

# Quality mentoring of Greek yoghurt fortified with pomegranate juice and arils (*Punica granatum L.*) during storage

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**Abstract** – Greek yoghurt is a highly nutritious fermented product characterized by its higher protein, low sodium and low carbohydrate contents, and has gained immense popularity compared to traditional yoghurt. It has a unique semi-solid texture due to manufacturing process. In this study, Greek yoghurt was fortified with pomegranate juice (PJ) and arils (PA) to investigate its thick-matrix effect on the availability of phenolics and dietary antioxidant during 18 days of storage at 4°C and to get indications about the best treatment that could sustain antioxidants during the storage period. PJ or PA in 20 % were mixed with cultured and dessert Greek yoghurts (CPJ, CPA, DPJ, DPA respectively). The total phenolics (TPC), using Folin-Ciocalteu, and antioxidant activity, using DPPH, FRAP, and luminal-induced chemiluminescence, were tested over four periods of storage (1; 6; 12; 18 days). Organoleptic quality was evaluated as well.

The obtained results indicated that both PJ and PA enhance the TPC and antioxidant activity of both yoghurts, and the cultured yoghurt appeared to be a better matrix to preserve pomegranate's bioactive molecules.

**Keywords:** Greek yoghurt; pomegranate; TPC; antioxidant activities; sensorial quality

## 1. Introduction

Pomegranate is a nutritional and healthy fruit that is consumed frequently. It is a desirable choice for patients suffering from high cholesterol levels as well as heart and kidney problems. It also has medicinal values which prevent intestinal disorder and diarrhea. It is known for its high phenolic contents, such as anthocyanins, phytoestrogenic, flavonoids, and tannins (Mousavinejad et al. 2009). In addition to that, pomegranate is an important source of other nutrients including dietary fibre, proteins, oligosaccharides, vitamins, and trace minerals. For this reason, pomegranate extracts are used as bioactive ingredients in several foods products (yoghurt, beef sausage, ice cream) (Sun-Waterhouse 2011; Robert et al. 2010; Çam et al. 2013; El-Nashi et al. 2015). Moreover, level of phenolic compounds is highly affected by harvesting time and ripening degree of fruit (Kelebek et al. 2015; Kelebek et al. 2016).

The diverse range of fruit-based functional food products available in the worldwide markets reflects the confidential using of such products as a delivery method for nutritional active compounds, e.g. flavonols, catechins, anthocyanidins, and isoflavones (Sun-Waterhouse 2011; De Carvalho Alves et al. 2013; Karimi et al. 2016). On the other hand, phenolic antioxidants in some cases tend to bind with the components of food matrix; hence phenolics wouldn't be available to exert its wanted beneficial effect as antiradical, e.g. protein binding to catechins can affect its scavenging ability by lowering the number of free hydroxyl groups (Trigueros et al. 2014). This criterion is less able to affect the antiradical activity of food supported by anthocyanins, where most of the reports indicated that it is the only group that



might not be hindered by binding effect of dairy proteins (Trigueros et al. 2014; Karaaslan et al. 2011). Different yoghurt-based functional foods are commercially available under diverse brand names. These products are including probiotic yoghurt drinks and yoghurt rich in fruits polyphenols (Sun-Waterhouse 2011; De Carvalho Alves et al. 2013; Trigueros et al. 2014). In this context, several reports discussed the beneficial addition of pomegranate juice and encapsulated pomegranate extracts to yoghurt (Robert et al. 2010; Trigueros et al. 2014). According to these reports, pomegranate based-yoghurt might have many advantages over plain yoghurt. Thus, the use of pomegranate in yoghurt making would not only improve the product quality but would provide one of the outlets for the fruit during the peak production period when its market rates are sharply lowered (Donkor et al. 2007).

Greek yoghurt is known to be in the top of European strained yoghurt products. It is obtained by removing part of the water from the yoghurt or by adding dried hydrocolloids, e.g. dairy protein, to obtain its unique and desired semi-solid texture (Desai et al. 2013). Moreover, in the US, the production of Greek yoghurt, with about 25% of the total sales, is the largest growing sector among all dairy products (Desai et al. 2013). There are two types of Greek yoghurt available on the market; the cultured yoghurt, that retains viable bacteria after processing, and the dessert yoghurt, where the culture bacteria have died after preparation; however, to the best of our knowledge, the use of pomegranate juice and arils (*Punica granatum L.*) to produce functional Greek yoghurt has never been discussed. For this reason, 20% of pomegranate juices or arils were added to Greek cultured and dessert yoghurt and the respective extracts were explored for their phenolic content and antioxidant activity during 18 days of storage to investigate the effect of viable bacteria as well as the thick-texture of the yoghurt on the availability of phenolic content and the antiradical activity in the fortified samples, in addition, to evaluate the ability of arils to retain the antiradical effect in comparison with samples based on juice.

