Physiochemical and sensory evaluation of yoghurt fortified with dietary fiber and phenolic compounds

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Abstract: The effect of adding different levels of dried grape pomace as a source of antioxidants and fiber at level 1.0% to 5.0% on physiochemical and sensory properties of yoghurt products. The physiochemical properties of yoghurt samples include pH, titratable acidity, syneresis, viscosity, dietary fiber, total phenolic content, antioxidant activity and sensory properties were determined during storage for21 days at 4 C. Increasing the ratios of dried grape pomace had no effect on yoghurt pH values and acidity. During storage periods, the pH values were decreased gradually and increased gradually in acidity in all treatments throughout storage period of 21 days. Dried grape pomace addition up to 2.0 % did not affect the synersis value, but the synersis value decreased, when 4.0 to 5.0 % dried grape pomace was added to the samples. Yogurt prepared with 1.0 and 2.0% dried grape pomace had similar textural properties (hardness, gumminess, adhesiveness, cohesiveness, and springiness) as control yogurts. Fortifying yogurt with 3.0, 4.0 and 5.0% dried grape pomace had significant effect on the textural properties. Hardness, gumminess, and springiness increased and adhesiveness and cohesiveness decreased significantly. The dietary fiber content of yoghurt increased with increased of dried grape pomace in the yoghurt samples. Total phenolic content increased along with increased dried grape pomace concentration in the product, 75, 102,132,168 and 190 mg GAE/100g yoghurt with 1%, 2%, 3%, 4% and 5% dried grape pomace, respectively. Yoghurt prepared with 5% dried grape pomace sample received the highest RSA of 84.72 mg AAE/100g yoghurt initially. Yoghurt fortified with up to 3% dried grape pomace DGP had similar appearance, flavor, texture, consistency, and overall acceptance ratings as control yoghurt. Increasing dried grape pomace fortification level to 4.0% and 5% decreased appearance, flavor, texture, consistency and overall acceptance ratings significantly compared with control yoghurt.
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1. Introduction

Yoghurt is an important dairy product, particularly for consumers with lactose intolerance. Yoghurt is considered a healthy food because it contains viable bacteria that are considered probiotics. Milk and dairy products do not contain fiber. Fiber is found in the cell wall of fruits, vegetables, and cereals, (Lunn and Buttriss, 2007). Fiber of different sources is added to products to increase cooking yield and water-holding capacity, reduce lipid retention, improve textural properties and structure, or reduce caloric content by acting as a bulking agent (Larrauri, 1999). Consumption of foods containing fiber may prevent or decrease gastrointestinal disorders (Elia and Cummings, 2007), hypertension, hypercholesterolemia, coronary heart disease and cancer (Pereira *et al.,* 2004 and Mann, 2007). The effects of different types of fruit as source of dietary fiber on the rheological properties of yoghurt have been studied, and showed stable physicochemical properties of fortified yoghurt during storage (Staffolo *et al.,* 2004; Sendra *et al.,* 2010). The concept of antioxidant dietary fiber was first proposed by Saura-Calixto (1998) with the criteria that one gram of antioxidant dietary fiber should have DPPH free radical scavenging capacity equivalent to at least 50 mg vitamin E and dietary fiber content higher than 50% dry matter from the natural constituents of the material. Grape pomace the residual seed and skins contain high phenolic compounds and dietary fiber (Deng *et al.,* 2011). Also found that fibers from grapes show higher reducing efficacy in lipid profile and blood pressure than that from oat fiber or psyllium due to combined effect of dietary fiber and antioxidants. Grape pomace as antioxidant dietary fiber not only retarded human low-density lipoprotein oxidation in vitro (Meyer *et al.,* 1998). There are increasing interests in applying fruit processing wastes as functional food ingredients since they are rich source of dietary fiber, and most of the beneficial bioactive compounds are remained in those byproducts (Balasundram *et al.,* 2006). Antioxidant dietary fiber may be incorporated with flour for making high dietary fiber bakery goods, while the polyphenols in antioxidant dietary fiber could contribute as antioxidant for improving color, aroma and taste of the product. For instance, mango peel powders were used for preparing macaroni to enhance the

