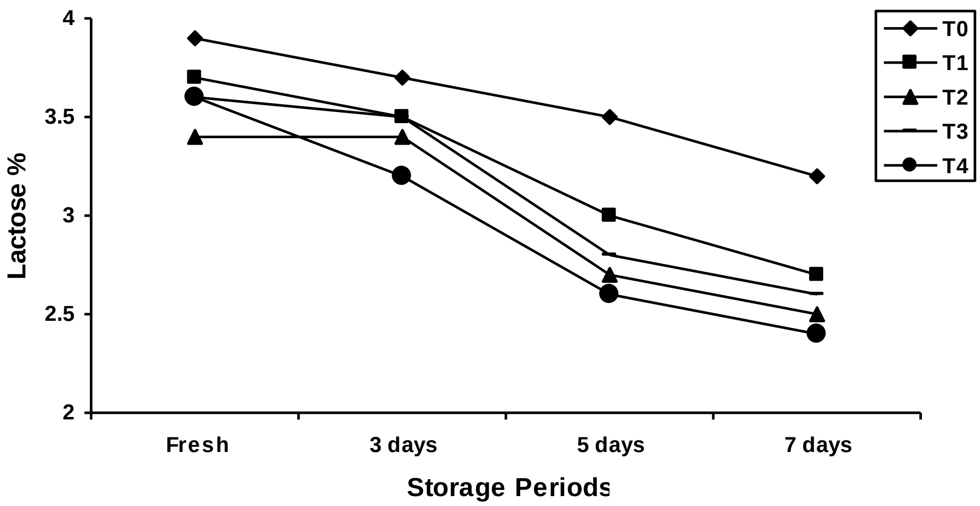

Fig. (2): Effect of adding iron salts on the lactose content of sheep yoghurt during storage.

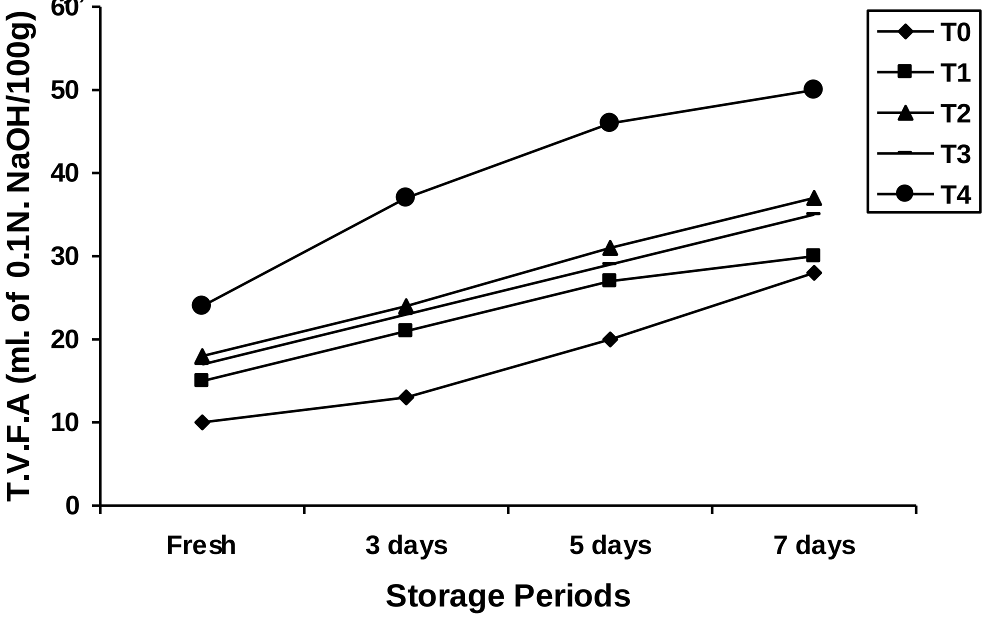

Fig. (3): Effect of adding iron salts on the total volatile fatty acids (FVFA) content of sheep yoghurt during storage.