## **2. Materials and methodes**

### **2.1. Chemicals**

Ethyl Acetate, Folin–Ciocalteu reagent, sodium carbonate,  $\text{FeC}_{13} \cdot 6\text{H}_2\text{O}$ ,  $\text{COCl}_2 \cdot 6\text{H}_2\text{O}$ , hydrogen peroxide solution ( $\text{H}_2\text{O}_2$ ) 30%, metaphosphoric acid was obtained from Merck (Germany). Ethanol, methanol (MeOH), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH<sup>\*</sup>), gallic acid, ascorbic acid, luminol, EDTA, sodium metabisulphate anhydrous, and TPTZ (2,4, 6-tripyridyl-s-triazine) were obtained from Sigma-Aldrich (Germany). Acetone, sodium acetate and ammonium sulphate were purchased from Fluka. Petroleum ether, diethyl ether, n-hexane and hydrochloric acid (37%) were obtained from Riedel-de Haën (Germany).

### **2.2. Fruit samples preparation**

The pomegranate fruits were bought from a local market in Zerkine, Gabes (Tunisia), which is well known of pomegranate fruit production. After throwing of any injured and/or sunburnt fruits, pomegranate fruits were peeled and then any skins around the seeds were removed manually. The arils were separated from the fruits and the juice was extracted via a pilot plant packaged-type press (Tefal Rondo 800 Multifunction). The juice samples were centrifuged (10 min at 3000 rpm at 4 °C). Before the experiments, the pomegranate juice was centrifuged at 10000 rpm for 10 min to remove methanol-insoluble particles. Pomegranate arils were incorporated directly in yoghurt samples.

### **2.3. Fortification of samples with pomegranate juice or arils**

Fresh prepared dessert and cultured yoghurts were purchased from a local Greek market for the experiment. Proportions of 20% yoghurt-flavoured pomegranate juice or arils were prepared in triplicates, with final weight of 100 g. This chosen percentage was selected based on multiple preparation experiments (data not shown). The yoghurt samples were then put inside glass jars covered with plastic cover and conserved in the refrigerator at 4 °C, pomegranate juice were also conserved in plastic tube. Samples of 10 g of yoghurt and 10 ml of juice were then taken in the 1, 6, 12 and 18 day to be analysed.

### **2.4. Extraction procedure**

Extraction of total phenolics was according to Amiot et al. (1986). 10 g of yoghurt enriched with pomegranate arils or juice extract were homogenized with 80% MeOH and 2% metabisulphite for 3 min. After agitation for 20 min, the sample was centrifuged. The procedure was repeated a second time to the pellet and the supernatants were combined. An extraction step with petroleum ether was performed

to remove the fat residues (2×50 mL petroleum ether). After evaporating the solvent, the sample extracted solvent was ethyl acetate in the presence of ammonium sulphate (20%), metaphosphoric acid (2%) and methanol was added when emulsion was formed. The ethyl acetate phase, after drying with sodium sulphate, was evaporated until dryness and re-dissolved in 2 mL MeOH and kept in  $-20^{\circ}\text{C}$  due to analysis.

## 2.5. Determination of total phenols content

The total polyphenol content (TPC) of pomegranate juices and the different extracts was determined via the Folin-Ciocalteu method adapted to a microscale (Arnous et al. 2013; Waterman et Mole 1994). Results were expressed as gallic acid equivalents (mg GAE/L).

## 2.6. Determination of total antioxidant activity

### 2.6.1. DPPH radical scavenging capacity

The free radical scavenging capacity was determined by the use of stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH $^{\cdot}$ ) as described by Arnous et al. (2013). An aliquot of 25  $\mu\text{L}$  of sample was added to 975  $\mu\text{L}$  of DPPH solution (60  $\mu\text{M}$  in MeOH) and vortexed. The absorbance was read at  $t = 0$  and  $t = 30$  min. Ascorbic acid was used as standards and the results were expressed as ascorbic acid equivalent mM/L.

