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Total polyphenol content and radical scavenging activity of functional yogurt enriched with dates

*Leila Arfaoui**

Clinical Nutrition Department, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia

**Corresponding author: leila.arfaoui@gmail.com*

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Abstract: Both yogurt and dates are functional foods known for their valuable nutrients and health benefits. Therefore, this study was conducted to produce two types of date-enriched yogurts (20% wt/wt) to enhance their nutritive value and health benefits. While yogurt A was made with dates blended with milk, yogurt B was produced using small pieces of dates that were added to milk before fermentation. Both date-enriched yogurts were assessed for their physicochemical composition, total polyphenol content (TPC) and radical scavenging activity (RSA). The results showed that the addition of dates significantly enhanced carbohydrate, mineral and total solids contents of yogurts A and B ($P < 0.05$). Furthermore, dates significantly enhanced the TPC (34 and 37 mg GAE 100 g^{-1} for yogurt A and B, respectively) and the RSA (51% for yogurt A versus 57% for yogurt B) of date-enriched yogurts especially when dates were added as small pieces. During cold storage, both TPC and RSA decreased in all yogurt samples; however, they were maintained higher in date-enriched yogurts.

Keywords: phenolic compounds; antioxidant activity; functional food

Chronic diseases are among the foremost healthcare problems that affect an increasing number of people in the world (Global Status Report on Noncommunicable Diseases, WHO, 2014). Among the risk factors associated with the expansion of these non-communicable diseases (NCDs), lifestyle including poor diet and lack of physical activity was shown to be the main factor leading to these diseases (Duncan et al. 2014). That is why diet-oriented prevention strategies including new functional foods and nutraceuticals were suggested to have protective effects against several chronic diseases. Functional foods are fortified, enriched or enhanced foods that provide health benefits when they are consumed at efficacious levels as part of a varied diet on a regular basis (Tsao 2010).

Among functional foods, dairy products and particularly yogurt are thought to be an essential food with tremendous nutritional and health benefits for both children and adults. In fact, yogurt contains numerous bioactive nutrients such as calcium (which is better absorbed from yogurt than milk), high quality proteins and peptides, phosphorus, B vitamins and many others beneficial substances (Pei et al. 2017). Besides its conventional bacteria (*Streptococcus thermophilus* and *Lactobacillus delbruekii* subsp. *bulgaricus*), yogurt may also contain other additional lactic acid bacteria (e.g. *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Lactobacillus rhamnosus*) and bifidobacteria (e.g. *Bifidobacterium bifidum* and *Bifidobacterium lactis*) known as probiotics which can promote the gastrointestinal tract environment (Pei et al. 2017). As yogurt consumption and production have been increasing significantly worldwide, new recipes have been developed using many types of fruits and flavours. The addition of fruits to yogurt makes it tastier, improves its flavour but also enhances its health benefits due to their natural contents of nutraceuticals especially polyphenols. Their incorporation into foods or other bioprocessed products allows them to acquire this beneficial property of high polyphenol content as well (Pandey & Rizvi 2009).

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As the demand for healthier food products with health-promoting effects has been increasing significantly worldwide, many studies have been undertaken to develop yogurts enriched with bioactive compounds such as polyphenols. The addition of fruits rich in phenolic compounds such as pomegranate, sweet cherries and grape to yogurt was shown to enhance its antioxidant activity and its polyphenol content (Trigueros et al. 2014; Sánchez-Bravo et al. 2018). However, and to the best of our knowledge, palm dates have not been studied as ingredient in yogurts. In fact, dates are a high source of energy that contain various nutrients such as dietary fibres, minerals and vitamins (Al-Shahib & Marshall 2003). Moreover, dates are a good source of polyphenols and antioxidants compounds (Yasin et al. 2015).

In this context this study was conducted to develop functional yogurts by combining the two health benefits of dates and yogurt. Two types of yogurts were produced when dates were added under two forms. While yogurt A was made with dates blended with milk, yogurt B was produced using small pieces of dates that were added to milk before fermentation. The date-enriched yogurts were assessed for their chemical composition, antioxidant activity as well as the total polyphenol content.