antioxidant properties (Ajila *et al.,* 2010). Apple pomace was incorporated into wheat flour as fiber source to improve the rheological characteristics of cake (Sudha *et al.,* 2007). Grape pomace was mixed with sourdough for rye bread (Mildner *et al.,* 2011). Aside from promoting human health, grape pomace as dietary fiber plays important role as antioxidant and antimicrobial agent to extend the shelf-life of food product. For example, grape pomace was added into minced fish and chicken breast to delay the lipid oxidation (Goni *et al.,* 2009). Also, grape pomace extract exhibited antimicrobial effect against foodborne pathogens when added into beef patties (Sagdic *et al.,* 2011). A few studies also reported good stability of the bioactive compounds from grape and other plant extract in fortified yoghurt (Karaaslan *et al.,* 2011).

The objective of this study was to evaluate of adding different ratios of (1.0, 2.0, 3.0, 4.0 and 5.0%) dried grape pomace as the source of dietary fiber and polyphenols, on physiochemical and sensory properties of yoghurt during storage periods at 4C for 0, 7, 14 and 21 days.

2. Material and Methods:

Fresh buffalo's milk was obtained from Dairy Dep., Fac. of Agri. Cairo Univ., Giza, Egypt.

Streptococcus thermophiles, Lactobacillus delbrueckii spp. Bulgaricus, and *Bifidobacterium lactis* were obtained from Chr. Hansens Lab. Copenhagen, Denmark. The cultures were propagated in sterilized skim milk and then incubated at 37 ^{o}C for 16 hrs.

The grape pomace *vitis viniferal*. Was obtained El- Gona Beverages Company

Gallicaeid 1.1 – diphenyl 1-2-picryhydrazl (DPPH) were purchased from sigma chemical co. USA.

Grape pomace was dried on 40 °C and under vacuum till no further weight loss was observed. Dried grape pomace (DGP) was then ground and passed through different sizes of sieves to obtain powders with particle size of 0.85 mm for the analysis of chemical composition and bioactive compounds, and the fortification in yoghurt. **Preparation of yoghurt**

Yoghurt was prepared with standardized buffalo's milk (3%fat, 12.5% total solids, 3.3% protein) as follows: the standardized milk was divided into 7 equal portions. The first portions served as control, the dried grape pomace was added at the level 1.0, 2.0.3.0, 4.0 and 5.0% to the second, third, fourth, five, sixth and seventh portion respectively, and added 0.3% lacta 525 (to prevent the sedimentation of the fibers) were added to each portion. All milk portions were heated at 90°C for 10 min., cooled to 42°C and inoculated with 2% *Streptococcus thermophiles, Lactobacillus delbrueckii spp. Bulgaricus, and Bifidobacterium lactis,* 3% honey and filled in plastic cups and incubated at 42°C for 3.0 to 3.5 hr. for coagulation, and then the yoghurt cups were cooled to 15 – 20°C and then transferred to the refrigerator (5±1°C). The chemical, microbiological analysis and sensory evaluation were carried out on fresh yoghurt and after 1, 2 and 3 weeks.

Moisture, ash, fat, protein, titratable acidity and dietary fiber were determined according to the Official Methods of Analysis **(AOAC, 2000).** The pH samples were measured using digital pH meter (Hanna, Italy).

Synersis

Degree of synersis, expressed as portion of free whey, was measured by a small modification of method by **Al-Kadamany** *et al.* **(2003).** A 10 g sample of mixed fruit yoghurt was placed on a filter paper resting on the top of a funnel. After 10 min. of drained in vacuum condition, the quantity of remained fruit yoghurt was weighed and synersis was calculated as follows:

Free whey (g/ 100 g): weight of initial sample - weight of sample after filtration / weight of initial sample X 100.

