RESEARCH ARTICLE

Influence of red grape pomace powder on physiochemical, antioxidant, nutritional, microbial, and sensory properties of probiotic yoghurt

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Abstract: In this study, we investigated the effect of 2 and 4 % grape pomace powder (GPP) on the physiochemical, nutritional, microbial and sensory properties of probiotic yoghurt (contains *Lactobacillus acidophilus* and *Bifidobacterium bifidum*) during the storage period every 7-day intervals (for 28 days). The results revealed that the pH, fat, protein, syneresis and sensory scores of samples decreased with elevation the grape pomace powder (GPP) level and also storage time. Ash, fiber, energy, total flavonoid, antioxidant activity, total phenol and the counting (log cfu/mL) of probiotic microorganisms decreased during storage, but the treatments with higher GPP had higher ash, fiber, energy, total flavonoid, antioxidant activity, total phenol content and probiotic counts. In converse, the acidity and viscosity increased with time storage and increase the GPP level. The probiotic yoghurt supplanted with 4% GPP in 0 day had the highest ash (1.2%), fiber (0.75%), energy (73.8 kcal/g), total flavonoid (4.9 mg Rutin equivalents/g) and antioxidant activity (42.4%). The highest viable count at the end of storage period (28 day) was obtained for probiotic yoghurt containing 4% GPP (8.03 log cfu/mL) and the lowest for control (6.2 log cfu/mL). Also the highest organoleptical scores belonged to yoghurt supplemented with 2% GPP at 0 day. The results of this research showed that grape pomace can be used in the production of probiotic yoghurt.

Keywords: Antioxidant, Grape pomace, Organoleptic, Probiotic yoghurt, Protein, Viscosity

Introduction

Yoghurt is a fermented dairy product that is fermented and acidified by addition of a starter culture containing fermenting lactic acid bacteria including *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus*. Yoghurt has gained widespread consumer acceptance as a healthy food providing health benefits. The functionality of yoghurt increases with the addition of probiotic microorganisms. Probiotics are living microorganisms having beneficial effect on host health (Pereira and Gibson 2002). The most commonly used probiotic microorganisms in dairy food are *L. acidophilus* and *B. bifidum*, which are chosen for their nutritive, therapeutic, and symbiotic characteristics (Hull et al. 1984, Gomes and Malcata 1999). Prebiotics are non-digestible carbohydrate substrates in the diet that are the preferred foods for *Bifidobacteria* and *Lactobacilli* and result in their increased number in the large intestine (Gibson and Roberfroid 1995). Grape (*Vitis* sp., Vitaceae) is one of the world's largest fruit crops, with an approximate annual production of 58 million metric tons (Zhu et al. 2014, Llobera and Cañellas 2007, Schieber et al. 2001). Winemaking process uses a considerable amount of fresh grape generating a huge mass of solid by-products. This by-product, usually referred to as grape pomace (GP), is generated after destemming and pressing grapes and is composed of grape seeds and skins (Cheng et al. 2010). In a study chemical composition of grape pomace evaluated. With regard to the compounds with functional properties, higher values of total dietary fiber (46.17 g/100 g), insoluble fiber (36.4 g/100 g), carbohydrate (29.2 g/100 g), protein (8.49 g/100 g), lipids (8.16 g/100 g), energy (224 kcal/100 g), vitamin C (26.25 mg/100 g), and anthocyanins (131 mg/100 g) were found. The minerals iron, potassium, zinc, manganese, and calcium were present in higher concentrations (Sousa et al. 2014). As mentioned above, grape pomace is a rich source of polyphenols and fibers. Agte et al. (2010) demonstrated prebiotic activity in grape varieties and hybrids that showed from 21.2 to 72.5% of the activity of fructo-oligosaccharide (FOS) used as a standard prebiotic. In this sense, Dairy products, such as yoghurts, are interesting foods in which grape pomace may be added, due to its positive effect on probiotic bacteria and consumers that prefer natural fibers instead of synthetic ingredients (Ramos et al. 2017). Some researchers studied the effect of the grape (juice, skin, seed, pomace, flour,