MATERIAL AND METHODS

Milk, starter and dates

Yogurt was produced from pasteurized low-fat fresh milk (3.2% protein, max 1.1% fat and min 8.5% nonfat solids) fortified using low-fat milk powder (26.5% protein, 16.2% fat and 46% carbohydrates). Low-fat plain yogurt (4.6% protein, max 1.1% fat and min 8.5% nonfat solids) was used as starter to inoculate milk. Sukkari ruttab dates at the final ripening stage (82% total solids, 2% protein, 0.5% fat and 2.5% ash) were added to yogurt in this study. All materials used in this study were purchased from local supermarkets.

Chemicals

Gallic acid and methanol were purchased from Sigma-Aldrich (Steinem, Germany), while sodium carbonate, Folin-Ciocalteu and DPPH (2,2-diphenyl-1-picrylhydrazyl) were purchased from Lobal chemie (New Delhi, India).

Manufacturing of low-fat set yogurt enriched with dates

Control yogurt as well as two types of date-enriched set yogurts were produced according to the diagram shown in Figure 1. Dates were washed with water and

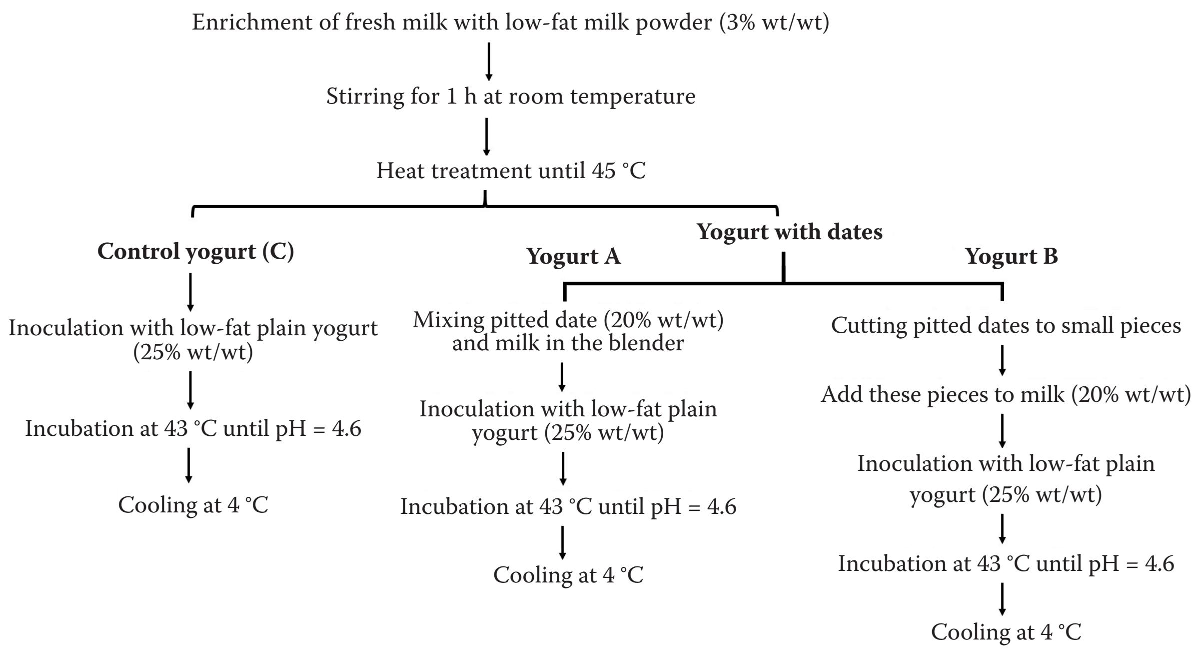

Figure 1. Steps involved in yogurt manufacture

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their seeds were removed. Low-fat milk powder was added (3% wt/wt) to low-fat fresh milk and the mixture was then stirred for 1 h at room temperature before usage. After that, the milk mixture was heated in a thermostatic water bath until 45 °C. Then, two types of yogurts were prepared. In the first type, dates were added to milk at the proportion of 20% (wt/wt), and blended with milk until fully homogenized (yogurt A). In the second type, dates were cut into small pieces (coffee bean size) and added to milk (20% wt/wt) without blending (yogurt B). After adding dates, milk was inoculated with low-fat plain yogurt at the proportion of 25% (wt/wt) and incubated at 43 °C until pH 4.6 ± 0.05. During fermentation, pH was continuously recorded using a pH meter (HI 2212; Hanna Instruments Inc., Woonsocket, USA). At the end of fermentation, yogurts were removed from the incubator and stored at 4 °C.

Chemical composition of yogurt

Dry matter, crude fat, protein and total minerals were determined as described by the official methods from AOAC (Official Method of Analysis, 2007) using oven drying, Soxhlet, Kjeldahl and muffle furnace methods, respectively. Carbohydrate content was determined by calculating the percentage remaining after all the other components were measured. The acidity was determined by titration using NaOH 0.1N and expressed as percentage of lactic acid as described by Nguyen & Hwang (2016).

Antioxidant activity determination

