Physicochemical, Sensory properties and antioxidant activity of yogurt flavored with some local fruit juice

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ABSTRACT

Interesting in dairy products fortified with fruits that have nutritive and healthy benefits increase globally, therefore the current study is focused to study the physicochemical properties, sensory evaluation and antioxidant activity of yogurt fortified with liquid extract of three kinds of local fruits including pomegranate, orange, and grape. The three kinds of selected fruits are characterized by their various phenolic compounds which contribute to their antioxidant activity, their high nutrition values and flavor. Three ratios (5, 10, and 15%) of liquid extract of each kind of fruit were prepared to mix with yogurt. Chemical Analysis of the yogurt samples include fat, protein, total solids, Ash, carbohydrate, and acidity beside pH. The rheological tests include the determination of yogurt gel hardness and syneresis. Antioxidant activity determined by DPPH (1,1-diphenyl2-picryl hydrazyl) free radical scavenging activity. Sensory parameters included flavor, body and texture, appearance, and overall acceptability. The results show significant differences among the samples in the most of the chemical properties, adding fruit extract to yogurt cause decrease in fat, protein, total solids percentage, and increase their acidity. Regarding to rheological properties adding pomegranate and grape juice had negative effect on the hardness of the yoghurt and increased of its syneresis, however orange juice had a positive effect on hardness of the yoghurt samples. All fruit fortified yogurt samples shows antioxidant activity and 15% grape juice yogurt showed the highest antioxidant capacity among the other treatments. Regarding to sensory evaluation, orange-fortified yogurt gained the highest scores by panelists in appearance and body texture, whereas grape-fortified yogurt gained the highest scores in flavor .

Keywords: Flavored Yogurt, Physicochemical ,Antioxidant, Sensory Evaluation.

.1INTRODUCTION

Yogurt is a major fermented dairy product that consumed in large amounts worldwide, due to its high nutritional value and benefits to human health. It improve lactose tolerance, strengthen the immune system and provide the health attributes associated with probiotic bacteria [1]. As consumers become more health conscious, their demands for functional dairy products are increasing rapidly. Yogurt can be considered functional dairy product as they have high nutritional value and they impart health benefits [2]. Supplementation of yogurt with functional components strength its functional activities, for this purpose some ingredient groups can be added to yogurt, among them phytochemicals are preferred globally due to their acceptability, palatability and minimal side effects on consumers' health [3], moreover, it is a natural source of antioxidants such as polyphenols. Hence yoghurt with added antioxidants from natural sources satisfy consumer interest in yoghurt nutrients, beneficial effects of LAB, and health benefits of added antioxidants [4]. Different properties of dairy products are extensively explored because these kinds of

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foods are the ideal medium for functional additives and ingredients [5]. To improve the nutritional characteristics of yogurt, many kinds of natural products incorporate to to the yogurt, such as pomegranate [1], Strawberry [6] , black, white and green tea [7], blueberry [8], grape [9], and orange [10 .[

Among them, pomegranate, orange, and grape are selected for this study due to their high contents of polyphenols and their prevalence in Iraq. Pomegranate (Punica granatum L.) is rich in phenolic compounds, flavonoids and other bioactive molecules. The phytochemical constituents of the pomegranate juice exhibit antioxidant and antimicrobial activity [11] as well as anti-cancer activity [12] and antihypertensive activity [13], hence the interest in pomegranate juices has increased in recent years. Regarding to orange (Citrus sinensis (L.), it considered as a rich source of some antioxidants, such as ascorbic acid and phenolic compounds (hesperidin and narirutin), carotenoids (violaxanthin, β-cryptoxanthin, and lutein) ,in which make in orange have anti-cancer, and anti-inflammatory activity. Grape (Vitis vinifera L.) is also rich in phenolic compounds, especially in catechin, epicatechins, anthocyanins, and phenolic acids hence have the ability to prevent the diseases associated with oxidative stress, including cardiovascular, neurodegenerative diseases and cancers [14]. Thus, the addition of the juice of these fruits to yogurt may provide some functional benefits for human health.The current study aims to explore the effect of incorporation the liquid extract of pomegranate, orange, and grape into yogurt on the chemical, rheological properties, and antioxidant capacity of yogurt as well as evaluate their sensory parameters.

.2MATERIALS AND METHODS

2.1Preparation of fruit juices

All the fresh fruits that used for Preparation of juices had been purchased from Sulaymaniyah local market, carefully washed, then the liquid extracted from the fruits using an electric juicer and homogenizer (Moulinex). After filtering the Juices by Cheesecloth, they kept in the refrigerator (4°C) in the sterilized glass bottle until use .