Texture Profile

Texture profile analysis of the yogurt samples was measured using QTS 20 texture analyzer (model QTS20, Brookfield Instruments, Harlow, UK) equipped with a 5-kg load cell. Texture profile analysis was carried out by a compression test that generated plot of force (g) versus time (s). A 25-mm-diameter perplex cylindrical probe was used to measure textural profile of the yogurt samples at 10 ± 0.5°C. In the first stage, the samples were compressed to 10 mm depth and the speed of the probe was fixed at 30 mm/min during the pre-test, compression, and relaxation of the samples. The typical textural profile (force–time) curve was obtained with one complete run. Hardness, gumminess, adhesiveness, cohesiveness, and springiness of yogurt samples were calculated by the software program (Texture Pro software, Brookfield Instruments). The data presented are average of 5 replications

Total phenolic contents (TPC)

Total phenolic compounds were determined According to Zheng & Wang (2001) by using Folin-Ciocalteu reagent and expressed as milligrams of Gallic acid equivalents (GAE) per 100 gm.

Antioxidant Capacity

Radical Scavenging Activity (RSA %) assay Free radical Scavenging activity (RSA) of the samples was measured using the method of Brand-Williams *et al.* (1995) and expressed as percentage inhibition of the DPPH radical and was determined by the following equation:

$$RSA = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Sensory Evaluation:

Ten panelists from Food Technology Research Institute Agricultural Research Center, Giza, Egypt were selected on the bases of their training and experience in the use and evaluation of control and yoghurt prepared with 1, 2, 3, 4 and 5% dried grape pomace DGP. They evaluated 20 g portions of each yoghurt sample and used a quality rating score card for evaluation of color & appearance (9 points), flavor (9 points), body& texture (9 points), consistency (9 points) and overall acceptability as described by Nelson and Trout (1981).

Statistical Analysis

Statistical analysis of the data was carried out by ANOVA using SAS statistical software (SAS, 1998). The significant differences among means were assessed by Duncan's multiple range tests (Duncan, 1955).

3. Results and Discussions

The chemical composition of dried grape pomace is presented in Table (1). Fat, protein, total sugar, ash and dietary fiber content of dried grape pomace were 10.40, 10.30, 7.80, 6.20 and 65.30 % respectively. On the other hand, total phenolic content and radical scavenge activity of dried grape pomace (DGP) were 75.80 mg GAE/g and 44.30 mg AAE/g. The phenolic compounds in DGP are influenced by many factors, including grape variety, growth climate and location, harvest time, as well as processing and storage conditions, extraction and analytical methods (Lafka *et al.,* 2007).

Table (1) Chemical composition of dried grape pomace

Components	%
Moisture content %	6.43
Total sugars %	7.80
Protein %	10.30
Lipids %	10.40
Ash %	6.20
dietary fiber	65.30
Radical scavenging activity mg AAE/g	44.30
Total phenolic content mg GAE/g	75.80

(GAE) Gallic acid equivalents. (AAE) Ascorbic acid equivalents

The effect of Different ratio of dried gape pomace on pH values and titratable acidity of yoghurt during storage periods at 4 °C are illustrated in table (2). The pH values and titratable acidity of fresh yoghurt prepared with different level of dried grape pomace ranged from (4.60 to 4.57) and (0.83 to 0.85) for pH values and acidity respectively. The pH value and acidity of yoghurt without dried grape pomace were 4.50 and 0.83%. Increasing the ratios of dried grape pomace had no effect on yoghurt pH values and acidity. It is clear that during storage periods the pH values were decreased gradually and increasing gradually in acidity in all treatments throughout storage period of 21 days. Staffolo *et al.* (2004) reported that addition of commercial apple, wheat, bamboo, or inulin fibers had no effect on yogurt pH. The decreased in pH values were an increase in acidity due to conversion lactose to lactic acid during storage period. These results were consistent with previous study in orange fiber fortified yogurt, in which about 0.2 units of pH 3 reductions was observed after 14 days of storage (Garcia *et al.,* 2005).