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oligofructose, extract) on the physiochemical, textural, sensory, nutritional, antioxidant and microbiological properties of yoghurt (Oztürk and Oner 1999, Karnopp et al. 2017, Dos Santos et al. 2017, Hervert-Hernández et al. 2009, Da Silva et al. 2017, Karaaslan et al. 2011, Chouchouli et al. 2013, Lachman et al. 2013, Gil-Sánchez et al. 2017). Also, consumption of fruits, prebiotics and yoghurt in combination has a potential to provide extra nutritional-physiological value that involve in synergetic effect on health such as cancer and cardiovascular disease (Espírito-Santo et al. 2010, Kourkoutas et al. 2006, Sendra et al. 2008, Karaaslan et al. 2011). Although the utilization of grape pomace in yoghurt was already the target of investigations (Marchiani et al. 2016), there is no comprehensive information about the physiochemical, microbial and sensory properties of fortified probiotic yoghurt with red pomace powder. Hence, we evaluated the combined effect of grape pomace powder and probiotic bacteria, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* on some quality and traits of yoghurt and produced a yoghurt formulation based on sensorial, nutritional, and functional perspectives. Therefore, the objectives of this study were to investigate grape pomace potential as a functional ingredient for yoghurt production, and to evaluate the chemical, physical, microbial and sensory properties of the product during refrigerated storage (4 °C).

Materials and Methods

Materials

Whole pasteurized cow milk used to prepare yoghurt formulations. Lyophilized pouches of commercial ABY culture (containing *Lactobacillus acidophilus* LA-5, *Bifidobacterium bifidum* BB-12, *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*) and Y culture (containing commercial yoghurt starter culture, *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*) supplied by Christian Hansen (Horsholm, Denmark). The cultures maintained according to manufacturer's instructions at -18 °C until use. Red wine grape pomace obtained from an industry of fruit pulp locate in the city of Gorgan (Iran). The pomace of grape fruit dried in oven under air flow at 60 °C until constant weight. The dried residue reduced to fine powder in a bimby processor. In order to make the mixture of the powder into the reconstituted milk easier, the particle size standardized to less than 0.42 mm, measured through sieves. The powder stored in clapped glass bottles and kept under refrigeration at 4 °C until use.

Yoghurt production

Milk standardized to 14% (w/v) total solids with skimmed milk powder. The mixture was pasteurized at 85 °C for 30 min and cooled to 43 °C and inoculated with the standard yoghurt culture (Y) and the working cultures of *L. acidophilus* and *B. bifidum* at the level of 1% (v/v). For the preparation of fruit incorporated

yoghurt, 2 and 4 % (w/v) red grape pomace powder added to the milk before adding skim milk powder during the standardization. The mixture was incubated at 42 °C until the pH reached 4.5. After the fermentation, the samples were transferred into a refrigerator at 4 °C and then stored at 4 °C for 28 days. pH, total titratable acidity, protein, fat, ash, total dietary fiber, energy, syneresis, viability of probiotic organisms, total phenolic content, total flavonoid content, antioxidant activity, viscosity and sensory properties were determined during the storage period every 7-day intervals.

Chemical and nutritional attributes

pH values of the samples measured using a pH meter. The titratable acidity (Dornic, °D) determined after mixing 10 mL of sample with 10 mL of distilled water and titrating with 0.1 N NaOH using phenolphthalein as indicator (Ahmadi et al. 2012). Ash content was detected according to Sowbhagya et al. (2007). Fat was measured gravimetrically by extraction with diethyl ether using a Soxhlet apparatus (AOAC 1990). Nitrogen content was detected by Kjeldahl method (Ayadi et al. 2009). Total dietary fiber content determined with using of the Megazyme International total DF assay (Sun-Waterhouse et al. 2010). The total energy was measured based on the energy nutrient results obtained using the conversion factors of Atwater, as described by Sousa et al. (2014) considering 4 kcal/g for carbohydrate, 4 kcal/g for protein, and 9 kcal/g for lipids. Syneresis determined by measuring the volume of separated whey (mL whey/ 100 mL yoghurt) after 30 min at room temperature (Abd El-Salam et al. 1991).

Total phenolic content

Total phenolic content was measured according to Zheng and Wang (2001) by using Folin–Ciocalteu reagent. The absorbance measured with a UV–Vis Spectrophotometer at 765 nm. The absorbance values were converted to the total phenolics and were expressed as µg gallic acid equivalents per gram sample (µg GAE/g).

Total flavonoid content

The amount of total flavonoid content (TFC) in the extracts was detected spectrophotometrically according to Djeridane et al. (2006). This method depends on the formation of a complex flavonoid aluminum, having the maximum absorbance at 430 nm. Rutin was used to make a calibration curve. TFC expressed as mg Rutin equivalents per g.