### 2.6.2. Determination of ferric-reducing antioxidant power (FRAP)

For the measurement of the reducing ability of pomegranate-flavoured yoghurt extracts, a protocol based on the ferric reducing antioxidant power (FRAP) assay was applied, as described by Benzie and Strain (1999). A freshly prepared working FRAP solution was prepared by mixing 50 mL of acetate buffer (0.3 M, pH 3.6), 5 mL of 10 mM TPTZ solution in 40 mM HCl, and 5 mL of 20 mM  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  solution. Pomegranate-flavoured yoghurt extracts (50  $\mu\text{L}$ ) were allowed to react with 1500  $\mu\text{L}$  of the FRAP solution and absorbance was measured at 593 nm ( $A_{(sample)t=0}$ ) at 0 min after vortexing. Thereafter, the samples were placed at  $37^{\circ}\text{C}$  in a water bath and absorption was measured again after 4 min ( $A_{(sample)t=4}$ ). Ascorbic acid was used as the calibration standard and results were expressed as ascorbic acid equivalent mM/L.

### 2.6.3. Determination of free hydroxyl radical scavenging activity

A chemiluminescence method was used as described by Parejo et al. (2000) and Mahmoud et al. (2013). In this method,  $\text{H}_2\text{O}_2$  was used as a radical source which reacts with Co (II) and generates hydroxyl radicals through a Fenton reaction. Then, luminol reacts with a generated hydroxyl radical to form 3-aminophthalate in an excited electronic state which returns to ground state by emission of light. EDTA, as a chelator, is used to decrease the speed of the Fenton reaction. The chemiluminescence intensity of this system reaches a plateau which drops in the presence of an antioxidant. Practically, 1 ml of borate buffer solution (50 mM, pH 9.00) containing  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  (8.4 mg  $\text{ml}^{-1}$ ) and EDTA (2.63 mg  $\text{ml}^{-1}$ ) was mixed in a test tube with 0.1 ml of luminol solution (0.56 mM in borate buffer 50 mM, pH 9.00), vortexed for 15 s and followed by the addition of 0.025 ml of  $\text{H}_2\text{O}_2$  aqueous solution (5.4 mM). After being vortexed for 30 s the mixture was rapidly transferred into a glass cuvette and the CL intensity (blank) was measured when it reached the plateau ( $I_0$ ). For the measurement of the samples an aliquot of 0.025 ml of sample (pure compound or mixtures) was added into the test tube containing the buffer, luminol and  $\text{H}_2\text{O}_2$  solutions as above and the CL intensity (I) was recorded at the plateau. The reactions in the glass cuvette were performed under magnetic stirring. The ratio  $I_0/I$  was calculated and plotted vs. concentration ( $\mu\text{mol l}^{-1}$ ) of the sample and correlations were established using linear, exponential and polynomial regression analysis. The concentration of sample ( $\text{IC}_{50}$ ), which is required to decrease  $I_0$  intensity by 50%, was also calculated. For all measurements, a fluorimeter (model 6200, Jenway Ltd., Gransmore, Essex, UK) was used, keeping the lamp off and using only the photomultiplier of the apparatus. Ascorbic acid was used as the calibration standard and results were expressed as ascorbic acid equivalent mM/L.

## 2.7. Sensorial Analysis

Ten trained panelists from the staff members of the, Mediterranean Agronomic Institute, Greece, used a quality rating scorecard for the evaluation of flavor (50 points) body and texture (40 points) and appearance (10 points). Samples were coded differently and presented randomly.

## 2.8. Statistical analysis

Data were presented as the mean  $\pm$  standard deviations ( $n=3$ ). Experimental data were analysed with the SPSS 20@programme, performing one-way analysis of variance (ANOVA), and the DUNCAN test was used (a  $p$ -value of 0.05 was considered significant).

## 3. Results and discussion

### 3.1. Total phenol content

Based on the present results of TPC (Table 1), pomegranate juice showed a high phenolic content. In fact, pomegranate juice (PJ) is well known as a highly rich source of anthocyanidins, e.g. cyanidin and pelargonidin, and other phenolics, e.g. gallic acid (Trigueros et al. 2014). On the other hand, the plain cultured and dessert yoghurts presented low phenolic content. These results are in agreement with the findings of El-Said et al. (2014) and Trigueros et al. (2014)

A significant decline in TPC in all samples was noticed during the 18 days of storage at 4° C. Such a phenomenon was also observed by Varela-Santos et al. (2012). In fact, the gel matrix of yoghurt might protect the phenolics during storage to some extent; however, the declination is thought to be triggered as a result of the degradation of phenolic compounds, mainly anthocyanins, which is influenced by the contact with oxygen (Pérez-Vicente et al. 2004; Trigueros et al. 2014; Mang et al. 2015).