The effect of different ratios of dried grape pomace on synersis

Synersis is defined as a spontaneous water release from the gel caused by contraction. The most important causes of synersis in fermented products are the use of high temperatures of incubation, low solid content or inadequate storage temperatures (Amaya-LIano *et al.,* 2008).The synersis of yogurt samples was affected by both storage time and dried grape pomace ratios. Dried grape pomace ratio x storage time interaction was detected for synersis.dried grape pomace addition up to 2.0 % did not affect the synersis value, but the synersis value decreased, when 4.0 to 5.0 % dried grape pomace was added to the samples. In general, synersis value of all yogurt samples decreased until 7 days of storage, and then increased for all samples. Synersis values of all yogurt samples except for sample with 4.0 to 5.0% dried grape pomace decreased up to 14 days then increased quickly at 21 days. The lowest synersis value observed in sample with 4.0 and 5.0% dried grape pomace was 22.0 and 20.5% respectively. The reason for this fluctuation could not be explained. Staffolo *et al.* (2004) reported that no synersis was occurred when yoghurt was fortified with 1.3% of wheat, inulin and apple fiber during 21 days of storage.

Table 2. The effect of Different ratio of dried gape pomace on pH values and titratable acidity of yoghurt during storage periods at 4 °C.

pH values							
ITEM	Control	D.G.P					± SE
		1%	2%	3%	4%	5%	
Fresh	4.50b	4.60a	4.59a	4.58a	4.58a	4.57a	0.21
7d	4.43b	4.53a	4.50a	4.49a	4.46a	4.36a	0.96
14d	4.38b	4.45a	4.44a	4.45a	4.43a	4.42a	0.15
21d	4.32b	4.40a	4.39a	3.37a	4.38a	4.37a	0.8
titratable acidity values							
ITEM	Control	D.G.P					± SE
		1%	2%	3%	4%	5%	
Fresh	0.83ab	0.83ab	0.83ab	0.84a	0.85a	0.85a	0.21
7d	0.88ab	0.87ab	0.89	0.89	0.90a	0.91a	0.16
14d	0.94a	0.92a	0.93a	0.94a	0.95a	0.96a	0.18
21d	1.08a	1.06a	1.07a	1.08a	1.09a	1.10a	0.301

Values with different letters in the same column are significant different at $P<.0.05$.

Table (3). The effect of different ratios of dried grape pomace on synersis

Synersis values							
ITEM	Control	D.G.P					± SE
		1%	2%	3%	4%	5%	
Fresh	28.60a	28.50a	27.30a	25.80ab	20.00b	19.70b	3.8
7d	25.30a	24.30a	24.00a	21.50ab	18.40b	17.20b	2.8
14d	28.80	26.40	25.00	23.00ab	19.00b	16.20b	2.1
21d	33.50a	31.10a	31.00a	29.60ab	22.00b	20.50b	3.6

Values with different letters in the same column are significant different at $P<.0.05$.

Texture properties

Texture properties of yogurt prepared with different ratios of dried grape pomace are presented in Table 4. Yogurt prepared with 1.0 and 2.0% dried grape pomace had similar textural properties (hardness, gumminess, adhesiveness, cohesiveness, and springiness) as control yogurts. Although the addition of 1.0 and 2.0 % dried grape skin had no effect on yogurt texture. Fortifying yogurt with 3.0, 4.0 and 5.0% dried grape pomace had significant effect on the textural properties. Hardness, gumminess, and springiness increased and adhesiveness and cohesiveness decreased significantly. Increasing the hardness may be related to DF absorbing more moisture because of its higher water-holding capacity.

Yogurt fortified with 3.0, 4.0 and 5.0% dried grape pomace had similar textural properties showing that increasing dried grape pomace level to 5.0% had no significant effect on yogurt texture.

Table 4.Texture properties of yogurt prepared with different ratios of dried grape pomace

Texture Values							
ITEM	Control	D.G.P					± SE
		1%	2%	3%	4%	5%	
Hardness, g	37.60b	36.90b	38.40b	54.80a	55.00a	56.50a	4.6
Gumminess, g	20.80b	20.90b	21.20b	27.60a	28.20a	29.20a	2.1
Adhesiveness	-78.60	-73.40	-79.20	-170.60	-175.80a	-180.40a	11.2
Cohesiveness	0.56	0.55	0.55	0.53	0.52	0.52	0.066
Springiness, mm	7.50b	7.40b	7.50b	7.60a	7.60a	7.70a	1.26

Values with different letters in the same column are significant different at $P<.0.05$.