Antioxidant activity

Antioxidant activities of yoghurt samples by 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) inhibition were determined. Yoghurt water extract (250 µL) was added into 3 mL of 60 µM DPPH in ethanol. The decrease in absorbance monitored at 517 nm until a

constant reading obtained. The readings compared with the control which contained distilled water (250 µL) (Apostolidis et al. 2007). The inhibition percentage calculated as follows:

$$\% \text{ Inhibition} = (A_{\text{control}} - A_{\text{sample}}) \times 100 / A_{\text{control}}$$

Viscosity

The apparent viscosity of samples was measured using a rotary Brookfield Viscometer (RVDV2, Brookfield, MA, USA) with RV4 spindle. Before starting the test, all samples were kept at 7°C in constant conditions to remove any stress or change in their texture. Viscosity of each treatment was carried out at 80 rpm shear stress during 60 s. The test type was as single point (Trachoo and Mistry 1998).

Probiotic Count

MRS-bile agar medium used for the selective enumeration of *L. acidophilus* and bifidobacteria (Sohrabvandi et al. 2012). The plates incubated anaerobically at 37°C for 72 h. Anaerobic conditions produced using the GasPack system. Number of probiotics calculated and expressed as cfu/mL (Shafiee et al. 2010).

Sensory Analysis

Yoghurt samples were subjected into a sensory evaluation with 10 untrained panelists. The yoghurt samples were served in white plastic pots in individual booths under light exposure. The sensory evaluation carried out using a five-point hedonic scale (5- like very much to 1- dislike very much) based on acceptance

to the product in order to evaluate the degree of likeliness for selected quality attributes i.e. flavor, appearance, consistency, taste, and overall acceptability (Senadeera et al. 2018).

Statistical analysis

The data obtained from the measurements were subjected to analysis of variance (ANOVA) to determine the significant differences among the samples, and the values were compared using the Duncan's test defined at $P < 0.05$. All measurements were carried out in triplicate and reported as the mean $\pm$ SD. The data analysis was performed using SPSS software version 16 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Effect of incorporation of grape pomace powder on physiochemical, antioxidant and nutritional properties of probiotic yoghurt

Chemical and nutritional properties of different yoghurt samples during 28 days storage is presented in Table 1. The pH of all yoghurt samples decreased throughout storage period, while the acidity increased. This was due to the growth of lactic acid bacteria and produced the lactic acid (Rasic et al. 1978). The values of pH and acidity recorded in this study were consistent with the results obtained by Mahmood et al. (2008) and Tarakci (2010).

The fat and protein of samples were decreased with increase the grape pomace powder (GPP) and also during storage time, while

Table 1 Physiochemical and nutritional parameters of different yoghurt formulations during storage period (mean $\pm$ SD)

