Physicochemical, rheological and sensory properties of yogurt flavored with sweet orange (citrus sinensis) marmalade

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Abstract. The current study was conducted to determine the effect of adding different ratios of orange marmalade on physicochemical, rheological and sensory properties of the yogurt that produced by using the whole bovine milk pH 6.7 and total acidity 0.16% which divided into four treatments: T2, T3, T4 with adding orange marmalade in ratios 2.5, 5.0 and 7.5% respectively beside control treatment. Physicochemical tests included determine the percentage of moisture, fat, protein, carbohydrates and ash as well total acidity and pH value. Rheological tests included viscosity, whey senerises exudation and water holding capacity, as well as sensory evaluation. These tests were carried out during the storage period at (5 ± 1) °C for 1, 7 and 14 day. The results showed an increase in the total solids percentage in proportion to increasing the added amount of orange marmalade compared to treatment T1 and the increase was observed with storage except for the percentage of carbohydrates that decreased. Also results showed an increase in the percentage of total acidity and decrease in pH of the added treatments directly after manufacturing and with storage. The results showed a decrease in the values of whey exudation, increased percentage of water holding capacity and viscosity of marmalade treatments. Results of sensory evaluation showed a decreased in scores with the increasing in marmalade addition compared to control treatment.

1. Introduction.
Yogurt is one of the fermented dairy products used widely in the world with special nutritional, therapeutic and sensory properties. Yogurt prepared by fermenting the milk through adding specific cultures of bacteria (Streptococcus salivarius Sub sp. thermophilus and Lactobacillus delbrueckii Sub sp.bulgaricus) [1; 2]. Converting lactose during fermentation lead to lactic acid formation which lead to protein gelation producing the known yogurt gel of a semi-solid firm body and its special and accepted taste with few amounts of collateral products which affect the flavor properties [3].
Although there is no predict file for yogurt first industry, using it goes back to many centuries. According to the myth, yogurt had been manufactured by Turkish ancient people in Asia. Yogurt is one of the most specific dairy products, and it is a functional product.
Yogurt’s uniqueness is attributed to combined fermentation which is involved during the process. It is a rich source for proteins and minerals like calcium, sodium, phosphor and potassium as well as it contains biologically active peptides like casomorphins, casokinins, immunopeptides, lactoferrin and phosphopeptides [4]. Yogurt has many therapeutic properties like lowering blood cholesterol, prevention tumor cells growth, preventing or reducing heart diseases, protection from osteoporosis and alimentary canal diseases like diarrhea and dysentery [5, 6]. Yogurt increasing consumption in many countries is attributed to the increasing diversity fruit yogurt, or that with added flavors and to the wide variety in product offerings. Flavors that are regularly ordered are amazingly little in number. Fruit flavored yogurt is produced by adding fruit concentrates or juice that has the flavor to the milk of starter before or after the incubation process, consequetively, the added flavor ingredients usuallu decreases product’s texture. On the other hand, adding flavor lead to increase in the nutritional value of the product and its variety in the market [7]. Adding different kinds of fruit to the yogur is a common thing around the world [8].

Kinds of fruit flavors include; fresh, preserved, canned, frizzed fruit and several fruit products [9]. The most common kinds of fruit used in yogurt mixture are plum, cherry, orange, lemon, purple plum, blackberry, spiced apple, apricot, pineapple, strawberries, raspberry and berry [10]. Orange is one of the most common fruit around the world. It contains too many healthy chemical compounds. In addition to that it is concentered as a principle source to enrich the body with vitamin C, fluoric acid, potassium, and pectin [11, 12, 13, 14]. Significance of orange health benefits depend on it form and content as in fresh fruit, fruit extract, adding to that, the flavonoids compounds of orange have high biological properties as they contain phenol compounds and antioxidant properties [15, 16, 17]. [9] found that the physical properties of fruit yogurt are affected by the heat treatment applied to milk, protein content, acidity, starter, additives, stabilizers, homogenizing and mechanical handling of yogurt. [18] reported that adding date pulp marmalade leads to decreased viscosity and increased syneresis fruit marmalade yogurts. [19] indicated that increasing marmalade percentage in yogurts decreased fat and ash contents because of increment the content of total solids of milk. Because of the remarkable lack of studies available on the internet about the effect of adding orange marmalade, not the fresh orange fruit, on quality properties of yogurt, this study has been conducted and aimed to investigate the effect of adding different ratios of orange marmalade to yogurt milk on the physicochemical, rheological and sensory properties of fruit flavored yogurt.