Although, the TPC decreased during the 18 days of storage, a considerable amount was remained in the fortified yoghurt with PJ and PA. This could be attributed to anthocyanins, which are the most stable among all phenolics in yoghurt matrix (Trigueros et al. 2014).

The results showed that PJ appears to be more efficient in increasing the TPC. Furthermore, Palma et al. (2015) found that ready to eat pomegranate arils lose its TPC during 10 days of cold storage at 5°C under modified atmosphere packaging. These results are in consistent with the later finding. Indeed, commercial pomegranate usually has more antioxidant power as it contains peels and rind constituent. During the storage time, the cultured yoghurt samples exhibit higher total phenol content than the dessert yoghurt ones, including those fortified with PA and PJ. The findings are in line with those found by El-Said et al. (2014) and Sun-Waterhouse (2011) who reported that bacteria transform polyphenolic compounds in health promoting extract like berry or pomegranate peel extract into smaller unites that are more extractable or stable. As bacterial culture could affect the anthocyanin content during storage which is probably associated with the production of antimicrobial compounds by the starter culture (Trigueros et al. 2014), the convenient culture for the production of yoghurt should be selected in order to guarantee a better stability of anthocyanins (Ścibisz et al. 2012).

**Table 1.** Effect of storage time and different treatments on total phenol content of permanganate juice and Greek yoghurts

Sample	Total phenol content (gallic acid equivalent mg/L)			
	Storage time (day)			
	1	6	12	18
<b>Cultured yoghurt</b>	116.00 $\pm$ 0.78 <sup>aD</sup>	112.34 $\pm$ 1.54 <sup>aC</sup>	110.16 $\pm$ 0.38 <sup>bB</sup>	104.15 $\pm$ 0.65 <sup>bA</sup>
<b>Dessert yoghurt</b>	116.225 $\pm$ 0.77 <sup>aD</sup>	111.28 $\pm$ 0.5 <sup>aC</sup>	99.38 $\pm$ 0.49 <sup>aB</sup>	95.28 $\pm$ 0.5 <sup>aA</sup>
<b>PJ</b>	915.39 $\pm$ 0.58 <sup>eD</sup>	853.9 $\pm$ 1.91 <sup>fC</sup>	764.50 $\pm$ 1.27 <sup>fA</sup>	785.11 $\pm$ 2.56 <sup>gB</sup>
<b>DYPJ</b>	277.53 $\pm$ 1.56 <sup>dD</sup>	211.77 $\pm$ 1.1 <sup>dC</sup>	176.86 $\pm$ 1.47 <sup>dB</sup>	144.39 $\pm$ 0.61 <sup>dA</sup>
<b>CYPJ</b>	279.39 $\pm$ 0.5 <sup>dD</sup>	250.21 $\pm$ 0.76 <sup>eC</sup>	208.25 $\pm$ 0.36 <sup>eB</sup>	198.66 $\pm$ 0.32 <sup>fA</sup>
<b>DYPA</b>	195.37 $\pm$ 1.61 <sup>bC</sup>	162.39 $\pm$ 0.5 <sup>bB</sup>	154.69 $\pm$ 0.9 <sup>cB</sup>	135.83 $\pm$ 0.75 <sup>cA</sup>
<b>CYPA</b>	209.56 $\pm$ 1.11 <sup>cC</sup>	195.82 $\pm$ 1.04 <sup>cC</sup>	174.19 $\pm$ 1.7 <sup>dB</sup>	166.8 $\pm$ 1.13 <sup>eA</sup>

*Each value is expressed as mean SD (n=3)  $\pm$  standard error. Means with different capital letter within a row and uppercase letter within line are significantly different (P < 0.05) according to Duncan's multiple range test.*

### 3.2. Antioxidant activity

The antioxidant activity of PJ, cultured yoghurt and dessert yoghurt mixed with PJ and PA were analysed during the 18 days of storage at 4 °C by mean of DPPH, FRAP and chemiluminescence tests (Tables 2-4). Several tests were used because the antioxidant capacities of samples might be influenced by several factors and could not be fully described by one single method. In addition, most natural antioxidants are multifunctional; therefore, a reliable antioxidant evaluation protocol requires performing different

antioxidant activity assessments to consider various mechanisms of antioxidant action (Zhang et al. 2009).