The effects of different ratio of dried gape pomace on the dietary fiber content of yoghurt

The effects of different ratio of dried gape pomace on the dietary fiber content of yoghurt are illustrated in Table (5). The dried grape pomace contained of 65.30 g dietary fiber/l00g dried grape pomace DGP. The yoghurt prepared with 5% dried grape pomace DGP had the highest of fiber content of 4.20%, followed by 4%,3%, 2% and 1% of DGP (w/w yogurt)respectively. The dietary fiber content

of yoghurt increased with increased of dried grape pomace in the yoghurt samples 0.70, 1.33, 2.20, 3.10 and 4.0 g dietary fiber /100 g yoghurt prepared with 1%, 2%, 3%, 4% and 5% dried grape pomace DGP respectively. The dietary fibers from fruit and vegetable byproduct may be developed as food ingredients to offer the physiological functionalities on solubility, viscosity, hydration property, oil-binding capacity and antioxidant activity on food products (Elleuch *et al.,* 2011) and (Staffolo *et al.,* 2004) used apple wheat, bamboo and inulin as source of dietary fiber for improving rheological properties of yoghurt. Sendra et al., (2010) fortified yoghurt with orange byproduct and showed increased viscosity and improved water absorption. Soukoulis *et al.* (2009) reported that dietary fibers from oat, wheat, apple and inulin are able to control the crystallization and re crystallization in frozen dairy products by elevating the glass transition temperature.

Table 5. The effect of Different ratio of dried gape pomace on the dietary fiber content of yoghurt

ITEM	D.G.P					± SE
	1%	2%	3%	4%	5%	
Fiber	0.70e	1.33d	2.20c	3.10b	4.20a	0.66

Values with different letters in the same column are significant different at *P*<.0.05.

Total phenolic content of yogurt prepared with different ratio of dried grape pomace

Total phenolic content of yoghurt prepared with different ratio of dried grape pomace are presented in Table (6).Total phenolic content of dried grape pomace DGP fortified products increased along with increased dried grape pomaceDGP concentration in the product, 75, 102,132,168 and 190 mg GAE/l00g yoghurt with 1%, 2%, 3%, 4% and 5% dried grape pomace DGP, respectively. On the other hand, the total phenolic content of yoghurt after 21 days of storage were 28, 44, 63, 75 and 88 mg GAE/l00g yoghurt with 1%, 2%, 3%, 4% and 5% dried grape pomace DGP respectively. Similar trend was found by, (Karaaslan *et al.,* 2011) that total phenolic content in 10% Merlot grape extract fortified yoghurt was 78 mg GAE/100 g on the first day of storage, but decreased remarkably after 14 days of storage. Wallace and Giusti (2008) also reported that total phenolic content degrades rapidly during the first week of storage, but is relatively stable after weeks in yoghurt fortified with berry and purple carrot extracts.

Table (6). The effect of Different ratio of dried gape pomace on total phenolic content of yoghurt during storage periods at 4 °C

ITEM	D.G.P					± SE
	1%	2%	3%	4%	5%	
Fresh	75c	102b	132b	168a	190a	5.61
7d	52c	83b	99b	130a	147a	7.31
14d	40c	66c	84b	98a	112a	6.91
21d	28c	44b	63b	75a	88a	4.7

Values with different letters in the same column are significant different at *P*<.0.05.
Total phenolic content (mg GAE/ 100g yoghurt with dried grape pomace

Radical scavenging activity of DGP fortified yoghurt

Radical scavenging activity of yoghurt prepared with different ratio of dried grape pomace is illustrated in Table (7). As expected, yoghurt prepared with 5% DGP (w/w yoghurt) sample received the highest RSA of 84.72 mg AAE/l00g yoghurt initially, followed by yoghurt with 4%, 3%, 2% and 1% of dried grape pomace DGP respectively. RSA of yoghurt prepared with 5% dried grape pomace DGP significantly (P<0.05) dropped during 21 days of storage was 71.10 mg AAE/l00g yoghurt at 21 days of storage, while the reduction rate after 21 days of storage was about 18.26, 18.95, 16.69 and 16.01% for yoghurt prepared with 1%, 2%, 3% and 4% dried grape pomace DGP respectively.