2.Materials and Methods:

2-1-Materials:
whole bovine milk used in yogurt manufacture was collected from the farms nearby the college of food science– Al-Qasim Green University. Also yogurt starters imported from Danisco company (France), the orange fruits were collected from Hilla city local markets.

2-2-Methods: Marmalade manufacturing:
Orange marmalade made according to [20], fresh fruit of orange was washed, cutted, blanched and the fruit pulp was extracted, then added to the other ingredients and mixed thoroughly, after that, the mixture was cooked till reaching to the wanted texture according to marmalade standard manufacturing method then the product was canned in a clean glass container and stored at room temperature till flavored yogurt manufacturing.

2-2-1-Yogurt Manufacturing:
Yogurt was made according to [1] as follows: whole bovine milk was divided into four treatments; control treatment (T1), and (T2, T3, T4) with orange marmalade addition in the following ratios (2.5, 5.0 and 7.5) % respectively. Milk was heat treated at 90 Cº for 10 minutes then cooled to 42 Cº and inculated by Streptococcus Salivarius thermophiles, Lactobacillus delbrueckii bulgaricus starter by direct addition with a suitable amount applied from Danisco company with ratio of 0.013
per liter and canned in plastic yogurt package (200 ml) and incubated until coagulation occurred then it was taken out from the incubation and transferred to the refrigerator for cooling and storage at 5 ± 1 °C for 14 days.

2-2-2-Yogurt Physicochemical tests:
Moisture percentage determined according to [21] where as ash determined by direct burning of samples in muffle furnace mentioned in [22], the total nitrogen determined according to Ling, [23]. Fat percentage determined according to[24]. Carbohydrates determined arithmetically according to [25].
\[
\% \text{ Carbohydrates} = (100 \times \frac{W_1}{W_2} - \% \text{ ash} - \% \text{ protein} - \% \text{ fat} - \% \text{ moisture})
\]
Total acidity determined according to [22]. pH determined by pH meter (model 211 type HANNA) after diluting the sample with distilled water before measuring.

2-2-3-Viscosity:
Apparent viscosity was determined after 1, 7 and 14 days under cooled storage conditions by using Brookfield DVII+ viscometer (Brookfield Engineering Lab Inc., Stoughton, Mass) according to [26] by using axial spindle No. 4 at a speed of 10 rpm/min for 60 seconds and a sample volume of 150 ml.

2-2-4-Water Holding Capacity:
Water holding capacity was determined according to [27] as follows: after fermentation the yogurt samples were kept refrigerated for 12 h, then we took 10 g of sample and transferred it to a centrifuge tube and were centrifuged at 9,800×g for 20 min at 4 °C. The separated whey was collected and weighed. WHC was calculated as bellow formula:
\[
\text{WHC} (\%) = \left(1 - \frac{W_1}{W_2}\right) 
\]
Where:
W1: pellet weight after centrifugation, W2: sample initial weight

2-2-5-Spontaneous Whey Separation: Whey separation was determined according to [28] by placing chilled yogurt cup at angle of 45° for 2 h at 5 °C and pulling out the clear whey from the surface using the syringe and re-weighting the cup again. The operation was perfumed within 10 seconds to avoid excess perfusion.

2-2-6-Sensory Evaluation: sensory tests were conducted by panelist group in college of food science according to organoleptic evaluation list on which [29] relied.

