Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 7010 - 7030
Received 25 April 2021; Accepted 08 May 2021.

The Effect of Adding Chemically Modified Whey proteins to low-fat Buffalo Milk as a fat replacers in the physicochemical, Rheological and Sensory Properties of Buffalo low-fat Yogurt

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Abstract

The current study was conducted and aimed to determine the effect of partial substitution of buffalo milk fat with whey protein concentrates (WPC) on the physicochemical ,rheological and sensory properties of low-fat yogurt by adding unmodified WPC in treatment T1, and modified WPC in treatments T2-T3-T4. These WPC were modified by three chemical methods: esterification, Succinylation, and deamination. These WPC were added to low-fat buffalo milk prepared for the yogurt manufacturing with 2.0% fat in addition to the control treatment C which made from full-fat buffalo milk without any WPC addition. Chemical tests were involved determination of moisture, protein, fat, ash, carbohydrates, total acidity percentage , in addition to the pH estimation ,while the rheological tests were included the viscosity, water holding capacity and spontaneous whey separation. Sensory evaluation was also performed for yogurt treatments. The results showed that the low-fat substituted treatments were distinguished by their higher moisture content compared to the control treatment. The percentages of protein, carbohydrates and ash were also increased for the substituted treatments, while the percentage of fat was decreased. Moreover, an improvement in the rheological and sensory properties of the substituted treatments was recorded.

Key words: whey protein concentrate, buffalo milk, low-fat yogurt

Introduction :

Many medical reports are pointing out that many diseases are directly related to fat consumption through the increased risk of many injuries and chronic diseases such as coronary heart diseases, atherosclerosis, hypertension and tissue injuries, which are related to fat oxidation. In addition, fat is one of the causes of obesity that has become a growing risk factor for many diseases around the world (Astrup *etal*., 2011; Baum *etal*., 2012). This has led to increase health awareness by consumers towards fat content of foods, and the high consumption of dairy products with low-fat content has been drawn much attention with a significant increase in the demand for products such as milk types. Yogurt and other dairy products with low-fat content (Katsiari *etal*., 2002). Buttermilk is one of the fermented dairy products with a good reputation and good consumer

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acceptance due to its nutritional importance and beneficial effects on health (Wang, 2013). In theory, only the starter cultures activities are needed to make the yogurt product. However, in practice the total solids content in this product must be increased to prevent exudation of whey (Aziznia *etal*., 2009 and Henriques *etal*., 2013). Increasing the temperature of heat treatment is also needed to enhance gel formation through the denatured whey proteins by this heat treatment (Xu ZM *etal*., 2008). The properties of yogurt can be improved by adding milk protein powders to increase the total solids content to desired levels (Bong and Moraru, 2014). However, studies pointed out the importance of controlling the texture of fermented products when preparing blends with low milk solids and added stabilizers. Several researchers have highlighted that excessive use of stabilizers can adversely affect the sensory properties of yogurt by producing a firmer texture and sensation of additives by mouth (Lee and Lucey, 2004a). A common practice for yogurt manufacturing is to make it from recovered dried milk with whey protein concentrates, along with heating treatment to induce denaturation of whey proteins (Bong and Moraru, 2014: Xu ZM *etal*., 2008). Casein protein can tolerate temperature of 160 ° C without decomposing, While the whey proteins begin to denature at temperatures above 70 ° C (Dissanayake *etal*, 2013), and since denaturing the whey protein (denaturation) enhances the structure of the yogurt gel, it will reduce the need to add dried skimmed milk. Therefore, fortifying the mixture or substrates of the yogurt industry may enhance the properties of the yogurt with better texture (Loveday *etal*., 2013). The present study aims to determine the effect of replacing part of the buffalo milk fat with concentrates of whey proteins after modifying them by methods (Esterification, Succinylation and Deamination). in the chemical, rheological and sensory properties of yogurt.

Materials and methods

Chemical methods for modulating whey proteins

Succinylation

According to the method described by Grant, (1973), WPC were succinate by preparing a suspension of WPC with distilled water at a ratio of 50% (weight / volume), then the pH of the suspension was adjusted to (8.5) using sodium hydroxide solution (2 N). Then, Succinic Acid Anhydride was added to at a rate of 0.5 g of succinic acid per 1 g of WPC with continuous stirring of the protein suspension; thereafter ,the pH was adjusted to 8.5 using sodium hydroxide solution (2 N). Dialysis was then conducted using dialysis tubes (MwCO:7000D)with distilled water for 24 hours, during which the distilled water is exchanged for four times.

Esterification

A suspension was prepared by dissolving the WPC with methanol alcohol at a ratio of (10 wt / 100 ml alcohol) according to the method described by Fraenlel, Conrate,

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Olcott(1945) and after dissolving, the suspension was cooled out to 4 ° C and (0.61 ml) HCL acid was added gradually to it and then left at 4 ° C for 2 hours. then the suspension is diluted with cooled non-ionic water in a ratio of (1: 1) and diluted by dialysis tubes type (MwCO: 7000D) against (0.001 N) HCl acid for of 24 hours, where the dialysis solution is changed every 6 hours. After the dialysis process was ended, the suspension was dried in freezdryer.

Deamination

A suspension of WPC was prepared by dissolving them in 0.5 N HCl acid at a ratio of (1 g protein / 20 ml acid) with continuous stirring, then the suspension was treated at a temperature of 70 ° C, in a water bath, the suspension was placed for a period of 2 hours after which the suspension was rapidly cooled and the pH was adjusted to (4.7) After that, the suspension was placed in tubes in the central centrifuge at a speed of (3000 rpm for 15 minutes) and the pellet was taken and the pH adjusted and then placed in dialysis tubes (MwCO: 7000 D) against non- ionic water for 24 hours and after the end of the dialysis process, the suspension was then dried (Mimouni et al., 1994).

Yogurt manufacturing

Yogurt was manufactured according to the method described by Tamime and Robinson, (1999) and as follows:

A quantity of raw buffalo milk was taken and divided into two Parts. The first part was left with full fat and used in the manufacture of control yogurt treatment (C). The second part was skimmed to obtain low-fat buffalo milk and was then divided into four parts (T1, T2, T3 and T4). Non modified-WPC was added to the milk of the T1 treatment, while the chemically modified WPC by esterification was added to the milk of theT2 treatment. Modified WPC by Succinylation method was added to the milk of the T3 treatment and modified WPC by deamination method was added to the milk of the T4 treatment. All WPC were added at 2% percent. All treatments were mixed with an electric mixer to ensure well mixed suspensions and to ensure complete dissolution. Then all treatments were heated at 90 ° C for 10 minutes, then cooled to 42 ° C and inoculated with the starter cultures containing *Streptococcus Salivarius* subsp *thermophilus* and *Lactobacillus delbrueckii* subsp *bulgaricus* by direct addition with 0.02% percent .Then, they were filled in 150 mL plastic containers and incubated at 42 ± 2 ° C until the coagulation process was completed within 4-5 hours and the pH decreased to 4.6. Thereafter, the samples were taken out and transferred to the refrigerator for cooling and preservation at a temperature of (5 ± 1) ° C until the necessary tests carried out after periods of 1, 7 and 14 days of storage.