2.2Preparation of the yogurt

Cow milk with 5.4% fat (supplied from the farms of College of Agricultural Engineering Science / Sulaimani University) was heated at 90˚C for 10min after adding 4% of skim milk powder (Rejelih), then cooled to 42ºC and divided to 4 parts. The solids content constant should keep at 16% by varying the amount of milk powder added using the following equation :

Solid content = (%milk powder× 0.96) + (%Milk solids) + (fruit juice %× 0.133.(

The first part used as control and pomegranate, orange, and grape juices were added to the other three parts of milk after they heated at 72ºC for 20 sec. and cooled to 42˚C and added to the milk and starter culture containing Streptococcus thermophilus and Lactobacillus delbrueckii sub sp. bulgaricus as freeze-dried (Danisco, China) were added and incubated at 42˚ C±1°C until complete curd formation. The resultant set yogurt samples were stored at 5˚C till analysis. This experiment was carried out in triplicates .

2.3Chemical Analysis

All materials and yogurt samples were chemically analyzed for fat(using Gerber method), protein (using Kjeldahl method), total solids (the remaining weight of yogurt samples after drying), titratable acidity (which expressed as % lactic acid) according to

AOAC [15]. The pH of yogurt samples was determined using the Wtw-pH530 pH meter at 4ºC. The carbohydrate contents were determined by the difference method given using the expression given below: Carbohydrates (%) = 100 − (protein % + fat % + moisture % + ash.(%

2.4Rheological Properties

2.4.1Determination of Yogurt Gel Hardness

The firmness or hardness of the yogurt was expressed in gm/cm2 indicating the force required to break the gel and is related to its structure and strength. It was measured using a texture analyzer with TRIGGER: 0.5, DEFORMATION: 50mm, SPEED: 1mm/s, SPENDIL: TA-PFS.

2.4.2Crud syneresis

Crud syneresis of yogurt samples, which is expressed as the proportion of free whey (Wheying-Off), was determined using draining methods as described by Amatayakul, Halmos [16], with some modifications. 40 gm of yogurt sample was filtered by a filter paper for nearly 3 hours for nearly 3 hours. The volume of drained whey was collected and measured by the equation: %Syneresis = [volume of separated water (ml) / weight of sample (g)]*100.

2.4.3Antioxidant activity by DPPH free radical scavenging activity :

The free radical scavenging activity of the samples was evaluated using DPPH (1,1-dipheny l2-picryl hydrazyl technique. For making the stock solution of DPPH, a total of 20 milligrams of DPPH were dissolved in 100 mL of methanol with an absorbance of around 0.973 at 517 nm. Three mL of this solutions were combined with 1.5 mL of the sample, mixed vigorously, and allowed to stand in the dark at room temperature for 30 min. Three milliliters of solution containing DPPH in 100 µL of methanol is often given as a standard. The absorbance was therefore determined at 517 nm. The percentage of antioxidants or the capability to scavenge the DPPH radical was calculated using the following equation [17[

%of antioxidant activity= [(Ac−As) ÷Ac] × 100

Where: Ac—Control reaction absorbance; As—Testing specimen absorbance

2.5Determination of Sensory Characteristics

The sensory evaluation of yoghurt samples was done by 10 panelists from the staff members of Department of Food Science and Quality Control/ College of Agricultural Engineering Sciences- University of Suleimani-Iraq. Sensory parameter included flavor (45 points), body and texture (30 points), appearance (25 points) and overall acceptability (100 points .(

2.6Statistical analysis: A 3×3 factorial experiment (two factors at three levels) was conducted according to Complete Randomized Design (CRD) with three replicates, the first factor was the type of juice added (pomegranate juice, orange juice, grape juice) and the second factor was the concentration of these juice (5%, 10%, and 15%). Data were analyzed in Excel 2011 and SPSS 24 software. Analysis of variance (ANOVA) and significant differences were performed using Duncan's multiple comparison procedure (p < 0.05.(

.3RESULTS AND DISCUSSION

3.1The chemical analysis

Table 1 shows the results of chemical analysis for each of fat, protein, carbohydrates, ash, acidity, pH values, and total acidity in the studied flavored yogurt with different levels (5%, 10%, and 15%) of pomegranates juice, orange juice and grape juice. Significant differences were found among the samples in the fat content, higher percentage of fat (5.74%) recorded in the control samples, and fat content decreased in most of fruit-flavored yogurt especially with an increase in the percentage of the fruit juices added to the yogurt, for instance, flavored yogurt with 5% pomegranate juice had a high-fat content (4.76%), compared to that (4.08%) of 15%pomagranat juice. These results are close to that of Wang, Kong [18], due to replacing part of the yogurt fat with fruit juices that contain a trace amount of fat or have no fat in them .