**Table 2.** Effect of storage time and different treatments on the antioxidant activity by mean of DPPH test of permanganate juice and Greek yoghurts

Sample	DPPH (ascorbic acid equivalent mM/L)			
	Storage time (day)			
	1	6	12	18
Cultured yoghurt	2.3±0.21 <sup>aD</sup>	2.07±0.01 <sup>aC</sup>	1.82±0.06 <sup>aB</sup>	1.62±0.04 <sup>aA</sup>
Dessert yoghurt	2.99±0.14 <sup>bD</sup>	2.72±0.05 <sup>bC</sup>	2.61±0.77 <sup>bB</sup>	1.89±0.09 <sup>aA</sup>
PJ	15.65±0.43 <sup>eD</sup>	14.07±0.04 <sup>eC</sup>	13.65±0.41 <sup>eB</sup>	11.50±0.16 <sup>dA</sup>
DYPJ	4.74±0.49 <sup>cdD</sup>	4.38±0.15 <sup>dC</sup>	2.94±0.13 <sup>cB</sup>	2.50±0.16 <sup>bA</sup>
CYPJ	5.07±0.03 <sup>dD</sup>	4.64±0.05 <sup>dC</sup>	3.97±0.08 <sup>dB</sup>	3.05±0.16 <sup>cA</sup>
DYPA	4.39±0.36 <sup>cD</sup>	3.81±0.05 <sup>cC</sup>	2.96±0.65 <sup>cB</sup>	2.51±0.05 <sup>bA</sup>
CYPA	4.94±0.05 <sup>cdD</sup>	4.23±0.05 <sup>dC</sup>	3.82±0.04 <sup>dB</sup>	2.93±0.5 <sup>cA</sup>

Each value is expressed as mean SD (n=3) ± standard error. Means with different capital letter and uppercase letter within a row are significantly different (P < 0.05) according to Duncan's multiple range test.

**Table 3.** Effect of storage time and different treatments on the antioxidant activity by mean of FRAP test of permanganate juice and Greek yoghurts

Sample	FRAP (ascorbic acid equivalent mM/L)			
	Storage time (day)			
	1	6	12	18
Cultured yoghurt	2.61±0.04 <sup>aD</sup>	2.08±0.02 <sup>aC</sup>	1.83±0.05 <sup>aB</sup>	1.28±0.55 <sup>aA</sup>
Dessert yoghurt	2.98±0.05 <sup>bD</sup>	2.56±0.05 <sup>bC</sup>	1.99±0.02 <sup>aB</sup>	1.55±0.55 <sup>aA</sup>
PJ	12.77±0.19 <sup>eBC</sup>	12.13±0.005 <sup>eB</sup>	11.70±0.04 <sup>dA</sup>	11.41±0.13 <sup>eA</sup>
DYPJ	4.72±0.05 <sup>dD</sup>	4.14±0.04 <sup>cC</sup>	3.5±0.055 <sup>cB</sup>	2.93±0.06 <sup>bA</sup>
CYPJ	5.06±0.04 <sup>cD</sup>	4.42±0.02 <sup>dC</sup>	3.81±0.04 <sup>cB</sup>	3.12±0.55 <sup>cA</sup>
DYPA	4.62±0.04 <sup>cD</sup>	4.09±0.01 <sup>cC</sup>	3.42±0.02 <sup>bB</sup>	2.88±0.01 <sup>bA</sup>
CYPA	5.00±0.01 <sup>dD</sup>	4.47±0.1 <sup>dC</sup>	3.82±0.12 <sup>cB</sup>	3.03±0.25 <sup>cA</sup>

Each value is expressed as mean SD (n=3) ± deviation. Means with different lowercase letter within a row and uppercase letter within line are significantly different (P < 0.05) according to Duncan's multiple range test

**Table 4.** Effect of storage time and treatments on the antioxidant activity by mean of Chemiluminescence test (IC<sub>50</sub>) test of permanganate juice and Greek yoghurts