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physicochemical tests:

The percentage of the yogurt moisture was estimated according to A. O. A. C, (2005), while the fat percentage was estimated using gerber method (Ling,2008). The ash percentage was estimated according to A. O. A. C, (2005). Total nitrogen (TN) was estimated according to the method described by FSSAI(2015) using a micro-Kjeldahl method (behr model – Germany)

The percentage of carbohydrates was estimated by the difference method according to Ihekoronye and Ngoddy (1985):

% Carbohydrates = 100 -% (protein + fat + moisture + ash).

Rheological examinations:

Viscosity was estimated using Brookfield DVII viscometer (Brookfield Engineering Lab Inc., Stoughton, Mass.) according to the method reported by Donkor *et al*., (2007). Spontaneous whey separation was measured , according to what was reported by Amatayakul *et al*., (2006).The water holding capacity (WHC) was estimated according to the procedure described by Harte *etal*., (2003). Where 10 gm of each sample was weighed and placed in a test tube, and centrifuged at 5000 rpm for 10 minutes. Then, the upper layer of whey was withdrawn and carefully weighed to know the amount of water excluded from it and the WHC was estimated using the following equation:

$$WHC\% = [1- (w_2 / w_1)] \times 100$$

Whereas: w_1: the weight of the used milk, w_2: the weight of the whey after the centrifugation.

Statistical analysis: The data were analyzed according to the complete random design (CRD). The significant differences between the averages were compared with the lowest significant difference test (LSD) according to the Statistical Analysis System (SAS , 2012).

Results and discussion

Chemical composition:

Moisture: Table 1 shows the moisture content of the yogurt for each of the control treatment C, whose reached 84.10%, and this result differs from what Sadiq (2019) found, which is 87.02%. On the other hand, the moisture percentages of the (WPC) substitution treatments values were 86.5, 86.4, 85.60 and 86.03 % respectively. The reason for this difference in the moisture percentage between the control and the (WPC) substitution treatments may be due to the fat skimming process that led to the reduction of the total solids percentage, although this reduction was compensated by adding 2.0%

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WPC, which are known for their high water binding capacity. This is consistent with what Al-Bedrani (2016), who found that adding WPC to yogurt bases materials increase the moisture content. It is also noticed from the results that the moisture level was decreased for all treatments with storage where after 7 days, the control treatment reached 83.79%, while the (WPC) substitution treatments values were reached 86.17, 86.17, 85.4 and 85.7% respectively .While after 14 days, moisture percentage reached to 283.6% for control and of the (WPC) substitution treatments values were 85.76,86, 85.17 and 85.42% respectively. This is consistent with what Qureshi *et al*., (2011) who found, a decrease in the moisture content of yogurt from 84.78 to 84.65% during refrigerated storage. The reason for this decrease may be due to the evaporation occurring during the storage period, which led to a decrease in the moisture content. These results also agree with what Aziznia et al.(2008)who pointed out that adding WPC can lead to an increase in the percentage of total solids and thus a decrease in the moisture content of low-fat yogurt. The results of the statistical analysis indicate that there are no significant differences between the different treatments at the end of storage period of 14 days.

protein : The results in Table 1 show the percentage of protein for the aforementioned yogurt treatments .It was reached immediately after manufacturing for C treatment 4.50%, and this result is consistent with what Al-Bedrani (2016) found for yogurt, which was 4.56% and close to what Sadiq (2019) found, that the percentage of protein in yogurt made from full-fat milk was 4.21%. While the (WPC) substitution treatments values were reached 4.06, 6.34, 5.06 and 4.06% respectively. It is noted from the results that the percentage of protein in the (WPC) substitution treatments were high, and this is consistent with what Al-Darwash *etal* (2014)found, who reported that the protein origin substances work to increase the percentage of total solids and then increase the protein content. It is also noted that the percentage of protein increased for all treatments during storage. After 7 days, it was for the control treatment 4.6% and for the (WPC) substitution treatments values were reached 6.5, 6.42, 6.60 and 6.5% respectively. After 14 days, the control treatment protein content reached 4.73%, and for the (WPC) substitution treatments values were reached 6.6, 6.50, 6.70 and 6.60% respectively. These results are in agreement with the findings of Qureshi *etal*. (2011), where who indicated an increase in the protein content in yogurt treatments from 4.76% to 4.80% at the end of the 15-day storage period. which led to an increase in the percentage of total solids, including protein, also this is consistent with what Aziznia *etal*. (2008) and Brodziak (2012) findings, who indicated that adding (WPC) to yogurt bases increases the content of non-fat dry matters, including protein. It also agrees with what Al-Bedrani (2016) found, which indicated an increase in the protein content in yogurt treatments from 4.34% immediately after manufacturing to 4.44% at the end of the 14-day storage period.

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The results of the statistical analysis indicate that there was no significant differences ($P \le 0.05$) in the protein ratio between the different yogurt treatments immediately after manufacturing and during the storage .

Fat: The results in Table 1 show the percentage of fat in the aforementioned yogurt treatments. The fat percentage of C treatment immediately after manufacturing was 6.70% and for the (WPC) substitution treatments were reached 1.82, 1.86, 1.85 and 1.83% respectively. It is noted from the results that the difference in fat percentage of the treatments in comparison to the control treatment may be due to the use of low-fat milk in making yogurt for these treatments.

It is also noted from the statistically analysis that there are significant differences ($P \le 0.05$) in the fat ratio between the control treatment C and all the (WPC) substitution treatments immediately after manufacturing. The increased in the fat percentage in all yogurt treatments was recorded during storage, as the value after 7 days for C treatment was 6.90% and for the (WPC) substitution treatments values were reached 2.0, 2.0, 1.9 and 2.1% respectively. Further, after14 days of storage, the value for the control treatment was 6.96%, and for the (WPC) substitution treatments values were reached 2.4, 2.20, 2.10 and 2.20% respectively. The reason for this increase in fat percentage may be due to a decrease in the moisture content, which led to an increase in the percentage of total solids, including fat. This is in agreement with the findings of Aziznia *etal*., (2008) and it is also consistent with what was found by Al-Bedrani(2016), who indicated that the percentage of fat increased from 3.63% immediately after manufacturing to 3.86% at the end of the 14-day storage period.