3. Results and Discussion:

3-1-Chemical composition: The results shown in Table 1 illustrate the chemical analysis of the yogurt samples of the above mentioned treatments. It shows the high percentage of total solids represented by protein, fat, carbohydrates and ash of the additive treatments directly after manufacturing and in direct proportion to the increase in the percentage of marmalade compared with the control treatment, it is also noted that the percentage of both protein and fat by the addition of marmalade is directly proportional to the increase in the percentage of addition and this is consistent with [19] who pointed that the increasing marmalade proportion added to yogurt leads to a decrease in the proportion of protein and fat. It is also noted that the proportions of protein, fat and ash are increased with storage for all treatments and these findings are in decrease in moisture percentage. Adecrease in carbohydrates is seen in all treatments during storage, this may be due to starter that continues to work even under refrigerated storage conditions, though slowly, to covert lactose into lactic acid. carbohydrates percentage was higher in treatments T2,T3,T4 significantly compared to the control treatment, this is due to the pectin and high carbohydrates content of the
orange peel used to make the marmalade added to these treatments. This results agree with results founded

**Table 1. Chemical composition of different yogurt treatments**

| Treatment                  | Storage period (day) | %Moisture | %Total solids | %Fat | %Protein | %Ash | %Carbohydrates |
|----------------------------|----------------------|-----------|---------------|------|----------|------|----------------|
| Control treatment (T1)     | 1                    | 86.80     | 13.20         | 3.90 | 4.68     | 0.76 | 3.90           |
|                            | 7                    | 86.65     | 13.35         | 4.00 | 4.70     | 0.77 | 3.88           |
|                            | 14                   | 86.49     | 13.55         | 4.20 | 4.73     | 0.78 | 3.80           |
| Yogurt treatments with orange marmalade T2 | 1               | 85.99     | 14.01         | 3.60 | 4.05     | 0.74 | 5.62           |
|                            | 7                    | 85.88     | 14.12         | 3.68 | 4.20     | 0.78 | 5.46           |
|                            | 14                   | 85.59     | 14.41         | 3.84 | 4.37     | 0.80 | 5.40           |
| Yogurt treatments with orange marmalade T3 | 1               | 84.50     | 15.50         | 3.42 | 3.58     | 0.80 | 7.70           |
|                            | 7                    | 84.31     | 15.69         | 3.48 | 3.73     | 0.82 | 7.66           |
|                            | 14                   | 84.18     | 15.82         | 3.57 | 3.98     | 0.84 | 7.43           |
| Yogurt treatments with orange marmalade T4 | 1               | 84.00     | 16.00         | 3.35 | 3.53     | 0.84 | 8.28           |
|                            | 7                    | 83.88     | 16.12         | 3.50 | 3.65     | 0.86 | 8.11           |
|                            | 14                   | 83.76     | 16.24         | 3.61 | 3.76     | 0.90 | 7.97           |

where: T1: control yogurt, T2: yogurt treatment with 2.5% orange marmalade, T3: yogurt treatment with 5.0% orange marmalade, T4: yogurt treatment with 7.5% orange marmalade.

By [30], the added sugar during marmalade production also contributes to the increment in carbohydrates.

3-2-pH: Results shown in Figure 1 illustrate that the pH value of the control treatment (T1) was 4.72, which is close to that of [31] for yogurt, which was 4.59. pH values for treatments (T2, T3 and T4) were reduced directly after manufacture in direct relation to the increment in marmalade addition compared to control treatments, this reduction in pH continued throughout the storage period, which may be due to the activity of the starter culture during storage and conversion of lactose to lactic acid, this is close to what [18; 33] found.

[34] refered that the decrease in pH is due to the microorganisms activity. [35] refered that the high acidity during storage is due to the activity of the beta-galactosidase enzyme which remains effective at 0-5° C. In this case, the pH may fall below 4.2. However, some studies suggest that the decrease in pH value during storage is due to the remaining enzymes produced by the starter culture during fermentation [36].