Sample	Chemiluminescence (ascorbic acid equivalent mM/L)			
	Storage time (day)			
	1	6	12	18
Cultured yoghurt	2.07±0.02 <sup>aD</sup>	1.73±0.06 <sup>aC</sup>	1.40±0.04 <sup>aB</sup>	1.17±0.05 <sup>aA</sup>
Dessert yoghurt	2.22±0.22 <sup>aD</sup>	1.93±0.04 <sup>aC</sup>	1.67±0.09 <sup>bB</sup>	1.48±0.07 <sup>bA</sup>
PJ	11.13±0.04 <sup>cD</sup>	10.49±0.15 <sup>cC</sup>	9.47±0.08 <sup>eB</sup>	9.84±0.06 <sup>eA</sup>
DYPJ	4.67±0.05 <sup>bD</sup>	4.09±0.01 <sup>bC</sup>	2.83±0.035 <sup>cB</sup>	2.42±0.02 <sup>cA</sup>
CYPJ	4.76±0.06 <sup>bD</sup>	4.27±0.07 <sup>bC</sup>	3.32±0.02 <sup>dB</sup>	2.84±0.04 <sup>dA</sup>
DYPA	4.49±0.49 <sup>bD</sup>	4.15±0.15 <sup>bC</sup>	3.02±0.02 <sup>cB</sup>	2.20±0.15 <sup>cA</sup>
CYPA	4.58±0.08 <sup>bD</sup>	4.14±0.15 <sup>bC</sup>	3.37±0.02 <sup>dB</sup>	2.78±0.11 <sup>dA</sup>

Each value is expressed as mean SD (n=3) ± standard deviation. Means with different lowercase letter within a row and uppercase letter within line are significantly different (P < 0.05) according to Duncan's multiple range test.

The antioxidant capacity of PJ and fortified yoghurt samples decreased during the storage time in the different antioxidant capacity measure tests. The antioxidant capacity of both dessert and cultured yoghurts mixed with PJ and PA were even less than the sum of the antioxidant capacity of PJ and plain yoghurt alone. Based on the work of Arts et al. (2009), this decrease is related to binding effect between polyphenol and dairy protein. For instance, addition of milk to tea reduced the antioxidant capacity of the antioxidants present in tea. The binding proteins and polyphenols reduce antioxidant activity by lowering the number of free hydroxyl (Amiot et al. 1986; Dubeau et al. 2010). Because of the

polyphenol-protein complexes, antioxidants bioavailability might be decreased. This phenomenon might also occur in other products containing both polyphenols and proteins.

The interaction, resulting in protein-polyphenol complexes, can be both reversible and irreversible depending on pH, temperature, and protein and flavonoid concentrations. Proteins which contain more proline groups and flavonoids such as epigallocatechin gallate (EGCG) and epicatechin gallate in green tea and gallic acid in black tea are responsible for the depletion of the antioxidant activity (Trigueros et al. 2014). In general, phenolic compounds have more antioxidant activity in acid solution. According to El-Said et al. (2014), at a basic pH, Gallic acid showed less absorbance at 765 nm due to the fact that the pH level can affect and change the ionization state of the phenolic compounds and, therefore, the absorbance maximum. These results could explain the higher antioxidant activities recorded for cultured yoghurt.

The correlation between the total phenol content and the different antioxidant activity parameters, DPPH, FRAP and IC<sub>50</sub> (Table 5) was very high for both dessert and cultures yoghurt mixed with pomegranate juice and arils at the end of storage period. These results are in consistent with previous studies which reported that the content of total polyphenols, as determined by the Folin–Ciocalteu reagent, is highly correlated with other antioxidant assays, such as ORAC, DPPH and FRAP ( Ozgen et al. 2008; Madrigal-Carballo et al. 2009).

**Table 5:** Correlation matrix between antioxidant capacity and total phenol content of PJ, CPA, CPJ, DPA, and DPJ

	TPC	DPPH	FRAP	IC <sub>50</sub>
TPC	1	0.992	0.996	0,99
DPPH	0,992	1	0,991	0,998
FRAP	0,996	0,991	1	0,988
IC <sub>50</sub>	0,99	0,998	0,988	1

### 3.3. Sensorial analysis

The organoleptic quality of yoghurt enriched with pomegranate juice and arils was assessed during the last day of the trial. The results presented in Table 6 show that both types of yoghurts enriched with pomegranate juice had the best flavor followed by that fortified with arils. Although, the juice has altered the semi-solid texture of Greek yoghurt. From the point of view of appearance, the addition of the juice or arils to both yoghurt was highly appreciated. Thus, the addition of arils and pomegranate juice improved the organoleptic quality of the yoghurt.