	Day	pH	Acidity	Protein	Fat	Ash	Fiber	Energy	Syneresis)
	Treatment		(°D)	(%)	(%)	(%)	(%)	(kcal/g)	(mL whey/ 100mL yoghurt
control	0	4.8 $\pm$ 0.1 ^g	80 $\pm$ 1.7 ^a	3.2 $\pm$ 0.1 ^a	3.4 $\pm$ 0.2 ^f	0.8 $\pm$ 0.1 ^a	0.07 $\pm$ 0.01 ^a	70 $\pm$ 1.1 ^{ab}	50 $\pm$ 2.2 ^k
	7	4.6 $\pm$ 0.1 ^{fg}	82.0 $\pm$ 2 ^b	3.2 $\pm$ 0.2 ^a	3.4 $\pm$ 0.2 ^f	0.8 $\pm$ 0.1 ^a	0.07 $\pm$ 0.01 ^a	70 $\pm$ 2.1 ^{ab}	49.9 $\pm$ 2.1 ^k
	14	4.6 $\pm$ 0.2 ^{fg}	83.0 $\pm$ 3.2 ^b	3.15 $\pm$ 0.2 ^a	3.3 $\pm$ 0.1 ^{ef}	0.78 $\pm$ 0.2 ^a	0.07 $\pm$ 0.01 ^a	69.6 $\pm$ 2.2 ^{ab}	46.7 $\pm$ 1.7 ^j
	21	4.4 $\pm$ 0 ^{efg}	85.0 $\pm$ 2.3 ^c	3.12 $\pm$ 0.1 ^a	3.3 $\pm$ 0.1 ^{ef}	0.78 $\pm$ 0.12 ^a	0.065 $\pm$ 0.01 ^a	69.2 $\pm$ 1.9 ^a	42.8 $\pm$ 1.8 ^g
	28	4.3 $\pm$ 0.07 ^{defg}	86.0 $\pm$ 4 ^{cd}	3.1 $\pm$ 0.23 ^a	3.28 $\pm$ 0.13 ^{def}	0.77 $\pm$ 0.06 ^a	0.06 $\pm$ 0 ^a	69.0 $\pm$ 1.7 ^a	39.8 $\pm$ 1.9 ^f
2% GPP	0	4.7 $\pm$ 0.3 ^g	83.0 $\pm$ 1.1 ^b	3.0 $\pm$ 0.3 ^a	3.2 $\pm$ 0.3 ^{cde}	1.08 $\pm$ 0.21 ^{bcd}	0.38 $\pm$ 0.08 ^b	72 $\pm$ 2.3 ^{def}	44.3 $\pm$ 2 ⁱ
	7	4.3 $\pm$ 0.11 ^{defg}	87.0 $\pm$ 2.2 ^d	3.0 $\pm$ 0.24 ^a	3.18 $\pm$ 0.21 ^{cde}	1.07 $\pm$ 0.04 ^{bc}	0.38 $\pm$ 0.06 ^b	72 $\pm$ 3.4 ^{def}	43.8 $\pm$ 3 ^h
	14	4.0 $\pm$ 0.21 ^{bcde}	90.0 $\pm$ 3.4 ^e	2.9 $\pm$ 0.34 ^a	3.16 $\pm$ 0.17 ^{bcd}	1.06 $\pm$ 0.06 ^{bc}	0.38 $\pm$ 0.02 ^b	71.7 $\pm$ 0.8 ^{de}	43.2 $\pm$ 0.9 ^g
	21	3.8 $\pm$ 0.14 ^{abcd}	93.0 $\pm$ 2.4 ^f	2.9 $\pm$ 0.17 ^a	3.13 $\pm$ 0.16 ^{bcd}	1.05 $\pm$ 0.09 ^b	0.37 $\pm$ 0.03 ^b	71.2 $\pm$ 4.1 ^{cd}	39.2 $\pm$ 1.5 ^e
	28	3.6 $\pm$ 0.32 ^{abc}	97.0 $\pm$ 2.8 ^g	2.8 $\pm$ 0.14 ^a	3.1 $\pm$ 0.2 ^{bc}	1.04 $\pm$ 0.1 ^b	0.37 $\pm$ 0.03 ^b	70.5 $\pm$ 2.2 ^{bc}	36.4 $\pm$ 1.2 ^d
4% GPP	0	4.5 $\pm$ 0.25 ^{efg}	87.0 $\pm$ 1.6 ^d	2.9 $\pm$ 0.15 ^a	3.1 $\pm$ 0.2 ^{bc}	1.2 $\pm$ 0.12 ^c	0.75 $\pm$ 0.04 ^c	73.8 $\pm$ 1.4 ^h	31.5 $\pm$ 0.8 ^c
	7	4.1 $\pm$ 0.30 ^{cdef}	92.0 $\pm$ 1.9 ^f	2.9 $\pm$ 0.09 ^a	3.1 $\pm$ 0.1 ^{bc}	1.2 $\pm$ 0.1 ^c	0.75 $\pm$ 0.02 ^c	73.3 $\pm$ 1.3 ^{gh}	31.3 $\pm$ 0.7 ^{bc}
	14	3.8 $\pm$ 0.42 ^{abcd}	96.0 $\pm$ 3.6 ^g	2.8 $\pm$ 0.08 ^a	3.0 $\pm$ 0.1 ^{ab}	1.17 $\pm$ 0.07 ^{de}	0.75 $\pm$ 0.01 ^c	73 $\pm$ 1.7 ^{fh}	31.1 $\pm$ 0.6 ^{ab}
	21	3.5 $\pm$ 0.33 ^{ab}	101.2 $\pm$ 3.8 ^h	2.8 $\pm$ 0.1 ^a	3.0 $\pm$ 0.13 ^{ab}	1.15 $\pm$ 0.05 ^{cde}	0.74 $\pm$ 0.04 ^c	72.6 $\pm$ 1.4 ^{efg}	30.9 $\pm$ 0.5 ^b
	28	3.4 $\pm$ 0.18 ^a	105.4 $\pm$ 2.5 ⁱ	2.7 $\pm$ 0.1 ^a	2.9 $\pm$ 0.08 ^a	1.13 $\pm$ 0.03 ^{bcd}	0.74 $\pm$ 0.01 ^c	72.1 $\pm$ 2.2 ^{def}	30.2 $\pm$ 0.8 ^a

Values are given as mean $\pm$ SD.

Different letters in the same column indicate significant differences ($p < 0.05$).