3-3-Total Acidity: The results shown in Figure 2 show values of the total acidity, ranging from 0.90% for T1 to 1.08% for T4. The total acidity of the
Figure 1. pH values of different yogurt treatments where: T1: control yogurt, T2: yogurt treatment with 2.5% orange marmalade, T3: yogurt treatment with 5.0% orange marmalade, T4: yogurt treatment with 7.5% orange marmalade

Addition treatments increased directly after manufacturing compared to the control treatment. It is also noted that the pH values of all treatments increase with the progress of the storage period and this is consistent with what [37] found, the cause for this the continued activity of the starter bacteria during storage, albeit slowly, and its consumption of lactose sugar, converting it to lactic and formic acids with small amounts of carbon dioxide, this is consistent with what [38] found. There are some other studies that indicated the same result [32;18]. In general, pH values decrease with storage and for all treatments due to the additional metabolic activity of starter during storage [39]. When the pH decreases, the acidic flavor and taste increase as a result of the decline in the original flavor characteristics of yogurt.
Figure 2. Acidity percentage of different yogurt treatments where: T1: control yogurt, T2: yogurt treatment with 2.5% orange marmalade, T3: yogurt treatment with 5.0% orange marmalade, T4: yogurt treatment with 7.5% orange marmalade.

Rheological Properties:

Spontaneous Whey Separation: The results shown in Figure 3 illustrate the spontaneous whey separation for the different yogurt treatments. The amount of whey separation was decreased directly after manufacturing with the increment of orange marmalade addition compared to control and this agrees with [38]. It also notes the decrease of syneresis quantities with storage for all treatments and this agrees with [33] who pointed that the rate of whey extrusion treatment of yogurt decreased from 55.8% on the first day to 53.3% on day 14 of storage, it also agrees with [40] who found that whey separation for control treatment decreased from 26.80% directly after manufacturing to 26.02% after 10 days of refrigerated storage meaning an increase in water holding capacity. This is due to the metabolic activity of the starter bacteria, as well as to the decrease in net pressure inside the protein matrix, which reduces the exudation [41] these findings are consistent with what [42] found who pointed out that the lactic fermenters exposed to high heat treatments have a more dense texture with extensive and more branched protein network. However, this result does not agree with [43] who pointed the direct correlation of the exudation rate to acidity.
Figure 3. Spontaneous whey separation values of different yogurt treatments, where: T1: control yogurt, T2: yogurt treatment with 2.5% orange marmalade, T3: yogurt treatment with 5.0% orange marmalade, T4: yogurt treatment with 7.5% orange marmalade.

3-5-Water Holding Capacity (WHC): results in Figure 4 shows the percentage of water holding capacity for different yogurt treatments. The water holding capacity was noticed to be increased directly after manufacturing with the increment in the percentage of the added marmalade compared to control treatment. This may be due to the increase in the percentage of total solids by the added marmalade, which contains a large proportion of carbohydrates with many hydroxyl groups, thus encouraging water binding, or may be due to the high phenolic and pectin compounds content of the orange peel help in water binding. This is consistent with [44] who pointed out the possibility of using the pectin extracted from orange fruit as a thickener and a binding agent for water and stabilizer agent in the products of yogurt. As it can be seen from the results, the (WHC) is affected by the storage period, being observed to rise for all treatments, this is consistent with what [31] found who pointed that (WHC) of yogurt made from skim milk was increased from 31.1% directly after manufacturing to 31.5% after 14 days of storage. It also agrees with [40] who found that (WHC) for control treatment was increased from 48.2% directly after manufacturing to 52.2% after 10 days of refrigerated storage. This may be due to the impact of the low moisture content of yogurt treatments.
Figure 4. Water holding capacity percentage of different yogurt treatments where: T1: control yogurt, T2: yogurt treatment with 2.5% orange marmalade, T3: yogurt treatment with 5.0% orange marmalade, T4: yogurt treatment with 7.5% orange marmalade.