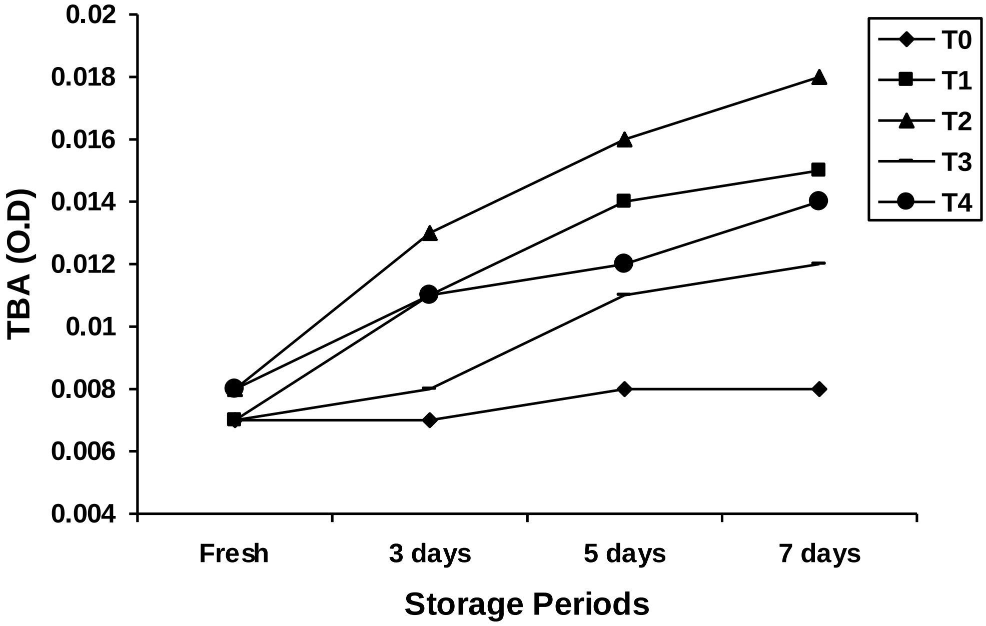

Fig. (4): Effect of adding iron salts on the thiobarbituric acid (TBA) content of sheep yoghurt during storage.

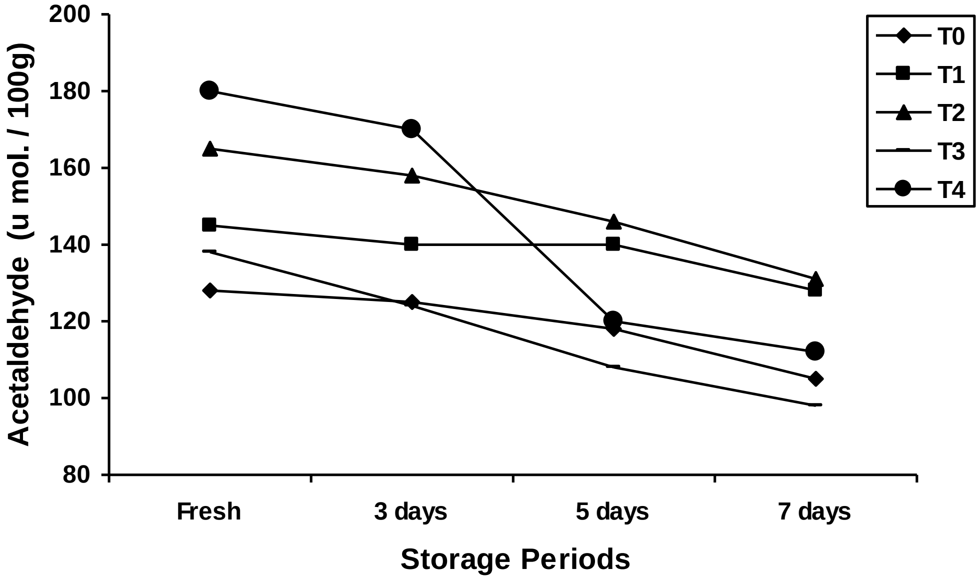

Fig. (5): Effect of adding iron salts on the acetaldehyde content of sheep yoghurt during storage.