Table 1: The chemical composition of different yogurt treatments, immediately after manufacturing and during storage at (5 ± 1) °C for 14 days

Treatment		Yogurt life (day)	Humidity %	Protein%	Fat %	Carbohydrates %	Ash %
Control treatment	C	1day	84.10	4.50	6.70	4.06	0.64
		7day	83.79	4.60	6.90	4.05	0.66
		14 day	83.62	4.73	6.96	4.03	0.68
(WPC) substitution treatments	T1 non-modified WPC	1day	86.50	5.40	1.82	5.63	0.65
		7day	86.17	5.60	2.0	5.55	0.68
		14 day	85.76	5.90	2.40	5.25	0.69
	T2 modified WPC by esterification	1day	86.40	5.56	1.86	5.52	0.66
		7day	86.17	5.72	2.0	5.43	0.68
		14 day	86.0	5.75	2.20	5.35	0.70

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	T3 modified WPC by Succinylation	1day	85.60	5.70	1.85	6.05	0.80
		7day	85.40	5.85	1.90	6.04	0.81
		14 day	85.17	5.92	2.10	5.99	0.82
	T4 modified WPC by deamination	1day	86.03	5.64	1.83	5.63	0.77
		7day	85.70	5.75	2.10	5.55	0,80
		14 day	85.42	5.90	2.20	5.25	0.90
LSD value		---	4.63 NS	1.077 *	1.694 *	1.,273 *	0.219 *
* (P≤0.05), NS: not significant.							

Carbohydrates: The results in Table 1 show the percentage of carbohydrates in the aforementioned yogurt treatments, as their ratio for control treatment C is 4.06%. This result is comparable to what Sengupta (2014) found, who found that the percentage of carbohydrates in full-fat yoghurt is 4.47%. However, this result differs from what Sheikh (2018) found, who indicated that the percentage of carbohydrates in full-fat yoghurt was 5.60%, while for the (WPC) substitution treatments values were 5.63, 5.52, 6.05 and, 5.73%, respectively. The results of the statistical analysis showed that there was a significant difference (P≤0.05) in the percentage of carbohydrates between C treatment and all other treatments immediately after manufacturing.

It is also noted that there is an increase in the percentage of carbohydrates in the yogurt WPC addition treatments and this result is consistent with the results of Al-Bedrani (2016), which indicated an increase in the percentage of carbohydrates with an increase in the added percentages of fat substitutes (WPC),as the WPC contains percentage of lactose and thus increase its concentration in WPC supported treatments.

Also, the percentage of carbohydrates decreased with the storage time for all treatments. The value after 7 days for the control treatment was 4.05% and for the (WPC) substitution treatments values were reached 5.55, 5.34, 6.04 and 5.65% respectively. The reason for this decrease may be due to the continued activity of the starter's cultures that converts the lactose into lactic acid. This is consistent with what Yilmaz-Ersan *etal*., (2014) findings that indicated a decrease in the percentage of carbohydrates in yogurt from 4.42% to 4.07% during the 25-day storage period. It also agrees with the findings of Sadiq, (2019) regarding the low carbohydrate content of iron-enriched yogurt when storage for 21 days. The results of the statistical analysis (P≤0.05) also indicate that there are significant differences in the percentage of carbohydrates at the end of the 14-day storage period between the C treatment and all other treatments. The reason for this may be due to the continued conversion of lactose to lactic acid due to bacterial activity, which continue to grow slowly under cooling conditions.

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Ash : The results in Table 1 show the percentage of ash for the aforementioned yogurt treatments, as its value immediately after manufacturing for C treatment was 0.64%, and this percentage is comparable to what Matter *et al*. (2016) and Stijepic *et al*. (2013) found, who indicated that the percentage of ash in the treatment of yogurt made from full-fat milk was 0.68%, while the ash percentage for the (WPC) substitution treatments values were reached 0.65, 0.66, 00.8 and 0.77% respectively. Also, it is noticed from the comparison of the results that the percentage of ash increased for the treatments to which the WPC was added. This is consistent with what Aziznia *etal*.(2008) found; however, there are no significant differences (P≤0.05) in the ash percentage between all milk treatments immediately after processing.

The results also show that there is an increase in the ash percentage for all treatments with storage, as the value after 7 days of C treatment was 0.66%, and the (WPC) substitution treatments values were reached 0.68, 0.68, 0.81 and 0.80% respectively. After 14 days, of C treatment was 0.68% and for the (WPC) substitution treatments values were reached 0.69, 0.70, 0.82 and 0.90% respectively. This result is in consistent with what Al-Bedrani (2017) found, who indicated that the percentage of ash in the yogurt increased from 0.81% immediately after manufacturing, to 0.86% at the end of the 14-day storage period. It also agrees with the findings of Aziznia *etal*. (2008) findings, where an increase in the ash content of low-fat yogurt treatments to which WPC were added. It is also noted that there are significant differences in the ash ratio of treatments during storage.

pH: The results in Figure 1 show the pH values of the aforementioned yogurt treatments, as these values were immediately after manufacturing for the C treatment 4.6, and this result is consistent with what Ibrahim (2015) found, who indicated that the pH value of full-fat milk yogurt is 4.59, while for the (WPC) substitution treatments values were 4.5, 4.5, 4.55 and 4.6 respectively. It is noticed that the pH values of the (WPC) substitution treatments are lower compared to the C treatment, and this is consistent with what Al-Bedrani (2016) found, who showed a lower pH value of skim milk with the addition of WPC compared to the control treatment. It is noted from the results of the statistical analysis that there was no significant difference (P≤0.05) in pH values immediately after manufacturing between different treatments.

It is also observed from the results that the pH values were decreased for all treatments during storage. The value after 7 days for C treatment was 4.5 and for the (WPC) substitution treatments values were 4.45, 4.4, 4.5 and 4.5 respectively. After 14 days, C treatment reached 4.45 and for the (WPC) substitution treatments values were reached 4.4, 4.35, 4.4 and 4.4 respectively. This result is consistent with the findings of Adriana Dabija *etal*., (2018), as well as with Habibi *etal*., (2018), who indicated a decrease in pH values during the storage period. This result is also comparable to what Pappa *etal*. (2018) found, who recorded a decrease in the pH value for the treated milk

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from 4.29 to 4.26 during the 21-day storage period. The reason for this decrease in the pH value may be due to the continuous fermentation of lactose and its conversion to lactic acid, as a result of the slow-fermenting activity of the starter's cultures during cold storage. The results of the statistical analysis indicate that there was no significant differences (P≤0.05) in the pH values between the different yogurt treatments during the 14-day storage period. It is also noticed from the results of the pH values that there was no effect of adding the modified WPC on the pH values.