**Tableau 6:** Organoleptical properties of cultured and dessert yoghurt fortified with pomegranate juice and arils

Samples	Flavor	Texture	Appearance	Overall quality
<b>Cultured yoghurt</b>	43,32 ± 1,4 <sup>a</sup>	38,56 ± 1,58 <sup>b</sup>	8,52 ± 0,56 <sup>a</sup>	90,4 ± 1,19 <sup>a</sup>
<b>Dessert yoghurt</b>	44,18 ± 1,98 <sup>a</sup>	38,15 ± 2,34 <sup>b</sup>	8,43 ± 1,32 <sup>a</sup>	90,76 ± 1.89 <sup>a</sup>
<b>CYPJ</b>	47,56 ± 1,54 <sup>c</sup>	37,23 ± 2,46 <sup>a</sup>	9,16 ± 0,98 <sup>b</sup>	93,95 ± 1,24 <sup>b</sup>
<b>CYPA</b>	46,32 ± 2,34 <sup>b</sup>	38,32 ± 1,89 <sup>b</sup>	9,57 ± 1,14 <sup>b</sup>	94,21 ± 1,34 <sup>c</sup>
<b>DYPJ</b>	47,13 ± 1,67 <sup>c</sup>	37,54 ± 3,6 <sup>a</sup>	9,32 ± 1,04 <sup>b</sup>	93,99 ± 1,57 <sup>b</sup>
<b>DYPA</b>	45,67 ± 2.03 <sup>b</sup>	38,13 ± 1,5 <sup>b</sup>	9,61 ± 1,24 <sup>b</sup>	93,41 ± 1,19 <sup>b</sup>

Each value is expressed as mean SD (n=3). Means with different lowercase letter within a row are significantly different (P < 0.05).

#### 4. Conclusion

Health-promoting Pomegranate juice appeared to be a promising rich antioxidant product that could improve the quality of dessert and cultured yoghurts. We conclude that the approaches for adding PJ to cultured and dessert yoghurts are feasible since both matrices could preserve a considerable antioxidant activity and TPC during the time frame of study (18 days), though cultured yoghurt exhibit better interaction. Furthermore, adding PJ to cultured yoghurt is preferred since the seed might cause problems for some consumers especially for elderly. As a future perspective, more focus on different product processing challenges, microbiological features and functionality advantages are needed to be studied.

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#### 6. Conflict of interest

The authors declared that there is no conflict of interest.

#### 7. References

- Amiot M, Fleuriet A, Macheix J (1986)** Importance and evolution of phenolic compounds in olive during growth and maturation. *J. Agric. Food* 34: 823-826.
- Arnous A, Makris D, Kefalas P (2002)** Correlation of pigment and flavanol content with antioxidant properties in selected aged regional wines from Greece. *J. Food Compos. Anal.* 15 : 655–665.
- Arts M. J. T. J, Haenen G. R. M. M, Wilms L. C, Beetstra S. A. J. N, Heijnen C. G. M, Voss H. P, Bast A (2002)** Interactions between flavonoids and proteins: effect on the total antioxidant capacity. *J. Agric. Food Chem.* 50 : 1184–1187.
- Benzie I. F. F , Strain, J. J (1999)** Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods Enzymol* 299: 15-27.
- Çam M, İçyer N.C, Erdoğan F (2014)** Pomegranate peel phenolics: Microencapsulation, storage stability and potential ingredient for functional food development. *LWT - Food Sci. Technol.* 55 : 117–123.
- De Carvalho Alves A. P, Corrêa A. D, Pinheiro A. C. M, De Oliveira F. C (2013)** Flour and anthocyanin extracts of jaboticaba skins used as a natural dye in yogurt. *Int. J. Food Sci. Technol* 48: 2007 -2013.
- Desai N, Shepard L, Drake M (2013)** Sensory properties and drivers of liking for Greek yogurts. *J. Dairy Sci* 96 :7454–7466.
- Donkor O. N, Henriksson A, Vasiljevic T, Shah, N. P (2007)** Rheological Properties and Sensory Characteristics of Set-Type Soy Yogurt. *J. Agric. Food Chem.* 55: 9868–9876.
- Dubeau S, Samson G, Tajmir-Riahi H. A (2010)** Dual effect of milk on the antioxidant capacity of green, Darjeeling, and English breakfast teas. *Food Chemistry* 122 : 539–545.
- El-Nashi H.B, Abdel Fattah A.F.A.K, Abdel Rahman N.R, Abd El-Razik M.M (2015)** Quality characteristics of beef sausage containing pomegranate peels during refrigerated storage. *Ann. Agric. Sci.* 60 : 403–412.
- El-Said M.M, Haggag H.F, Fakhr El-Din H.M, Gad a.S, Farahat A.M (2014)** Antioxidant activities and physical properties of stirred yoghurt fortified with pomegranate peel extracts. *Ann. Agric. Sci.* 59: 207–212.
- Karaaslan M, Ozden M, Vardin H, Turkoglu H (2011)** Phenolic fortification of yogurt using grape and callus extracts. *LWT-Food Science and Technology* 44: 1065-1072.
- Karimi N, Sani A. M, Pourahmad R (2016)** Influence of carboxy methyl cellulose (CMC) and pectin on rheological, physical stability and sensory properties of milk and concentrated jujuba mixture.