Protein content was also reduced in all of the fruit-flavored yogurt samples by increasing the percentage of the fruit juices added to the yogurt, compared with the control sample which was the highest. Similar results were obtained by other studies [18, 19]. The decreases of proteins in yogurt samples after adding of fruit juice have been explained as a result of the complexes formation between polysaccharides at low pH (of the fruit juice) with yoghurt proteins mainly with b-lacto globulin, leading to the strong decrease of this protein –in yogurt [6]. Moreover, generally, fruits are poor and not good source of proteins. On the other hand, Carbohydrates and ash percentages increased in the all fruit flavored yogurt by increasing of the percentage of the juice added to the yoghurt samples this is due to that usually moisture proportions of these juices are less than that of yogurt, which lead to increase the minerals contain in the flavored yogurt and consequently increase their ash content .

Regarding to the pH values of the studied samples, the results showed that the yogurts become more acidic with the addition of all the studied fruit juice, orange juice yogurt recorded the lowest value (4.31) and the highest acidity (0.96%). Similar results was obtained by Mani-López, Palou [20]. Adding passion fruit juice to yogurt also reduce the pH of the yogurt [21]. Decreasing pH value could be explained as the nutrient-enriched yogurt which provides the optimum incubation environment that supports the rapid growth of the bacterial strains (L. bulgaricus and S. thermophiles), the probiotic yogurts, and enhances their high metabolic activity in fermenting lactose to lactic acid.

Table 1: Chemical analysis of flavored yogurt with different fruit juice

Chemical analysis	Treatments	Control	Fruit juice 5%	Fruit juice 10%	Fruit juice 15%
Fat%	Pomegranate yogurt	5.75^{a}	4.76^{b}	4.01^{I}	**4.08^{h}**
	Orange yogurt	5.74^{a}	4.65^{d}	4.40e	**4.31^{f}**
	Grape yogurt	5.75^{a}	4.67^{d}	4.72^{c}	**4.22^{g}**
Protein%	Pomegranate yogurt	4.420^{a}	4.28^{bc}	4.23^{bcd}	**4.20^{d}**
	Orange yogurt	4.440^{a}	4.27^{bc}	4.21^{d}	**4.01^{e}**
	Grape yogurt	4.430^{a}	4.28^{b}	4.21^{d}	**4.03^{e}**
Total Solids%	Pomegranate yogurt	16.01^{a}	14.82^{a}	11.12^{b}	**15.04^{a}**
	Orange yogurt	16.0b0^{a}	15.10^{a}	14.58^{a}	**14.73^{a}**
	Grape yogurt	16.01^{a}	14.10^{a}	15.06^{a}	**14.81^{a}**

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Ash%	Pomegranate yogurt	0.71^{g}	0.73^{f}	0.83^{e}	**0.93^{b}**
	Orange yogurt	0.71^{g}	0.70^{g}	0.90^{c}	**0.87^{d}**
	Grape yogurt	0.71^{g}	0.72^{g}	1.02^{a}	**0.90^{c}**
Carbohydrate%	Pomegranate yogurt	4.40^{e}	4.42^{e}	4.61^{c}	**4.91^{b}**
	Orange yogurt	4.42^{e}	4.45^{de}	4.50^{d}	**4.85^{b}**
	Grape yogurt	4.41^{e}	4.01^{f}	4.40^{e}	**4.99^{a}**
pH	Pomegranate yogurt	4.68^{b}	4.66^{b}	4.90^{a}	**4.93^{a}**
	Orange yogurt	4.70^{b}	4.31^{g}	4.38^{f}	**4.46^{e}**
	Grape yogurt	4.69^{b}	4.48^{de}	4.54^{c}	**4.52^{cd}**
Acidity	Pomegranate yogurt	0.87^{ab}	0.85^{c}	0.75^{ab}	**0.83^{ab}**
	Orange yogurt	0.87^{ab}	0.96^{a}	0.61^{c}	**0.73^{bc}**
	Grape yogurt	0.69^{bc}	0.76^{bc}	0.64^{c}	**0.69^{bc}**