Fig 1. Variation in the viscosity of different probiotic yoghurt formulations during storage period. Values are given as mean $\pm$ SD. Different letters indicate significant differences ($P < 0.05$).

Table 2 Total phenol and flavonoid contents and Antioxidant activity of different probiotic yoghurt formulations during storage period (mean $\pm$ SD)

Treatment	Day	Total phenolic ($\mu\text{g GAE/g}$)	Total flavonoid (mg Rutin equivalents/g)	Antioxidant activity (%)
control	0	9.21 $\pm$ 0.70 ^{ab}	1.0 $\pm$ 0.03 ^c	21.40 $\pm$ 1.30 ^e
	7	9.30 $\pm$ 0.40 ^c	0.90 $\pm$ 0.04 ^{bc}	18.10 $\pm$ 1.10 ^d
	14	9.12 $\pm$ 0.30 ^a	0.80 $\pm$ 0.05 ^b	16.70 $\pm$ 1.00 ^c
	21	9.18 $\pm$ 0.28 ^{ab}	0.60 $\pm$ 0.02 ^a	14.30 $\pm$ 0.80 ^b
	28	9.16 $\pm$ 0.32 ^{ab}	0.50 $\pm$ 0.01 ^a	12.20 $\pm$ 0.60 ^a
2% GPP	0	12.82 $\pm$ 0.21 ^d	2.44 $\pm$ 0.10 ^g	28.50 $\pm$ 2.10 ⁱ
	7	13.10 $\pm$ 20.22 ^e	2.10 $\pm$ 0.10 ^f	26.10 $\pm$ 2.00 ^g
	14	12.76 $\pm$ 0.41 ^d	1.80 $\pm$ 0.09 ^c	23.40 $\pm$ 1.40 ^f
	21	12.70 $\pm$ 0.10 ^{cd}	1.70 $\pm$ 0.06 ^c	21.00 $\pm$ 1.00 ^e
	28	12.60 $\pm$ 0.18 ^c	1.50 $\pm$ 0.12 ^d	18.30 $\pm$ 0.50 ^d
4% GPP	0	16.40 $\pm$ 0.25 ^f	4.90 $\pm$ 0.22 ⁱ	42.40 $\pm$ 1.20 ^j
	7	16.70 $\pm$ 0.30 ^g	4.60 $\pm$ 0.25 ^k	37.60 $\pm$ 2.30 ^k
	14	16.60 $\pm$ 0.27 ^g	4.20 $\pm$ 0.27 ^j	33.20 $\pm$ 2.10 ^j
	21	16.40 $\pm$ 0.30 ^f	3.60 $\pm$ 0.30 ⁱ	27.50 $\pm$ 1.40 ^h
	28	16.30 $\pm$ 0.27 ^f	3.20 $\pm$ 0.26 ^h	23.10 $\pm$ 1.00 ^f

Values are given as mean $\pm$ SD.

Different letters in the same column indicate significant differences ($P < 0.05$).

the syneresis was decreased. The differences between protein of treatments were not significantly difference ($p > 0.05$). Ash, fiber and energy decreased during storage, but the treatments with higher GPP had higher ash, fiber and energy contents. The yoghurt supplemented with 4% GPP in 0 day had the highest ash (1.2%), fiber (0.75%) and energy (73.8 kcal/g) (Table 1). This result is due to low fat and protein, and high ash, fiber and energy content of grape pomace. Similarly, in research carried out by Karnopp et al. (2017), grape skin flour (GSF) increased the ash and total fiber contents of yoghurts that were in parallel with our findings. Conversely, Da Silva et al. (2017) reported that pH, titratable acidity, ash, fat and moisture content of probiotic

yoghurt supplemented with 1.5 and 3.0 g L⁻¹ of grape extract that stored at 4 °C were not significantly different ($P < 0.05$).

Syneresis is the leakage of liquid from yoghurt. Syneresis is one of the key quality parameters for yoghurt. Higher level of syneresis revealed that yoghurt is of low quality (Lee and Lucey 2010). The syneresis of yoghurts were affected significantly ($P < 0.05$) by both grape pomace concentration and storage time and the changes are presented in Table 1. Syneresis values of different types of probiotic yoghurt varied from 30.2 to 50.0%. This result was matched with (Tarakci and Kucukoner 2003). In the first day, the highest mean value (50%) of syneresis was related to control and the lowest mean value (31.5 %) in sample containing 4% GPP. The addition of fruit pomace powder caused

a decrease of syneresis in all samples of yoghurts and the differences between the control and these samples were statistically significant ($P < 0.05$). It could be related to the capacity to absorb water by solids (fiber, carbohydrate and protein) that present in pomace powder which leads to a decrease of syneresis (Mahmood et al. 2008). But also controversial results also reported. For example, Da Silva et al. (2017) noted that syneresis increased with high concentration of grape extract. While Dos Santos et al. (2017) observed no differences for syneresis in yoghurt samples fortified with Pinot Noir grape juice and concentrated grape skin extract.