Total polyphenol content (TPC). Total polyphenols were extracted from dates and date-enriched yogurts using methanol-water (50%/50%) as described by Saleh et al. (2011) with some modifications. Briefly, 10 g of the sample was mixed with 90 mL of methanol-water (50%/50%) and left under gentle agitation at room temperature for 2 h. Then the mixture was filtered using Watman filter paper No. 1 (Watman, Maidstone, Great Britain) and centrifuged at 5 300 g for 30 min. The supernatant was collected and the TPC was determined using Folin-Ciocalteu according to El-Din et al. (2017) and expressed as mg gallic acid equivalent (GAE) per 100 g of sample. The absorbance was measured at 765 nm using a spectrophotometer (Genesys 20; Thermo Spectronic, Rochester, USA). A calibration curve was determined using gallic acid as a reference material for the determination of polyphenols.

Radical scavenging activity. The supernatant collected as described in methodology part was used for DPPH radical scavenging activity determination following the method described by Brand-Williams et al. (1995). Briefly, 0.1 mL of the extract was mixed with 2.9 mL DPPH solution (0.1 mM in methanol). The mix was vortexed for 1 min, left in dark at room temperature for 1 h, then the absorbance was read at 517 nm wavelength using a spectrophotometer. The blank solution was prepared without the extract. Methanol was used to zero the spectrophotometer. The percentage of inhibition was determined using the following equation:

$$\text{Inhibition} = \frac{Ac - As}{Ac} \times 100\,(\%) \qquad (1)$$

where: Ac – absorbance of the control; As – absorbance of the sample.

Statistical analysis

Data analysis was performed by ANOVA (GraphPad prism 8, GraphPad software) using a completely randomized design to compare the three types of yogurt (the control and two types of date-enriched yogurts) using the following variables: pH, acidity, total minerals, protein, fat, carbohydrates, TPC and RSA. Tukey's test was thereafter used to compare the treatments' means. Differences were considered as statistically significant at $P \leq 0.05$. All experiments were done in triplicate.

RESULTS AND DISCUSSION

Chemical composition, pH and titratable acidity

The measured acidity was around 1.03% of lactic acid for all yogurt types. Both yogurt B and control yogurt reached the target pH of 4.6 ± 0.5 after 4 h 30 min ± 15 min. However, yogurt A reached a pH of 4.6 ± 0.5 only after 3 h 30 min ± 15 min. These differences in incubation time may be explained by the step of blending in yogurt A which allowed better exposure of the date polyphenols to caseins, increasing therefore polyphenol-casein interactions and bonding compared to yogurt B when dates were added as pieces. These interactions may accelerate casein gelification as suggested in previous studies (Jobstl et al. 2006, Yuksel et al. 2010).