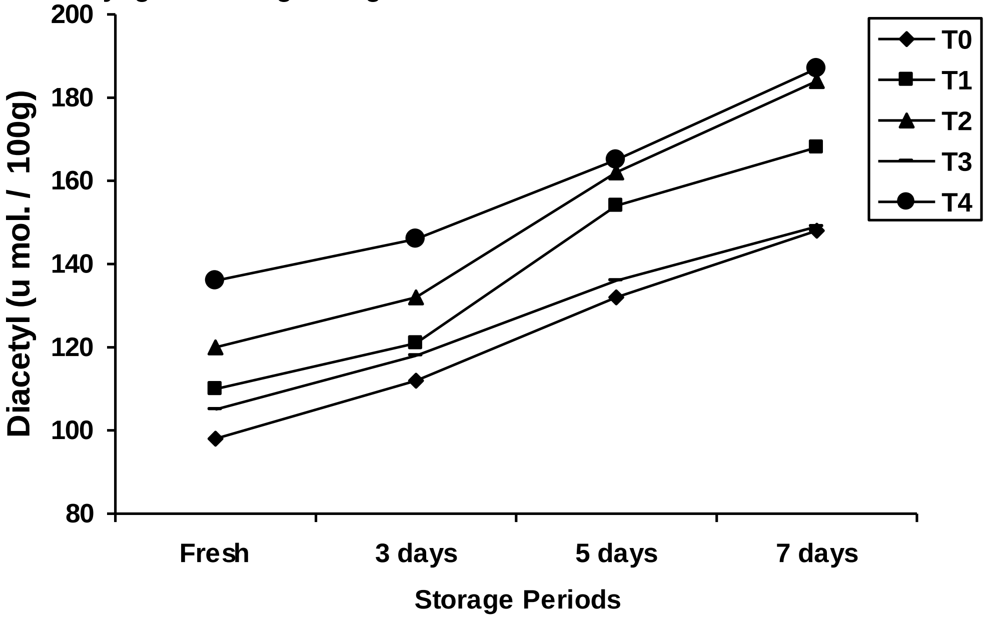

Fig. (6): Effect of adding iron salts on the diacetyl content of sheep yoghurt during storage.

CONCLUSION

It could be concluded that the addition of iron (especially ferrous gluconate) at rate of 10 and 20 mg/Litre milk, for sheep yoghurt during storage gives the highest scores for evaluated organoleptically with good flavour and consistency, texture of the resultant yoghurt. Moreover, its produce yoghurt fortified with iron which was important for each children as well as for adult as a good nutrient dairy products (yoghurt) which the consumers prefer it in Egypt).

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تأثير التدعيم بالحديد على جودة الزبادى

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يحتوى اللبن ومتجاته على نسبة عالية من البروتين والكالسيوم وبعض المعادن مثل الفسفور والمغنسيوم ولكن يوجد نقص بهما فى عنصرى الزنك والحديد. ولأنهما عنصران مهمان من الناحية الغذائية للإنسان اتجهت الأبحاث الآن إلى تدعيم بعض هذه المنتجات بالزنك والحديد ونظراً لأن الزبادى منتج لبنى مهم ويفضله أغلب المستهلكين فى مصر كان لابد من إجراء أبحاث لتدعيم الزبادى بالحديد لأن ذلك يقضى على الأنيميا الناتجة عن نقص الحديد ويمنع ظهور الطعم المؤكسد للدهن والغير مرغوب في الزبادى الناتج.

تم تصنيع زبادى من لبن الغنم الطازج والذى يحتوى على نسبة عالية من الدهن فتم عمل 5 معاملات من الزبادى المعاملة الأولى (T0) كنترول بدون إضافات، والمعاملات (T1, T2) أضيف لهما 10، 20 مللجم كلوريد حديدوز / لتر لبن وكذلك المعاملات (T3,T4) أضيف لهما 10، 20 مللجم جلوكونات حديدوز / لتر لبن.

تم تخزين الزبادى الناتج فى الثلاجة على حرارة 5 -8°م لمدة 7 ايام وتم تحليله على فترات طازج وبعد فترة 3 ، 5 ، 7 يوم من حيث % للجوامد الكلية TS، البروتين الكلىTP%، الدهن%، واللاكتوز % ورقم الـ pH كما تم تقدير محتواه من حمض الثيوباربيتوريك (TBA) والأحماض الدهنية الكلية الطيارة (T.V.F.A)، الاسيتالدهايد والداى اسيتايل كما تم أيضاً تقييم عينات الزبادى الناتج حسياً من حيث النكهة والتركيب والمظهر الخارجى كذلك قابلية الزبادى الناتج لدى المستهلك.

أظهرت النتائج المتحصل عليها فى هذا البحث عدم وجود اختلافات فى التركيب الكيماوى للزبادى من حيث % لـ (Fat , T.P, T.S) خلال مراحل التخزين المختلفة فى الـ 5 معاملات ومن جهة أخرى أظهرت النتائج وجود اختلافات معنوية جداً فى محتوى الزبادى الناتج الطازج والمخزن والكونترول والمعامل بالحديدوز من ناحية محتواه من الـ TBA ، رقم الـ pH ، T.V.F.A، اللاكتوز، الاسيتالدهايد، الداى أسيتايل كما أوضحت النتائج أيضاً أن تأثير جلوكونات الحديدوز واضح وملموس على هذه الخواص أكثر من تأثير كلوريد الحديدوز ماعدا الـ TBA فكان تأثير كلوريد الحديدوز واضح وملموس..

أظهر التقييم الحسى للزبادى الناتج المعامل بالحديد وخلال مراحل التخزين المختلفة أن أفضل مصدر لتدعيم الزبادى بالحديد هو استخدام جلوكونات الحديدوز يليه فى ذلك كلوريد الحديدوز من حيث إعطاء النكهة الحمضية الخفيفة المرغوبة (Flavour) فى الزبادى والقوام المتماسك (Compact consistency) وعدم التشريش (Wheying off) والمظهر الخارجى (Appearance) وكذلك قابلية الزبادى الناتج لدى المستهلك (Total acceptability) خلال مراحل التخزين المختلفة.