3-6- Viscosity: Figure 5 shows the viscosity values of the different yogurt treatments, the results showed that the viscosity values of the additive treatments are increased by increasing the percentage of marmalade directly after manufacturing compared to the control treatment. This is consistent with [45] who pointed that the viscosity of yogurt is usually improved by the addition of stabilizers and thickeners such as natural starch or modified starch, algens, agar, carrageenan, edible gums, cellulose and pectin. This also conforms with [46] who indicated the increment in viscosity of the yogurt treatments by increasing the proportion of additives. Viscosity values are also noted to get down for all treatments with storage and this is consistent with [33] who found a decrease in the viscosity of yogurt treatments with red agate cherry marmalade. However, this result is not consistent with [47] who indicated that there was an increase in the viscosity of yogurt treatment from 2123 centipoise directly after manufacturing to 2244 centipoise after 14 days of storage, and also does not agree with [31].

Figure 5. Viscosity values of different yogurt treatments.
where: T1: control yogurt, T2: yogurt treatment with 2.5% orange marmalade, T3: yogurt treatment with 5.0% orange marmalade, T4: yogurt treatment with 7.5% orange marmalade.

3-7- Sensory Evaluation: Table 2 shows the results of the sensory evaluation of the mentioned models, it is possible to approximate the scores attributed to the qualities of treatment T2 with 2.5% addition of orange marmalade with the scores given for the same characteristics of the control treatment T1 directly after manufacturing with preference given to control treatment qualities, this comes in line with [19] results, who pointed the panalists’ preference for the control treatment yogurt over the yogurt treatments produced by adding kiwi marmalade. The evaluation of T3 and T4 treatments was influenced negatively, in context of taste and flavor characteristics, by the increment of marmalade addition ratio compared to control.
Concerning body, treatment T4 got the highest scores, and that is due to the increase in total solids percentage in comparison with the control treatment, giving it a more firm body. It is also noticed that the sensory evaluation of all treatments was getting lower with the progressive storage, and this may be due to panelists feeling the sweet taste, especially in the treatment T4 with 7.5% of marmalade, adding to that, is the affected body and appearance of the last two treatments by the clear whey separation and unstable texture due to their high acidity. A decrease in the evaluation scores granted to most of the attributes as the storage period progresses is also noted due to the high acidity, and this is consistent with [48 ;49].

### Table 2. Organoleptic evaluation of yogurt

| Treatments                      | Storage period (day) | Appearance | Acidity | Texture and consistency | Flavor | Total |
|--------------------------------|----------------------|------------|---------|-------------------------|--------|-------|
| Control                        | 1                    | 44.0       | 34.0    | 10.0                    | 9.50   | 97.50 |
|                                | 7                    | 40.0       | 31.0    | 9.50                    | 9.37   | 89.87 |
|                                | 14                   | 33.0       | 29.0    | 8.75                    | 8.00   | 78.75 |
| Yogurt treatments with orange marmalade addition | T2 | 1 | 44.0 | 33.0 | 9.00 | 10.0 | 96.00 |
|                                | 7                    | 41.6       | 32.0    | 9.00                    | 9.60   | 92.20 |
|                                | 14                   | 33.0       | 30.0    | 9.0                     | 8.00   | 80.00 |
|                                | T3                   | 1          | 40.0    | 33.0                    | 9.00   | 8.75  | 90.75 |
|                                | 7                    | 36.0       | 29.0    | 8.50                    | 9.00   | 82.50 |
|                                | 14                   | 30.0       | 28.0    | 8.00                    | 8.00   | 74.00 |
|                                | T4                   | 1          | 36.0    | 35.0                    | 8.50   | 8.60  | 88.1  |
|                                | 7                    | 34.4       | 30.5    | 8.30                    | 8.00   | 80.57 |
|                                | 14                   | 25.0       | 20.3    | 7.75                    | 7.32   | 60.07 |

where :T1:control yogurt,T2:yogurt treatment with 2.5% orange marmalade,T3: yogurt treatment with 5.0% orange marmalade,T4: yogurt treatment with 7.5% orange marmalade characteristics probably because of the high concentrations used . On the good side, there was an improvement in all rheological properties after manufacture and this improvement lasted through out the storage period for all but viscosity.

### 4.Conclusions
Although orange marmalade yogurt is rich in many nutritional compounds ,yogurt made from this marmalade was not better than the control yogurt treatment especially in terms of sensory

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