Results showed higher total solids content in both date-enriched yogurts (12.8% versus 24.8%) compared to the control (C). Additionally, the addition of dates to both yogurts A and B significantly increased the total minerals compared to the control (1.1% and 0.8%, respectively). Such findings are expected since palm dates

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are a rich source of several minerals mainly potassium, magnesium, copper and others (Al-Farsi & Lee 2008).

The protein content was around 4% for yogurts A and B compared to the control (4.4%) and the fat concentration was about 1.4%, 1.38% and 1.6% for yogurts A, B and C, respectively. Both proteins and fat contents decreased slightly but not significantly ($P < 0.05$) after the addition of dates since dates are poor sources of proteins and mainly fat (Al-Farsi & Lee 2008).

Antioxidant Activity

Total Polyphenol Content (TPC). The TPC levels in the control yogurt were 8.5 mg GAE $100g^{-1}$. These phenolic compounds (PCs) are naturally present in cow's milk and may derive from the feed and/or the products of amino acid catabolism as reported elsewhere (Lopez & Lindsay 1993). The TPC in dates was around 135 mg GAE $100g^{-1}$, which is in line with previous studies where dates were shown to be a good source of polyphenols (Yasin et al. 2015). Their addition to yogurt significantly enhanced its polyphenol content ($P < 0.05$) to 34 and 37 mg GAE $100g^{-1}$ for yogurt A and yogurt B, respectively, compared to the control (8.5 mg GAE $100g^{-1}$) (Figure 2).

Interestingly, data showed that yogurt made with small pieces of dates (B) exhibited a significantly higher TPC than yogurt made with date-blended milk (A) ($P < 0.05$). This could be explained by the blending process that enhanced the interaction of date polyphenols with caseins compared to those in dates present as small pieces. These phenolic compounds (PCs) can therefore easily diffuse inside the casein-gel network. In fact, previous studies reported that phenolic compounds have a higher affinity to milk proteins, mainly caseins, near their isoelectric point (pH = 4.6) (Trigueros et al. 2014; Yildirim-Elikoglu & Erdem 2018). Since caseins are proline-rich proteins, they have a strong affinity to the hydroxyl (–OH) group of PCs (Yuksel et al. 2010). These polyphenol-casein interactions occur via covalent and non-covalent bonding which may trap polyphenols in the yogurt polymer network and gel matrix making their extraction and quantification harder (Trigueros et al. 2014; Yildirim-Elikoglu & Erdem 2018). Moreover, these casein-polyphenol interactions were reported to accelerate casein aggregation and precipitation (Trigueros et al. 2014; Yildirim-Elikoglu & Erdem 2018), which could speed up the gelification process in blended-dates yogurt A (pH = 4.6; 3 h 30 min) compared to both B and C yogurts (pH = 4.6; 4 h 30 min).

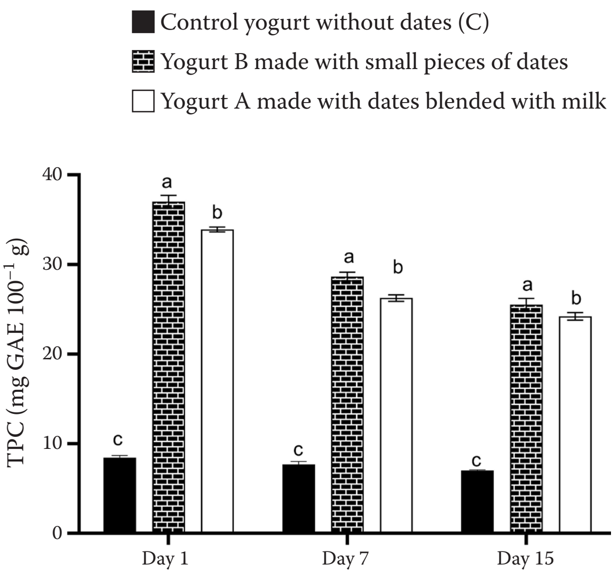

Figure 2. TPC of different yogurt samples during cold storage

Results sharing a common lowercase letter did not differ significantly between trials for the same sampling day ($P > 0.05$). Time effect was significant ($P < 0.05$)

The TPC decreased proportionally during cold storage for all yogurt samples (Figure 2). These results are in line with other studies where TPC in yogurts enriched with high polyphenol content fruits was reported to decrease during storage (El-Din et al. 2017; Sánchez-Bravo et al. 2018). In the study carried out by Trigueros et al. (2014) in a pomegranate yogurt, they reported that 85% of the total anthocyanins were bound to proteins on the first day compared to 90% after 28 days of cold storage. These continuous and increasing PC-milk protein interactions during storage may explain the slight TPC decrease observed in this study.