Sensory evaluation

Sensory evaluation of yoghurt prepared with different ratio of dried grape pomace DGP during storage periods are illustrated in Table (8). Significant differences in appearance, Flavor, texture, consistency, and overall acceptability for both control and yoghurt samples prepared by added different ratio of dried grape pomace. Sensory attributes ratings depended on the level of fortification. Yoghurt fortified with up to 3% dried grape pomace DGP had similar appearance, Flavor, texture, consistency, and overall acceptability ratings as

control yoghurt. Increasing dried grape pomace DGP fortification level to 4.0% decreased appearance, Flavor, texture, consistency, and overall acceptability compared with control yoghurt. A noticeable decrease in all sensory characteristics was showed during storage periods could be due to proteolytic activity of bacteria and the production of higher acidity **Bakirci and Kavaz (2008).**

Table (7). DPPH radical scavenging activity of yoghurt prepared with different ratio of dried grape pomace during storage at 4 °C.

ITEM	D.G.P.					± SE
	1%	2%	3%	4%	5%	
Fresh	42.33c	55.40c	65.67c	77.30c	84.72	3.81
7d	40.10c	52.60c	61.40b	74.10a	79.96a	4.73
14d	37.30c	48.70b	59.20b	70.20a	75.50a	5.07
21d	34.60c	44.90b	54.80b	65.40a	71.10a	4.10

Values with different letters in the same column are significant different at $P<.0.05$.
RSA (mg AAE / 100 g yoghurt prepared with dried grape pomace DGP

Table 8. Sensory properties of yoghurt prepared with different ratio of dried grape pomace DGP

properties	ITEM	D.G.P						± SE
		Control	1%	2%	3%	4%	5%	
Appearance	Fresh	8.40a	7.50b	7.40b	7.30b	6.40c	6.00c	1.35
	7d	8.20a	7.40 b	7.40b	7.20b	6.30c	6.00c	0.40
	14d	8.00a	7.20b	7.20b	7.10b	6.20c	5.90cd	0.089
	21d	7.80b	7.00b	7.00b	6.90b	6.00c	5.80d	0.070
Flavor	Fresh	7.50a	7.30a	7.20ab	7.10ab	6.40b	6.20b	0.49
	7d	7.40a	7.20ab	7.20ab	7.00b	6.30b	6.10bc	0.38
	14d	7.20a	7.00b	7.00b	6.80b	6.10bc	6.00c	0.51
	21d	7.10a	6.80b	6.70b	6.60b	5.90c	5.80c	0.152
Texture	Fresh	7.80a	7.20ab	7.30ab	7.40ab	6.50b	6.10b	0.33
	7d	7.60a	7.10ab	7.20ab	7.20ab	6.30b	6.00bc	1.08
	14d	7.50a	7.00ab	7.10ab	7.10ab	6.10bc	5.80c	0.22
	21d	7.30a	6.80b	6.90b	6.90b	5.80c	5.60c	0.490
Consistency	Fresh	7.60a	7.40a	7.20ab	7.30ab	6.60b	6.40bc	1.02
	7d	7.50 a	7.30a	7.10ab	7.20ab	6.40b	6.20b	0.76
	14d	7.30a	7.10ab	6.90b	7.00ab	6.20b	6.00bc	0.38
	21d	7.10a	6.90b	6.70b	6.90b	6.10bc	5.80c	0.631
Overall acceptability	Fresh	8.10a	7.60ab	7.70a	7.80a	6.40b	6.20b	0.099
	7d	8.00a	7.40a	7.50a	7.60a	6.30b	6.00bc	0.402
	14d	7.80a	7.20ab	7.30a	7.40a	6.20b	5.80c	0.92
	21d	7.50a	7.00b	7.10ab	7.20a	6.00bc	5.70c	0.80

Values with different letters in the same column are significant different at P<.0.05.

Conclusions

Fortifying yoghurt or dairy products with dried grape pomace is of great interest to improve the functionality and create functional foods with health benefits. The addition of dried grape pomace DGP to yoghurt would complement its healthy characteristics. This study has shown that fortifying yoghurt with **3%** dried grape pomace DGP produced an acceptable product with potential beneficial health effects.

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