Total acidity : The results of Figure 2 show the total acidity values of the aforementioned treatments, as these values for C treatment immediately after manufacturing was 0.8% and this is a agreement with Al-Bedrani (2016), who found that the total acidity value of full-fat yogurt is 0.90%. As for the the (WPC) substitution treatments values were 0.95, 0.8, 1.0 and 1.0% respectively. As it can be noted from the results, the values of the total acidity of all treatments during storage were higher . It was reached after 7 days for C treatment 0.85% and for the (WPC) substitution treatments reached 1.1, 0.85, 1.2 and 1.1 % respectively ,and after 14 days for C treatment was 0.9% and for the (WPC) substitution treatments values were 1.2, 0.87, 1.25 respectively. These results were in agreement with what Kaur and Riar, (2020) found, which indicated an increase in the acidity of the yogurt treatments from 1.22% to 1.41% during cold storage. There were no significant differences (P≤0.05) in the total acidity ratio between all treatments during storage.

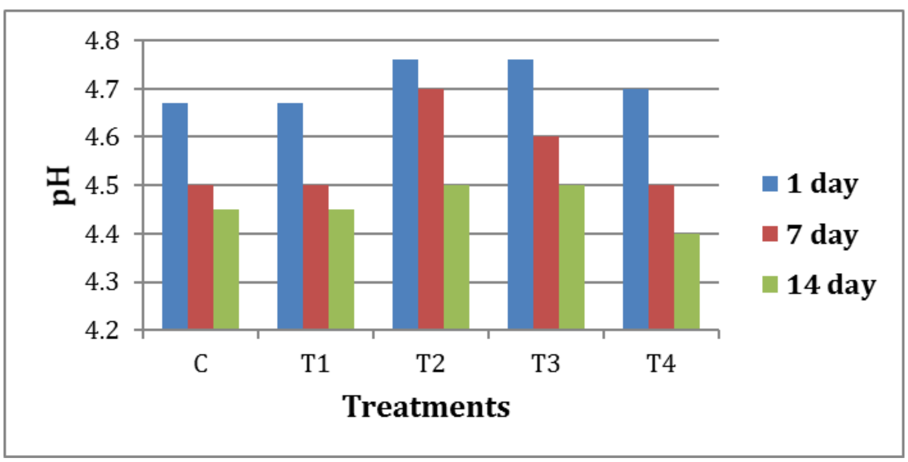

Figure 1: pH values for different yogurt treatments, immediately after manufacturing and during storage at (5 ± 1) °C for 14 days.

LSD value = 0.511. NS(P≤0.05)

An increase in the acidity values of the substituted treatments to which WPC were added compared to the control treatment is also observed, and the reason for this increase

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may be due to the production of propionic and acetic acids (Nikoofar etal., 2013). This result is consistent with what were found by Sady *etal*. (2007) and Al-Dabbas *etal*. (2011), which was due to the increase in the lactic acid level during lactic formation due to the fermentation of the lactose, as the lactic acid bacteria strains used in the production of yogurt produce lactic acid in the range of 0.6% to 1.0%, in addition to acetaldehyde, acetoin and diacetyl, which gives the yogurt specific sensory properties and a long shelf life. The addition of WPC also increases storage properties and that would affect potential acidity (Al-Dabbas et al. 2011).

This may be due to the acidic nature of WPC ,and being a source for providing amino acids and peptides for microorganisms on the other hand when exposed to heat during yogurt manufacturing (Dave and Shah, 1998). Amatayakul *etal*. (2006) indicated that it has an effect on the growth and reproduction of the starter's bacteria, while Sanli, (2015) also indicated an increase in the rate of total acidity of low-fat yogurt when adding (Dairy-Lo) substance, which is a product made from WPC, when compared with the yogurt treatment made from full-fat milk. The results of the statistical analysis also indicate that there was no significant difference ($P \leq 0.05$) in the percentage of total acidity between all treatments immediately after manufacturing and during the storage period of 14 days.

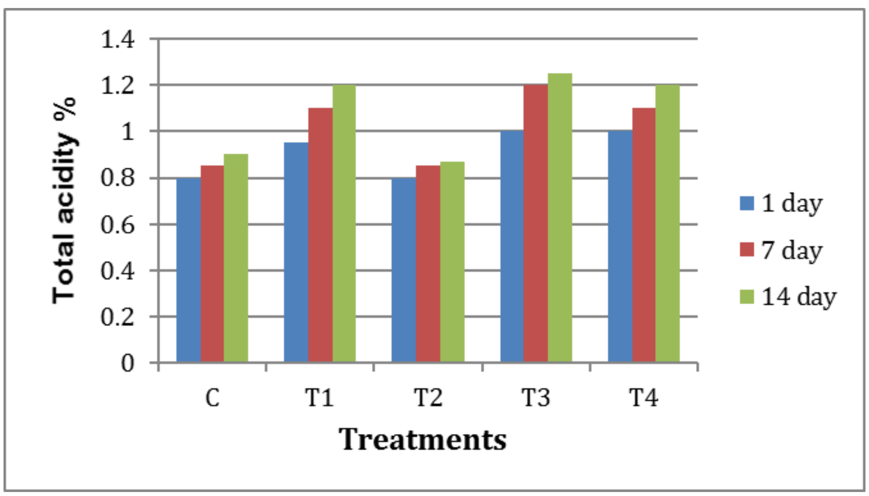

Figure 2: The total acidity values for different yogurt treatments, immediately after manufacturing and during storage at (5 ± 1) °C for 14 days.

LSD value = 0.288 ,NS = (P≤0.05)

Rheological properties:

Viscosity: The viscosity is one of the most important factors that determine the quality properties of the yogurt, which is related to both the stability and the taste of the fermented milk (Lewis, 1996).

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According to Rawson and Marshal, (1997), *Streptococcus salivarius* subspp *thermophilus* plays a major role in producing the texture of the product and this comes from exo-cellular products called exo-polysaccharides that interfere with the protein content in milk and increase its viscosity and improve its quality characteristics. The results in Figure 3, show the viscosity values of the aforementioned yogurt treatments, where C treatment immediately after manufacturing was 1820 centipoise while for the T1 treatments was 2360 centipoise. The increase in the viscosity ratio for this treatment is in consistent with what Al-Bedrani (2016) found. The results indicated that there were significant differences between the two treatments. The other treatments with the modified WPC, they were recorded 1000, 1200 and 1800 centipoise respectively. Also, a decrease in the viscosity values is observed in these treatments compared to each of the two previous treatments C and T1. The reason for this may be due to the modification process WPC that led to the decrease in the viscosity.

As storage proceed, it is noted that the viscosity values increased for all treatments. After 7 days, for C treatment it was 2000 centipoise, and for the (WPC) substitution treatments were reached 2450, 1100, 1300 and 2100 centipoise respectively. The value after 14 days for C treatment was 2250 centipoise and for the (WPC) substitution treatments values were 2575, 1200, 1450, and 2290 centipoise respectively. and this result is consistent with what Al-Bedrani (2016) found, which indicated that there was an increase in the viscosity values of the yogurt treatments after storage for 14 days. This may be due to the lowering of the acidity of the milk, which led to an increase in its hardness and thus an increase in the viscosity (Walstra et al. 2005).