DPPH radical scavenging activity (RSA). Results of DDPH showed that the RSA of dates was about 88%. The addition of dates significantly increased the RSA especially in yogurt B (57%), followed by yogurt A (51%) compared to the control yogurt C without dates (31%) ($P < 0.05$) (Figure 3). In fact and as discussed before, it seems that, when dates are blended with milk (yogurt A), the binding of polyphenols to milk proteins increased which may reduce their antioxidant activity compared to yogurt where dates were added as small pieces (yogurt B). The RSA results are also in agreement with several studies showing that protein-polyphenol interactions reduced the antioxidant activity of polyphenols (Trigueros et al. 2014; Yildirim-Elikoglu & Erdem 2018). For instance, Arts et al. (2002) showed that the antioxidant activity of tea flavonoids and milk pro-

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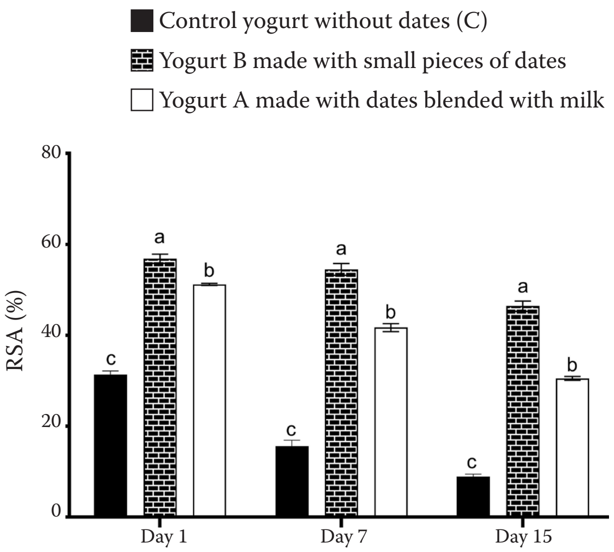

Figure 3. RSA of different yogurt samples during cold storage

Results sharing a common lowercase letter did not differ significantly between trials for the same sampling day ($P > 0.05$). Time effect was significant ($P < 0.05$)

teins separately was higher than the antioxidant activity of the tea-milk mixture. This study demonstrated that a part of the total antioxidant capacity is masked by the tea PC-milk protein interaction.

The RSA decreased during cold storage for all yogurt samples as shown in Figure 3. In fact, control yogurt C lost rapidly its RSA which decreased from 31 (Day 1) to 9% (Day 15 of cold storage) compared to both date-enriched yogurts. Additionally, among the two types of date-enriched yogurts, yogurt A exhibited a faster RSA decrease (from 51% to 31%) compared to yogurt B (from 57% to 46%) after 15 days (Day 15) of storage (Figure 3). It seems that polyphenol-milk protein interactions continue during cold storage especially when dates were blended with milk, which decreased faster their antioxidant activity.

CONCLUSION

Two types of date-enriched yogurts were successfully produced in this study and the showed a similar nutritive value. Among the nutrients analysed, both mineral and carbohydrate contents were significantly increased by the addition of dates. The TPC as well as the antioxidant activity of date-enriched yogurts were also highly improved (both on the first day as well as during 15 days of cold storage) especially when dates were added as pieces.

Additional studies are required to demystify the polyphenol functions and interactions with milk proteins in model systems and complex dairy matrices. The understanding of these interactions as well as the PC nutraceutical and functional roles will pave the path towards the optimization of both polyphenol bioavailability and health benefits.

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