3.2Determination of Antioxidant activity (Scavenging DPPH(

Using the analytical techniques of DPPH assay to determine the antioxidant activity of food extracts is based on its free radical property which is lost, when it reacts with any food containing antioxidant compound, and its color changes from violet or deep purple to yellow in a concentration dependent manner [22]. The results of this study as illustrated in table 2 shows that the antioxidant capacity of all the treated yogurt (DPPH scavenging) increased with increasing the percentage of the added juice in a concentration-dependent manner, and there are significant differences between these treatments of yogurt. Fortified yogurt with 15% grape juice recorded the highest value of DPPH among the other treatments, while 5% pomegranate recorded the lowest value of antioxidant capacity. [23] reported that the antioxidants capacity of grape is higher than that of pomegranate, and this is due to the high level of flavonoid compounds in grape juice compared to pomegranate juice. Aliakbarian, Casale [24] also found that fermented milk fortified with extracts of grapes have higher phenolic compounds and then higher antioxidant activity. The antioxidant capacity of some foods returns to their contents the phenolic compounds which considered as the most important radical scavengers compounds. There are different kinds of these kinds of compounds in the added fruits juice to the yogurt. These include rutin, methoxy benzoate, catechin, epicatechin, ferulic acid, P-coumaric acid, and cinnamic acid in pomegranate juice are responsible for their antioxidant power [11]. The phenolic compounds in grape juice are farandol, caftaric acid, chlorogenic-acid, gallic acid, caffeic -acid, syringic acid, coumaric acid, hesperidin, and epicatechin compounds [25]. The Orange juice also contains an array of potent antioxidants including vitamin C and some flavonoids which can be further categorized into flavanones, flavones, flavonols (hesperetin and naringenin), carotenoids (xanthophylls, cryptoxanthins, carotenes) and ,Phenolic acids [26]. The phenolic compounds convert peroxy radicals into hydroperoxides then themselves converted to phenoxy radicals which can react with another peroxy radical producing nonradical products [27]. This mean that the

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antioxidants substances can donate a hydrogen atom or an electron to DPPH, to neutralizing it and forming the reduced, yellow-colored DPPH-H and Spectrophotometric measurement at 517 nm allows quantification of this reaction. Its worth to metion that antioxidant activity in some food is very important due it help the body to protect itself against the oxidative damage of the Free radicals that can react with biomolecules of living cells, such as DNA or protein causing various diseases such as cardiovascular diseases and cancer.

The DPPH method is rapid, simple, accurate and inexpensive assay for measuring the antioxidant activity of any kind of food by determine their compounds to act as free radical scavengers (hydrogen donors). Many studies have recommended the DPPH assay as an accurate/easy method to measure the antioxidant activity of fruit products [28, 29.[

Table 2: DPPH radical scavenging activity of yogurt treatments

DPPH	Control	Fruit Juice 5%	Fruit Juice 10 %	Fruit Juice 15 %
Pomegranate juice yogurt	23.58 [I]	10.19 [j]	29.25 [e]	31.00 [d]
Orange juice yogurt	23.58 [I]	24.61 [g]	27.16 [f]	39.11 [b]
Grape juice yogurt	23.58 [I]	23.82 [h]	35.45 [c]	50.44 [a]

3.3Rheological Properties

The rheological properties and texture characteristics of yogurt play a very important role in consumer acceptability. They are affected by the composition and manufacturing processes. As seen from the results of this study in Table 3, addition of 5, 10, 15% pomegranate and grape juice had negative effect on the hardness of the yoghurt and increased of the syneresis of the yoghurt , however adding orange juice with all their level had a positive effect and improve the hardness of yoghurt and reduced the syneresis or whey off of the yoghurt ,this is due to the organic acids contained in orange juice to yogurt ,which cause lowering the pH of the yogurt, so the voluminosity of casein micelle increases at lower pH value due to increased hydration [30]. Moreover adding some fruits juice lead to none covalently interaction between the phenolic compounds in these juice and the yogurt protein (casein) since casein is a proline-rich protein that has a strong affinity for the hydroxyl group of phenolic compounds [31]. The firm gel of set-type yogurt is based on milk coagulation which is induced by the action of lactic acid bacteria, where casein micelles aggregate to form a gel structure. Incorporating any compound into yogurt can contribute to gelling-texturizing capability through interaction with the casein matrix, thus altering the milk gelation process which depends on the kind of the add substance and it's interaction with the casein matrix during fermentation. Adding different kinds of fruit juice to yogurt influences the viscoelastic parameters of yogurt through the different components present in their composition. Syneresis is a textural defect in which the whey separated from yogurt leading to a loss of moisture and the formation of a watery layer. Hence there is an inverse relationship between yogurt hardness and syneresis, any factor decreases the firmness of yogurt, it increases it's syneresis his result is close to the results of other studies [32, 33 .[