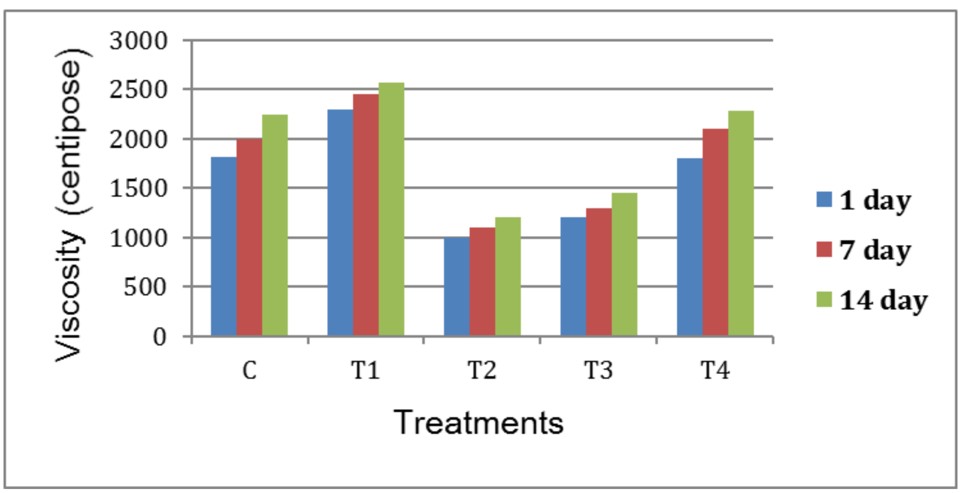

Figure 3: Viscosity values for different yogurt treatments, immediately after manufacturing and during storage at (5 ± 1) °C for 14 days.

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LSD value = 307.41, NS ($P \leq 0.05$).

Spontaneous whey separation: The most important factor indicating yogurt quality during storage is whey separation (Shakerian *etal*., 2015). and that has an inverse relationship with the water holding capacity of the protein network (Niamah et al., 2016). Exudation occurs either due to lack of solids or due to insufficient heat treatment or lower pH than 4.4 (Konhorst,2007). The results in figure 4 show the quantity of whey exuded for the aforementioned treatments, as immediately measured after manufacturing, it was 1.80 ml / 50 ml for C treatment and for the (WPC) substitution treatments the values were 1.40, 1.20, 1.10 and 1.20 ml / 50 ml yogurt respectively. It is noticed that the quantities of pure whey from the treatments to which the modified and non-modified WPC were added is less than it is quantities in the control treatment, and this is consistent with what Kailasapathy and Supriadi (1998), Remeuf *etal*. (2003) and Aziznia *etal*., (2008) found, as well as with Barkallah *etal*. (2017), who indicated that there was a decrease in the amount of whey extracted from low-fat yogurt by an increase in the amount of added WPC, and this characteristic may give it physical properties similar to those possessed by full-fat yogurt.

The strength of the yogurt is directly proportional to the amount of WPC added, which results in a decrease in the ratio of casein to the WPC, and thus; the yogurt would reach its highest strength, and consequently, this would result reduced perfusion (Puvanenthiran *etal*., 2002). Lucey *etal*., 1990) stated that adding WPC to the yogurt mixture increases the number of bonds that bind (whey protein - whey protein) on those bonds that bind (casein protein - whey protein), which increases the stiffness of the clot and reduces perfusion. It is also noticed that the quantities of perfused whey with storage decreased, so the values after 7 days for C treatment were 1.5 ml / 50 ml yogurt, and for the (WPC) substitution treatments the values were 1, 1.2, 1, and 1.12 ml / 50 ml yogurt respectively. This is in agreement with what Kaur and Riar, (2020) have reported, which may be due to the molecular weight. The higher concentration of WPC would improve the ability of milk to retain water and prevent the excretion of whey to the surface (Zhao *etal*., 2015). After 14 days ,for C treatment was 1.04 ml / 50 ml yogurt, and for the (WPC) substitution treatments the values were 0.95, 1, 0.98 and 1 ml. / 50 ml yogurt respectively.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 7010 - 7030
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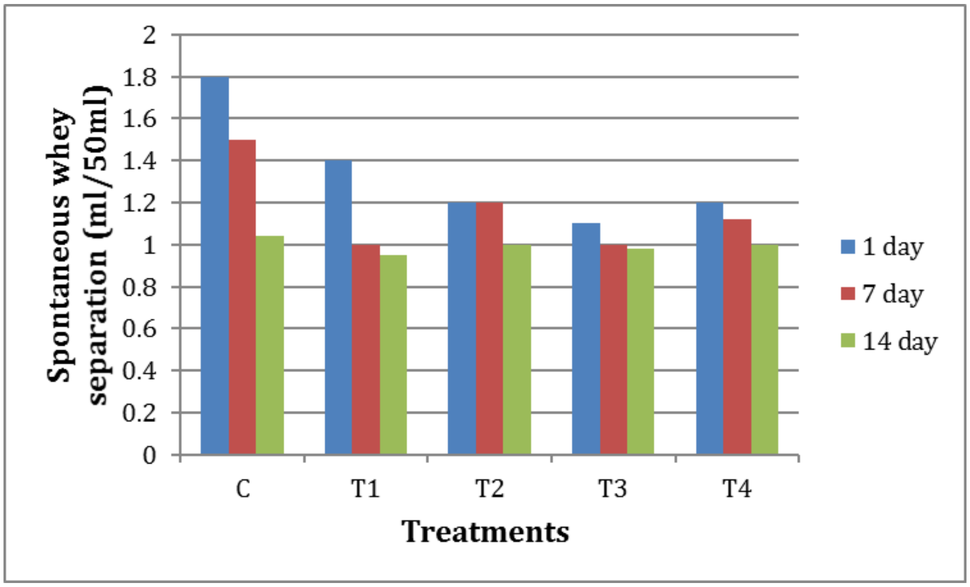

Figure 4: Spontaneous whey separation values for different yogurt treatments, immediately after manufacturing and during storage at (5 ± 1) °C for 14 days. LSD value = 0.379, NS (P≤0.05)

Celik, (2007) indicated that the decrease in the amounts of whey exuded with storage may be due to the metabolic activity of the starter's cultures and to the decrease in the net pressure within the protein matrix, which reduces the perfusion (Guler-Akin and Akınm, 2007) and due to the increase in protein complexes that prevent penetration large quantities of whey and this is consistent with what El-Sabie *etal*., (2010) findings. The results of the statistical analysis showed that there were significant differences (P≤0.05) between the control treatment and the replacement treatments immediately after manufacturing and during storage for 14 days.