Since grape pulp contains a wide variety of phenolic compounds (Chung et al. 1998), its regular consumption reduces the risk of diseases such as cancer and cardiovascular diseases (Jang et al. 2010). Total phenolic, total flavonoid contents and antioxidant activity of probiotic yoghurt samples are shown in Table 2. There were significant differences in the total phenolic, total flavonoid contents and antioxidant activity of the samples ($P < 0.05$). All these factors were decreased throughout storage period. But also these factors for supplemented yoghurt samples were significantly ($p < 0.05$) higher than control samples. In a study, yoghurts supplemented with red grape and callus extracts displayed high phenolic and anthocyanin content and thus exhibited higher antioxidant power compared to yoghurts containing chardonnay extracts and control samples. The storage time significantly affected the free radical scavenging capacity of the yoghurts. The yoghurts supplied with grape callus extract displayed the greatest antioxidant power on the first day of storage compared to all the assayed samples (Karaaslan et al. 2011).

In our study, the highest total flavonoid (4.9 mg Rutin equivalents/g) and antioxidant activity (42.4%) recorded for yoghurt enriched with 4% GPP at 0 day that had significant differences with other treatments ($p < 0.05$). But the higher total phenolic (16.7 μg GAE/g) content was obtained for yoghurt enriched with 4% GPP at 7 day. The higher values of these factors were for enriched probiotic yoghurts with grape pomace powder compared to control, it may be due to the high concentrations of antioxidant compounds of grape pomace (Leong and Shui 2002) have observed the highest activity using DPPH. Similar studies describe that the antioxidant activity of yoghurts was enhanced by the presence of natural extracts, for example, in studies with yoghurts fortified with white and red dragon fruit (Zainoldin and Baba 2009), grape seed (Chouchouli et al. 2013) or with wild blackberry (Martins et al. 2014) extracts. Generally, the development of dairy foods containing polyphenols and fibers from grape pomaces are a technological trend that can improve the nutritional and functional value of foods (Dos Santos et al. 2017, Lachman et al. 2013).

The viscosities of liquid and semisolid foods have a large impact on their quality properties (Karaman et al. 2014). Figure 1 is

presented the viscosity of yoghurt samples during storage time. It was obvious that the apparent viscosity of all fruit yoghurts increase in proportion to increase percentage of pomace powder addition and also storage time. The lowest and highest viscosity recorded for control sample at 0 day (714 Pa.s), and yoghurt supplemented with 4% GPP at 28 day storage (1644 Pa.s), respectively (Figure 1). In a study, purple grape juice (PGJ) increased the viscosity of yoghurt, but the combinations 50% grape skin flour (GSF) and 50% oligofructose (OLI) and 66.7% GSF, 16.7% OLI and 16.7% PGJ presented the lowest mean values for viscosity (Karnopp et al. 2017). A similar trend was found by Öztürk and Öner (1999) for yoghurt containing concentrated grape juice. The fibers in Grape acted as stabilizer, forming colloidal solution (three dimensional network) that prevented water mobility, and so yielding high values of viscosity (Cruz et al. 2013).

Effect of incorporation of grape pomace powder on microbial counts of probiotic yoghurt

A sufficient number of viable microorganisms must be present throughout the entire shelf life of the product in order to produce therapeutic benefits. Although there is no world-wide agreement on the minimum of viable probiotic cells per gram or milliliter of probiotic product until the time of consumption, generally, the values of 10^6 and 10^7 - 10^8 cfu/mL or cfu/g have been accepted as the minimum and satisfactory levels, respectively (Korbekandi et al. 2011, Tamime et al. 2005, Ahmadi et al. 2012, Mortazavian et al. 2008). Usually due to high acid content, presence of bacteriocins, and fermentation conditions, the survival ability of probiotic bacteria in yoghurt decreased before consumption (Ferdousi et al. 2013). Therefore, utilization of prebiotic compounds is a common method in order to increase the survival ability of these bacteria at the consumption time (Pharmaceutiques 1995). Prebiotic activity of grape previously reported (Agte et al. 2010). Viability (log cfu/mL) of probiotic microorganisms in different treatments during refrigerated storage (4°C) for 28 days is presented in Figure 2. The probiotic microorganisms (log cfu/mL) in different treatments during refrigerated storage (4°C) decreased by time, but also this decrease was lower in samples contain GPP. Although counts of probiotic bacteria decreased gradually during storage time, yoghurt samples held probiotic values of 10^6 log cfu/mL until day 28. Vinderola and Reinheimer (1999) also stated that probiotic microflora counts decrease during storage. The rate of this loss in cell viability depended on the yoghurt type and the use of lactic starter. In current study the highest viable counts at the end of storage period (28 day) were obtained for probiotic yoghurt contain 4% GPP (8.03 log cfu/mL) and the lowest for control (6.2 log cfu/mL) (Figure 2). In a research carried out by Da Silva et al. (2017), bacterial strains were found in at least 10^9 cfu/g of yoghurt showing the possibility of probiotic yoghurt production with grape extract that overlapped with current study as yoghurt containing 4% GPP had 8.03 log cfu/mL at the end of storage time (Figure 2). Also Ehsani et al. (2015)