Table (3): Rheological analysis of flavored yogurt with different fruit juice

Rheological analysis%	Treatments	Control	Fruit juice 5%	Fruit juice 10%	Fruit juice 15%
Hardness	Pomegranate yogurt	196.00 f	188.25 g	125.51 I	156.50 j
	Orange yogurt	198.00 d	223.51 b	241.76 a	205.26 c
	Grape yogurt	197.00e	169.75h	130.05 k	160.75
Syneresis	Pomegranate yogurt	38.59 d	37.41 g	45.70 a	43.54 b
	Orange yogurt	38.59 d	38.29 e	33.68 h	38.13 e
	Grape yogurt	38.60 d	32.64 I	37.81 f	42.88 c

3.4Sensory evaluation

Using sensory tests tools is a powerful technique for determine the specific sensory effects of the ingredients on the acceptability of yogurt. The most sensory parameters of yogurt that determines consumer acceptance are appearance, texture, and flavor [1]. Table 4 shows the sensory properties of flavored yogurt with different fruit juice. The results indicated that flavored yogurt with 5, 10, 15 % orange juice gained the highest scores by panelists in appearance, body texture, and over acceptability. Whereas flavored yogurt with grape gained the highest scores in flavor. Pomegranates gained the lowest scores by the panelists in most of the sensory properties compared to other treatments of yogurt. This means that control yogurt was negatively affected by the addition of pomegranate juice. Fortifying yogurt with pomegranates also recorded low sensorial scores in the study of Mohamed, Magied [34], these results may return to the high acidity of the pomegranates compared to orange and grape juice , because of the high content of phenolic, flavonoids in pomegranates, which affect the texture of the yogurt [35]. Hence, fortifying yogurt with grape juice positively affects its overall acceptability, with nearly equal overall acceptability to the yogurt fortified with orange with no significant differences between them .

Table (4): Sensory evaluation of flavored yogurt with different fruit juice

Sensory Analysis (score)	Treatments	Control	Fruit juice 5%	Fruit juice 10%	Fruit juice 15%
Appearance 25	Pomegranate yogurt	16.25^{d}	18.63^{c}	12.50^{e}	10.75^{e}
	Orange yogurt	16.25^{d}	21.25^{ab}	23.38^{a}	21.63^{ab}
	Grape yogurt	16.25^{d}	20.00^{bc}	20.88^{b}	21.88^{ab}
Body & Texture 30	Pomegranate yogurt	26.25^{a}	20.40^{c}	18.00^{d}	18.60^{d}
	Orange yogurt	26.25^{a}	18.45^{d}	26.10^{a}	25.50^{a}
	Grape yogurt	26.25^{a}	24.60^{a}	22.50^{b}	18.00^{d}
Flavor 45	Pomegranate yogurt	33.75^{e}	22.50^{g}	26.44^{f}	24.75^{f}
	Orange yogurt	33.75^{e}	36.00^{d}	37.13^{cd}	38.25^{bc}
	Grape yogurt	33.75^{e}	39.38^{b}	39.38^{b}	41.63^{a}

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Overall acceptability 100	Pomegranate yogurt	70.00[a]	50.00[b]	40.00[bc]	30.00[c]
	Orange yogurt	70.00[a]	80.00[a]	80.00[a]	80.00[a]
	Grape yogurt	70.00[a]	70.00[a]	80.00[a]	80.00[a]

.4CONCLUSIONS

It is concluded from this study that fortification of yogurt with aqueous extracts from local Pomegranate, orange, and grape which have a high content of phenolic component, increase the antioxidant activity of yogurt fortified with these fruit juice with increasing of the ratio of the added juice in a concentration-dependent manner. Fortified yogurt with 15% grape juice recorded the highest antioxidant activity. Hence they can be used successfully in making functional fruit yogurt, due they also enhanced its nutritional value, as they could be used as a source of bioactive components in manufacture fruit fortified yogurt. These products are also get promising organoleptic characteristics which assumed to be critical for consumer acceptability and therefore for final adaptation from the food industry. Future research on the development of these kinds of products is needed.

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