%Water holding capacity: The results in figure 5 show the percentage of water holding capacity for the aforementioned yogurt treatments, where the water retention capacity immediately after manufacturing for C treatment was 35%, and this result is comparable to what Ibrahim (2015) found, who indicated that the water holding capacity for yogurt treatment reached Immediately after manufacturing, 31.1%. As for the (WPC) substitution treatments the values were 58, 45, 60 and 55% respectively, this is consistent with what Al-Bedrani (2016) finding, which indicated an increase in the water holding capacity for the treatments of curd with added WPC by 2.0%. The current results are consistent with what was found by Kozioł *etal*., (2014), that stated the higher water holding capacity in products containing WPC may result from increased cross-linking of the curd compared to milk not supplied with protein modifications. Lee and Lucey, (2010) also mentioned that the dry matter content and the ratio of casein to WPC are important factors affecting the ability of curd to retain water. And & Guo (2006) also

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indicated that the percentage of yogurt content of total solids increases its water retention capacity.

According to Puvanenthiran *etal*., (2002), the decrease in the ratio of casein protein to whey protein in yogurt made from milk along with WPC, may lead to an increase in the hardness of the gel. It is also noticed that this ability to WPC added parts has substantially increased in comparison with the control treatment.

It is also noted from the results that the ability to retain water is affected by the duration of storage, as it is noticed that it increases for all treatments, as the percentage values of water retention percentage after 7 days for C treatment reached 38% and for the (WPC) substitution treatments the values were 60, 47, 62 and 60% respectively and this is consistent with what Landge, (2009) finding, which indicated an increase in water retention capacity for treating yogurt enriched with WPC from 18.68% immediately after manufacturing to 20.95% on the 25th day of storage, and the reason for this may be due to the effect of low moisture content of the yogurt treatments. After 14 days, C treatment was 40%, and for the (WPC) substitution treatments the values were 62, 49, 63, and 61%, respectively. Akalin *etal*., (2012) noted that adding WPC to formed yogurt to enhance the water holding capacity more than caseins as increased water retention potential could occur. Due to the increased amount of WPC added as a stabilizer that interferes with the casein network.

It is also noticed from the results of the statistical analysis that there are significant differences (P≤0.05) between the control treatment and all treatments that contain WPC.

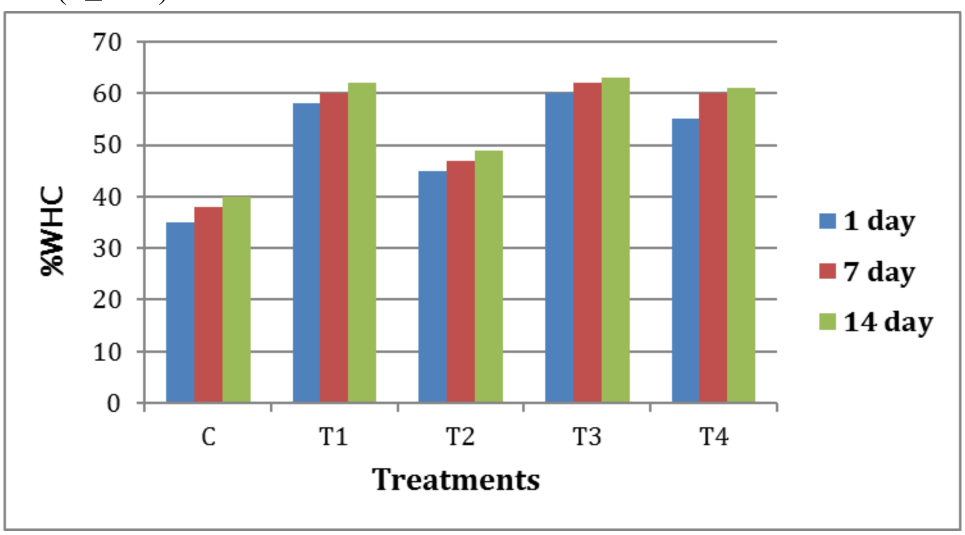

Figure 5: % of water holding capacity for different yogurt treatments, immediately after manufacturing and during storage at (5 ± 1) °C for 14 days.
LSD value = 6.911, NS (P≤0.05).

Sensory evaluation:

Table 2 shows the results of the sensory evaluation of the aforementioned yogurt treatments, as it becomes clear from them the convergence of the scores given to yogurt,

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the treatments to which the modified WPC were added, compared to the scores given to the yogurt for the control and T1 treatments to which the non-modified WPC was added.

The results of the evaluation of the taste and flavor characteristic show a great convergence between the yogurt treatments to which the modified WPC were added with the C treatment yogurt and with the T1 treatment for yogurt made from low-fat buffalo milk and the non-modified WPC added to it because of the distinct flavor characteristics of the WPC. The flavor of WPC is one of the most important factors for its increased use in many food products (Quach et al.,1999). Also, increasing the addition ratios of compounds that contain the α-Lactoalbumin protein or that are manufactured from partially hydrolyzed protein that given to low-fat yogurt, which is supported by sensory characteristics that are completely identical to whole milk (Matumoto-Pintro *etal*., 2011). It is noted from the results of the statistical analysis that there are no significant differences ($P \leq 0.05$) in the degrees granted for this characteristic among all the treatments.

Regarding the characteristic of the texture, it is noted that the (WPC) substitution treatments obtained high evaluation scores compared to the control treatment. There are no significant differences in the scores conferred on this trait. As for the acidity characteristic, which is also considered one of the important taste characteristics, the results showed that yogurt treatments made from low-fat buffalo milk and with the addition of modified WPC on high evaluation scores in this trait compared to the evaluation scores of yogurt t in control and T1treatments. It is noticed that there are no significant differences between all treatments for this characteristic immediately after manufacturing. However, significant differences were found within a single treatment between the first and fourteenth days of storage.

Table 2: The sensory evaluation of different yogurt treatments, immediately after manufacturing and during storage at (5 ± 1) °C for 14 days.

Treatment		storage life	flavor 45 ˚	Texture ˚30	Acidity ˚10	Appearance ˚10	Package 5	Total ˚100
Control treatment	C	1day	45	30	10	10	5	100
		7day	43	30	10	10	5	98
		14 day	40	25	9	9	5	88
(WPC) substitution treatments	T1 non-modified WPC	1day	41	29	9	10	5	94
		7day	40	28	9	9	5	91
		14 day	39	27	8	9	5	88
	T2 modified WPC by	1day	40	22	9	9	5	85
		7day	39	20	8	8	5	80

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	esterification	14 day	38	20	8	8	5	79
	T3 modified WPC by Succinylation	1day	42	29	9	10	5	95
		7day	41	29	9	9	5	93
		14 day	40	28	8	8	5	89
	T4 modified WPC by deamination	1day	45	30	10	10	5	100
		7day	44	30	9	9	5	97
		14 day	43	29	8	8	5	93
LSD value			3.015 *	2.982 *	1.669 *	1.705 *	0.75 NS	6.371 *
* (P≤0.05), NS: not significant.								