Fig 2. Viability (log cfu/mL) of probiotic microorganisms in different yoghurt samples during refrigerated storage (4°C) for 28 days. Values are given as mean $\pm$ SD. Different letters in the same column indicate significant differences ($P < 0.05$).

Table 3 Sensory analysis of the probiotic yoghurt treatments using score methodology

Treatments	Day	Appearance	Taste	Consistency	flavor	Overall acceptability
control	0	5.0 $\pm$ 0.20 ^c	4.5 $\pm$ 0.20 ^e	4.7 $\pm$ 0.10 ^{gh}	4.7 $\pm$ 0.20 ^{edc}	4.7 $\pm$ 0.00 ^g
	7	4.8 $\pm$ 0.10 ^{bc}	4.3 $\pm$ 0.10 ^d	4.5 $\pm$ 0.20 ^{ef}	4.5 $\pm$ 0.10 ^{bcd}	4.4 $\pm$ 0.00 ^f
	14	4.5 $\pm$ 0.12 ^{abc}	4.0 $\pm$ 0.08 ^c	4.2 $\pm$ 0.08 ^{bc}	4.2 $\pm$ 0.20 ^{abc}	4.2 $\pm$ 0.10 ^{de}
	21	4.3 $\pm$ 0.22 ^{abc}	3.8 $\pm$ 0.06 ^b	4.0 $\pm$ 0.06 ^a	4.0 $\pm$ 0.00 ^{ab}	4.0 $\pm$ 0.10 ^{bc}
	28	4.2 $\pm$ 0.14 ^{ab}	3.7 $\pm$ 0.06 ^{ab}	4.0 $\pm$ 0.05 ^a	3.8 $\pm$ 0.08 ^a	3.8 $\pm$ 0.20 ^a
2% GPP	0	5.0 $\pm$ 0.20 ^c	4.8 $\pm$ 0.40 ^f	4.9 $\pm$ 0.17 ⁱ	5.0 $\pm$ 0.07 ^c	4.9 $\pm$ 0.10 ^h
	7	4.7 $\pm$ 0.30 ^{bc}	4.5 $\pm$ 0.30 ^e	4.8 $\pm$ 0.14 ^{hi}	4.9 $\pm$ 0.20 ^c	4.7 $\pm$ 0.30 ^g
	14	4.6 $\pm$ 0.24 ^{bc}	4.2 $\pm$ 0.10 ^d	4.6 $\pm$ 0.15 ^{fg}	4.7 $\pm$ 0.10 ^{edc}	4.4 $\pm$ 0.40 ^f
	21	4.5 $\pm$ 0.26 ^{abc}	4.0 $\pm$ 0.00 ^c	4.4 $\pm$ 0.27 ^{de}	4.3 $\pm$ 0.30 ^{abcd}	4.3 $\pm$ 0.00 ^{ef}
	28	4.3 $\pm$ 0.27 ^{abc}	3.8 $\pm$ 0.08 ^b	4.3 $\pm$ 0.18 ^{cd}	4.0 $\pm$ 0.20 ^{ab}	4.2 $\pm$ 0.10 ^{de}
4% GPP	0	4.7 $\pm$ 0.28 ^{bc}	4.6 $\pm$ 0.12 ^{ef}	4.8 $\pm$ 0.16 ^{hi}	4.8 $\pm$ 0.20 ^{de}	4.7 $\pm$ 0.10 ^g
	7	4.5 $\pm$ 0.21 ^{abc}	4.3 $\pm$ 0.14 ^d	4.6 $\pm$ 0.31 ^{fg}	4.7 $\pm$ 0.20 ^{edc}	4.6 $\pm$ 0.30 ^g
	14	4.3 $\pm$ 0.18 ^{abc}	4.0 $\pm$ 0.09 ^c	4.5 $\pm$ 0.24 ^{ef}	4.5 $\pm$ 0.10 ^{bcd}	4.4 $\pm$ 0.10 ^f
	21	4.1 $\pm$ 0.19 ^{ab}	3.8 $\pm$ 0.10 ^{bc}	4.3 $\pm$ 0.20 ^{cd}	4.2 $\pm$ 0.10 ^{abc}	4.1 $\pm$ 0.20 ^{cd}
	28	3.8 $\pm$ 0.18 ^a	3.6 $\pm$ 0.10 ^a	4.1 $\pm$ 0.11 ^{ab}	3.9 $\pm$ 0.30 ^a	3.9 $\pm$ 0.20 ^{ab}