- J. Food Meas. Charact. 10 (2): 396-404.
- Kelebek H, Selli S, Kola O (2016)** Quantitative determination of phenolic compounds using LC-DAD-ESI-MS/MS in cv. Ayvalik olive oils as affected by harvest time. J. Food Meas. Charact 11 (1): 226-235.
- Kelebek H, Kesen S, Selli S (2015)** Comparative Study of Bioactive Constituents in Turkish Olive Oils by LC-ESI/MS/MS, Int. J. Food Prop. 18 : 2231- 2245.
- Madrigal-Carballo S, Rodriguez G, Krueger C. G, Dreher M, Reed J. D (2009)** Pomegranate (*Punica granatum*) supplements: authenticity, antioxidant and polyphenol composition. J. Funct. Foods 1(3): 324-329.
- Mahmoud M. A. A, Chedea V. S, Detsi A, Kefalas P (2013)** Ascorbic acid modifies the free radical scavenging behaviour of catechin: An insight into the mechanism. Food Research International Food Res. Int. 51 : 907–913.
- Mang D. Y, Abdou A. B, Njintang N. Y, Djiogue E. J. M, Loura B. B, Mbofung M. C (2015)** Application of desirability-function and RSM to optimize antioxidant properties of mucuna milk. J. Food Meas. Charact. 9(4): 495-507.
- Mousavinejad G, Emam-Djomeh Z, Rezaei K, Khodaparast M. H. H (2009)** Identification and quantification of phenolic compounds and their effects on antioxidant activity in pomegranate juices of eight Iranian cultivars. Food Chemistry 115(4) :1274–1278.
- Ozgen M, Durgaç C, Serçe S, Kaya C (2008)** Chemical and antioxidant properties of pomegranate cultivars grown in the Mediterranean region of Turkey. Food Chem. 111(3): 703-706.
- Palma A, Continella A, La Malfa S, Gentile A (2015)** Overall quality of ready-to-eat pomegranate arils processed from cold stored fruit. Postharvest Biology and Technology 109: 1–9.
- Parejo I, Codina C, Petrakis C, Kefalas P (2000)** Evaluation of scavenging activity assessed by Co (II)/EDTA-induced luminol chemiluminescence and DPPH·(2, 2-diphenyl-1-picrylhydrazyl) free radical assay. Journal of Pharmacological and Toxicological Methods 44: 507-512.
- Pérez-Vicente A, Serrano P, Abellán P, García-Viguera C (2004)** Influence of packaging material on pomegranate juice colour and bioactive compounds, during storage. J. Sci. Food Agric. 84 (7): 639-644.
- Robert P, Gorena T, Romero N, Sepulveda E, Chavez J, Saenz C (2010)** Encapsulation of polyphenols and anthocyanins from pomegranate (*Punica granatum*) by spray drying. International Journal of Food Science & Technology 45(7): 1386–1394.
- Ścibisz I, Ziarno M, Mitek M, Zaręba D (2012)** Effect of probiotic cultures on the stability of anthocyanins in blueberry yoghurts, , LWT-Food Sci. Technol. 49 (2): 208-212
- Sun-Waterhouse D (2011)** The development of fruit-based functional foods targeting the health and wellness market: a review. Int. J. Food Sci. Technol 46: 899-920.
- Trigueros L, Wojdyło A, Sendra E (2014)** Antioxidant activity and protein–polyphenol interactions in a pomegranate (*Punica granatum* L.) yogurt. J. Agric. Food Chem. 62: 6417–6425.
- Varela-Santos E, Ochoa-Martinez A, Tabilo-Munizaga G, Reyes J. E, Pérez-Won M, Briones-Labarca V, Morales-Castro J (2012)** Effect of high hydrostatic pressure (HHP) processing on physicochemical properties, bioactive compounds and shelf-life of pomegranate juice. Innov. Food Sci. Emerg. Technol. 13:13-22.
- Waterman P. G, Mole S (1994)** Analysis of Phenolic Plant Metabolites. Blackwell Scientific Publications, Oxford, pp 238.
- Zhang Y, Wang D, Lee R, Henning S, Heber D (2009)** Absence of pomegranate ellagitannins in the majority of commercial pomegranate extracts: implications for standardization and quality control. J. Agric. Food Chem. 57:7395–7400