It is also noted from the results that the yogurt in T4 treatment to which the modified WPC was added by the (deamination) method over the yogurt in the T1 treatment to which the non-modified WPC was added in all characteristics, especially on the first day of storage, as it was characterized by a very acceptable appearance. It is noticed that there are no significant differences in the degrees granted to the characteristic of the external appearance between all treatments immediately after manufacturing and after the 14-day storage period.

References

1. Al-Badrani, Diaa Ibrahim Gro Haidar. (2016). Manufacture of low-energy milk products using fatmimetics alternatives and studying their physiochemical and nutritional properties. PhD thesis - College of Agriculture - University of Baghdad.

2. Akalin A.S., Unal G., Dinkci N., Hayaloglut A.A. Microstructural, textural, and sensory characteristics of probiotic yogurts fortified with sodium calcium caseinate or whey protein concentrate. *J. Dairy Sci.* 2012;95:3617–3628.

3. Akin M., Güler-Akin M. Effect of cystein and different incubation temperatures on the microflora, chemical composition and sensory characteristics of bio-yogurt. *Food Chem.* 2007;100(2):788–793.

4. Al-Dabbas M.M., Al-Ismail K., Al-Abdullah B.M. Effect of chemical composition on the buffering capacity of selected dairy products. *Jordan. J. Agric. Sci.* 2011;7:690–700.

5. AL-Darwash, Amir K.; AL-Mosawey, A.J. and AL-Sultan, A.M. (2014).Study of chemical, microbiological and organoliptic properties of high protein Iraqi soft cheese.J. Biol. Chem. 9: 365- 376.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 7010 - 7030
Received 25 April 2021; Accepted 08 May 2021.

6. Amatayakul, T.; Sherkat, F. and Shah ,N. P. (2006). Syneresis in set yogurt as affected by EPS starter cultures and levels of solids.Int. J.Dairy Tech. 59 (3): 216–221.

7. And JL, Guo M (2006) Effects of polymerized whey proteins on consistency and water-holding properties of goat's milk yogurt. J. Food Sci 71: 34-38.

8. Association of Official Analytical Chemists- A.O.A.C.(2005). Official methods of Analysis of AOAO International, th18 ed. Maryland : AOAO International.

9. Astrup, A.; J. Dyerberg ; P. Elwood; K. Hermansen; F.B. Hu; M.U. Jakobsen; W.C. Willett (2011). The Role of Reducing Intakes of Saturated Fat in the Prevention of Cardiovascular Disease: Where Does the Evidence Stand in 2010. The American Journal of Clinical Nutrition, 93:684-688.

10. Aziznia, S.; Khosrowshahi, A.; Madadlou, A. and Rahimi, J. (2008). Whey protein concentrate and gum tragacanth as a fat replacers in nonfat yogurt: Chemical, physical and microstructural properties, J. Dairy Sci, 91(7): 2545-2552.

11. Barkallah M., Dammak M., Louati I., Hentati F., Hadrich B., Mechichi T. Effect of Spirulina platensis fortification on physicochemical, textural, antioxidant and sensory properties of yogurt during fermentation and storage. *LWT Food Sci. Technol.* 2017;84:323–330.

12. Baum, S.J.; P.M.Kris-Etherton; W.C. Willett; A.H. Lichtenstein; L.L.,Rudel; K.C. Maki; J.Whelan; C.E. Ramsden. and R.C. Block. (2012).Fatty acids in cardiovascular health and disease: A comprehensive update. Journal of Clinical Lipidology, 6:216-234.

13. Bong DD, Moraru CI (2014) Use of micellar casein concentrate for Greek-style yogurt manufacturing: Effects on processing and product properties. J Dairy Science 97: 1259-1269.

14. Brodziak A. Gelling properties and texture of gels obtained from whey proteins derived from milk of different cow breeds. Zywnosc-Nauka Technologia Jakosc. 2012;83:161–174.

15. Çelik, E. S. (2007). Determination of aroma compounds and exopolysaccharides formation by lactic acid bacteri isolated from traditional yogurts (Master's thesis, Izmir Institute of Technology).

16. Dabija A., Codină G.G., Ropciuc S., Gâtlan A.-M., Rusu L. Assessment of the antioxidant activity and quality attributes of yogurt Enhanced with wild herbs Extracts. J. Food Qual. 2018.P.1-12.

17. Delikani, B.and Ozcan, T. (2014). Effects of various whey proteins on the physicochemical and textural properties of set type nonfat yoghurt. Int. J. Dairy Tech, Vol 67, No 4.

18. Dissanayake M, Ramchandran L, Piyadasa C, Vasiljevic T (2013) Influence of heat and pH on structure and conformation of whey proteins. Int Dairy Journal 28: 56-61.

19. Donkor , O.N.; Nilmini S.L.I.; Stolic P., Vasiljevic T.; and Shah N.P., (2007). Survival and activity of selected probiotic organisms in set-type yoghurt during cold storage. Int. Dairy J. 17, 657-665.

20. El-Sabie, W. B., El-Abd, M. M., Al-Assar, M. A., and Hassan, M. A. (2010). physicochemical propareties of yoghurt fortification with different zinc salts. Journal of Food and Dairy Sciences, 1(11), 669-679.

21. Habibi Najafi M.B., Fatemizadeh S.S., Tavakoli M. Release of proteolysis products with ACE-inhibitory and antioxidant activities in probiotic yogurt containing different levels of fat and prebiotics. Int. J. Pept. Res. Therapeut. 2018:1–11.

22. Harte ,F.; Luedecke ,L.; Swanson, B.G. and Barbosa-Cánovas,G.V. (2003).Low-fat set yogurt made from milk subjected to combinations of high hydrostatic pressure and thermal processing.J Dairy Sci.86:4,1074-1082.

23. Henriques MF, Gomes DGS, Pereira CD, Gil MM (2013) Effects of liquid whey protein concentrate on functional and sensorial properties of set yogurts and fresh cheese. Food Bioprocess Tech 6: 952-963.

24. Ibrahim, K.J. (2015). Purification and Characterization of Karadi Sheep's Milk Protein and its Relationship with Yoghurt Quality. M.S.Thesis. Sulaimani University.

25. Ihekoronye, A. I., and Ngoddy, P. O. (1985). Integrated food science and technology for the tropics. Macmillan press Ltd London.

26. Kailasapathy ,K. and Supriadi, D .(1998). Effect of partially replacing skim milk powder with whey protein concentrate on the sensory qualities of lactose hydrolyzed acidophilus yoghurt. Milchwissenschaft.53 :385–389.

27. Kaur, R., & Riar, C. S. (2020). Sensory, rheological and chemical characteristics during storage of set type full fat yoghurt fortified with barley β-glucan. Journal of food science and technology, 57(1), 41-51.

28. Katsiari, M. C.; L. P. Voutsinas. And E.Kondyli.(2002). Improvement of
29. Sensory Quality of Low-fat Kefalograviera Type Cheese with Commercial Adjunct Cultures.Inter. Dairy J., 12: 757-764.