Values are given as mean $\pm$ SD.

Different letters in the same column indicate significant differences ($P < 0.05$).

noted that the viability of both probiotic bacteria were significantly greater in the treatments containing artichoke extract compared to control yoghurts during storage period of 28 days. Similarly, Mocanu et al. (2010) observed that highest number of probiotic bacteria at the end of refrigerate storage, belongs to yoghurt containing mixture of bilberry and liquorice extract compared to control yoghurt.

The antioxidant compounds in grapes can affect the survival of probiotics in yoghurt. But in some studies, the authors did not find an inhibitory effect (Hervet-Hernandez et al. 2009, Chouchouli et al. 2013). It is possible that phenolic compounds are transformed into more active derivatives (e.g. aglycones) under certain conditions, which might enhance starter culture activity (Sun-Waterhouse et al. 2013). In our study, after 28 days

of storage, in all cases (even control treatment) the viable numbers of probiotics remained in values higher than 10^6 cfu/mL that is necessary to confer the probiotic character of the products.

Effect of incorporation of grape pomace powder on sensory properties of probiotic yoghurt

Fruit mixes improve the nutritional value and the taste of yoghurt, and fruit enhancement plays a considerable role in yoghurt consumption and sales (Kailasapathy et al. 2008). Sensory properties of yoghurt samples prepared in this study are shown in Table 3. Sensory scores decreased during storage period. This could be attributed to the development of acidity. Similar results reported by Cakmakci et al. (2012) about probiotic banana

yoghurts. Regarding appearance attribute there was not significant differences between samples (Table 3).

The lowest appearance (3.8) and taste (3.6) scores were obtained for sample containing 4% GPP at 28 day storage, and the lowest consistency (4.0), flavor (3.8) and overall acceptability (3.8) scores were obtained for control sample at the end of storage period. While the highest sensory scores belonged to yoghurt supplemented with 2% GPP at 0 day (5.0, 4.8, 4.9, 5.0 and 4.9 respectively). In our study, the addition of grape pomace has no negative effect on the color of yoghurt samples. In parallel with our results, Dos Santos et al. (2017) observed the higher sensory scores for flavor, color, and overall acceptability about fermented goat milk added with grape pomace extract. In the study done by Chouchouli et al. (2013) the fortification of full-fat and non-fat yoghurts with grape seed extracts did not cause major defects in consistency, color and flavor compared to controls. Also in a research carried out by Da Silva et al. (2017), sensory attributes (color, flavor, taste, texture and appearance) of probiotic yoghurt supplemented with grape extract were acceptable by panelists. These findings proved that fruity yoghurt contains both the refreshing flavor and taste of fruit and beneficial effect of yoghurt (Mahmood et al. 2008). In current study, storage period had slightly effect on all attributes. According to this result grape pomace yoghurt at 2% has the most acceptability in point view of consumer (Table 3). Since grape pomace can be used in the production of probiotic yoghurt.

Conclusions

Grape pomace is a nutritious, but underused, by-product of winemaking containing fiber and antioxidants. Using a suitable production design, a new probiotic fortified yoghurt formulation with grape by-product could be optimized to enhance consumers' daily intake of antioxidants. The use of grape pomace powder in the development of value added food products will be a step toward making new functional foods, and partially solving waste management problem from wine production. As consumption of fruits, prebiotics and yoghurt in combination has a potential to provide extra nutritional-physiological value that involve in synergetic effect on health. The results of this study would provide an opportunity of dairy producer to develop a novel product in agreement with consumers' preferences. This research represents a new approach in the development of novel probiotic yoghurt with high nutritional quality for human and with great potential applications on food industry.

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