30. Kozioł J., Gustaw W., Waśko A., Skrzypczak K., Sławińska A., Sołowiej B. Effect of selected milk protein preparations on growth and survival of *Lactobacillus acidophilus* as well as on rheological properties of fermented milk beverages. *Food Sci. Technol. Quality*. 2014;94:41–55.

31. Landge, V.L. (2009). Quality of yogurt supplemented with whey protein concentrate and effects of whey protein denaturation. Thesis: MSc Thesis. Kansas state university.

32. Lee ,W.J. and Lucey ,J.A.(2004a).Rheological properties, whey separation and microstructure in set type yogurt: effects of heating temperature and incubation temperature.J.Texture Studies, , 34(5):515-536.

33. Lee, W. J. and Lucey, J. A. (2010). Formation and physical properties of yogurt. Asian-Australian J of Anim. Sci. 23(9):1127-1136.

34. Lewis, M. J. (1996). Physical properties of food and food processing systems. Cambridge: Wood head Publishing Limited.

35. Ling, E.R.(2008). "A textbook of dairy chemistry". Vol. II practical, Chapman and Hall. LTD, (London).

36. Loveday SM, Sarkar A, Singh H (2013) Innovative yoghurts: Novel processing technologies for improving acid milk gel texture. Trends Food Sci Tech 33:5-20.

37. Matumoto-Pintro, P.T.; Rabiey, L.; Robitaille, G.; Britten, M. (2011). Use of modified whey protein in yoghurt formulations. Int. Dairy J. 21: 21-26.

38. Niamah ,Alaa Kareem, Shayma Thyab Gddoa Al-Sahlany and Alaa Jabbar Al-Manhel , Gum Arabic Uses as Prebiotic in Yogurt Production and Study Effects on Physical,Chemical Properties and Survivability of Probiotic Bacteria During Cold Storage, World Applied Sciences Journal 34 (9): 1190-1196, 2016.

39. Nikoofar, E., Hojjatoleslamy, M., Shakerian, A., Molavi, H., & Shariaty, M. A. (2013). Surveying the effect of oat beta glucan as a fat replacer on rheological and physicochemical characteristics of non fat set yoghurt. International Journal of Farming and Allied Sciences, 2(20), 861-865.

40. Pappa, E. C., Kondyli, E., MacNaughtan, W., Kakouri, A., Nesseris, K., & Israilides, C. (2018). Quality and sensory properties of reduced fat yoghurt made with addition of β-glucans. Food and Nutrition Sciences, 9(4), 390-402.

41. Puvanenthiran A., Williams R.P.W., Augustin M.A. Structure and viscoelastic properties of set yoghurt with altered casein to whey protein ratios. *Int. Dairy J.* 2002;12:383–391.

42. Qureshi, A.M.; Hassan,Y .;Sulariya, M. and Rashid, A.(2011). Preparation and nutritional evaluation of garlic Based yogurt. Sci. Int. Lahore 23: 59-62.

43. Quach, M .L.; Chen ,X. D. and Stevenson ,R. J .(1999). Headspace sampling of whey protein concentrate solutions using solid-phase microextraction.Food Res. Int., 31 :371–379.

44. Rawson, H.L. and Marschall.V.M. (1997). Effect of 'ropy' strains of Lactobacillus delbrueckii ssp. bulgaricus and Streptococcus thermophilus on rheology of stirred yogurt. Int. J. Food Sci. Tech, 32: 213-220.

45. Remeuf, F., Mohammed, S., Sodini, I., and Tissier, J. P. (2003). Preliminary observations on the effects of milk fortification and heating on microstructure and physical properties of stirred yogurt. International Dairy Journal, 13(9), 773-782.

46. Sadiq, Israa Haider (2019). Study the effect of fortifying curd with iron coated on its physiochemical, rheological and nutritional properties. Master Thesis - College of Agricultural Engineering - University of Baghdad.

47. Sady M., Domagała J., Grega T., Najgebauer-Lejko D. Quality properties of non-fat yoghurt with addition of whey protein concentrate. *Biotechnol. Anim. Husb.* 2007;23:291–299.

48. Şanli,T. (2015). Effects of Using Transglutaminase and Fat Replacer on Functional Properties of Non-Fat Yoghurt. Journal Home-Page: 907-913.

49. Sengupta, S.; Ankita, C. and Jayati, B. (2014). Production and evaluation of yogurt with watermelon (*Citrullus lanatus*) juice. J. int. academic research for multidisciplinary. Vol. 2, Issue 5.

50. Shakerian M., Razavi S.H., Ziai S.A., Khodaiyan F., Yarmand M.S., Moayedi A. Proteolytic and ACE-inhibitory activities of probiotic yogurt containing non-viable bacteria as affected by different levels of fat, inulin and starter culture. *J. Food Sci. Technol.* 2015;52(4):2428–2433.

51. Stijepic. M. Glušac. J.; Đurdević- Milošević. D. and Pešić-Mikulec. D. (2013). Physicochemical characteristics of soy probiotic yoghurt with inulin addition during the refrigerated storage. Romanian Biotech Letters, Vol. 18, No.2.

52. Serge-Olivier, . K. K., Mathieu , B. ., Dje Bi, Y. B. T. ., Kamagate, S. ., & Angoué, P. . (2021). Influence Of Crude Protein Diet on Growth Performance and Some Blood Biochemical Parameters of Growing Male Japanese Quail In Côte d'Ivoire. Journal of Scientific Research in Medical and Biological Sciences, 2(1), 10-18. https://doi.org/10.47631/jsrmbs.v2i1.217

53. Tamime, A .Y. Robinson, R.K. (1999).Yogurt Science and Technology, 2n .edition, CRC Press, Boca Raton, FL, USA, pages 11-108.

54. Wanga, H., Livingston, K. A., Fox, C. S., Meigs, J. B., & Jacques, P. F. (2013). Yogurt consumption is associated with better diet quality and metabolic profile in American men and women. Nutrition Research, 33, 18e26.

55. Walstra, P., Walstra, P., Wouters, J. T., & Geurts, T. J. (2005). Dairy science and technology. CRC press

56. Xu ZM, Emmanouelidou DG, Raphaelides SN, Antoniou KD (2008) Effects of heating temperature and fat content on the structure development of set yogurt.J Food Eng 85: 590-597.

57. Yilmaz-Ersan, L.and Kurdal,E. (2014). The Production of Set-Type-Bio-Yoghurt with Commercial Probiotic Culture. Int.J .Chem Eng and Appl. 5:402 -408.

58. Zhao, C. B., Wu, F., Li, Y. P., & Liu, X. L. (2015). Effects of β-glucans on properties of soya bean protein isolate thermal gels. International Journal of Food Science & Technology, 50(2), 347-355.