



Formulation and storage effects on pomegranate smoothie phenolic composition, antioxidant capacity and color



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ARTICLE INFO

Keywords:

Punica granatum L.
Mollar de Elche
Wonderful
Anthocyanins
Polymeric procyanidins
FRAP
ABTS

ABSTRACT

Smoothies are an increasingly popular way of consuming fruits and the industry is focusing on the increment of shelf life and the maintenance original color and the content of bioactive compounds. The aim of the present study was to evaluate how formulation and storage conditions (6 months at 4 or 20 °C) of different pomegranate smoothies affected on functional compounds. Phenolic compounds, antioxidant capacity (ABTS, FRAP) and color of 12 different smoothies were studied. The study was completed evaluating the effect of ratio purée:juice (60:40 or 40:60), pomegranate cultivar (*Mollar de Elche* or *Wonderful*) and fruit purée (quince, jujube, or fig) on studied smoothies. The smoothies before storage presented high values of total polyphenolic content (TPC): 247–314 mg/100 g fresh weight (fw), 2939–3920 mg/100 g fw, and 3809–5324 mg/100 g fw, in fig, jujube and quinces pomegranate smoothies, respectively. A positive effect of the 40:60 ratio purée:juice, the *Wonderful* pomegranate juice storing at 4 °C was found on total polyphenolic content [sum of anthocyanins, flavanols, flavan-3-ols (as monomeric and dimeric), polymeric procyanidins and phenolic acids] and quality of smoothies (a^* coordinate) being only a reduction of 30.1%, 13.1% and 9.5% in fig, jujube and quinces smoothies, respectively.

1. Introduction

In the last years, smoothies are an increasingly popular way of consuming fruits. They are a non-alcoholic creamy drink made from fruit purée and fruit juice (or less commonly vegetables), and optionally ice, yoghurt and/or milk. Fruits and vegetables are rich in polyphenols, which do not only play physiological roles in plants but also act as antioxidants by donating a hydrogen atom or an electron to other compounds, scavenging free radicals, quenching singlet oxygen, and maintaining a balance between oxidants and antioxidants to improve human health (Wolfe, Wu, & Liu, 2003). Various studies concluded that high consumption of fruits and vegetables promotes health, and it is associated with reduced risk of degenerative diseases (Miller et al., 2017). Therefore, smoothies are one of the many ways that consumers have to include fruits and vegetables in their diets (Castillejo, Martínez-Hernández, Gómez, Artés, & Artés-Hernández, 2016).

The shelf life of non-pasteurized smoothies is relatively short due to microbial growth as a result of the minimal level of processing associated with these products. These are normally consumed fresh or

preserved for short periods (1–3 weeks) by storing them under refrigeration. Besides, storage time can affect the color and polyphenolic composition of the smoothies. Some researchers suggested that it could be worth to use a mild thermal pasteurization (Walkling-Ribeiro, Noci, Cronin, Lyng, & Morgan, 2010) or a thermal and high hydrostatic pressure processing to increase their shelf life and for better color retention, polyphenols and other quality attributes, guaranteeing consumers acceptance and food safety (Keenan, Brunton, Gormley, & Butler, 2011; Tiwari, 2018, pp. 261–278).

There are some pomegranate cultivars which extreme sourness precludes their fresh consumption, although they are rich in functional ingredients; similarly, several quince cultivars, especially the most known cannot be used as fresh fruits because of their excessive astringency (Szychowski, Munera-Picazo, Szumny, Carbonell-Barrachina, & Hernández, 2014). However, these non-edible-as-fresh fruits could be used as fruit purée to develop novel products, such as smoothies, and will have intense and interesting flavor and taste, and will help in getting a high consumer acceptance. Pomegranate fruit contains many phenolic compounds including flavonoids - anthocyanins, and other

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complex flavanoids and hydrolyzable tannins (punicalagin, gallic acid, and ellagic acid), which have high antioxidant capacity that may offer beneficial health properties (Aloqbi et al., 2016).

Therefore, the aim of the present research was to study how storage conditions (6 months at 4 or 20 °C) affected color, polyphenolic profile, and antioxidant activity of pomegranate smoothies. The study was completed by evaluating the effect of ratio purée:juice (60:40 and 40:60), pomegranate cultivar (*Mollar de Elche* and *Wonderful*), and fruit purée (quinces, jujubes, and figs) on the quality of the studied smoothies.

2. Materials and methods

2.1. Sample preparation

Pomegranate trees (*Punica granatum* L., cultivar *Mollar de Elche* and *Wonderful*) were cultivated in a farm located in Murcia (Spain) under regulated deficit irrigation (RDI) (Cano-Lamadrid et al., 2018). Pomegranate (cv. *Mollar de Elche* and *Wonderful*), figs (*Ficus carica*, cv. *Colar*), jujubes (*Ziziphus jujube*, cv. *Grande de Albaterra*) and quinces (*Cydonia oblonga*, cv. *Gigante de Vranja*) were hand-harvested at a commercial maturity stage.

The different stages of the smoothie preparation were:

i) Purée preparation: The figs (F), jujubes (J), or quinces (Q) were peeled, ground, and heated at 80 °C in a Thermomix device (Vorwerk, Wuppertal, Germany). Rhubarb juice (5%) was added to prevent enzymatic browning. Purées were cooled to room temperature. The purees were subjected to analyses right after preparation and after 6 months of storage at 4 or 20 °C. These same formulation ratio purée:juice and storage conditions used were based on previous smoothies studies (Nowicka, Wojdyło, Teleszko, & Samoticha, 2016). Rhubarb juice was added in the same amount to all smoothies, the effect for preserve polyphenolic compounds before oxidation (Oszmiański & Wojdyło, 2008).

ii) Juice preparation: Pomegranate fruits [*Mollar de Elche* (Mo) and *Wonderful* (W)] were cut in halves, and arils were manually separated and juices were prepared using only arils.

iii) Smoothies preparation: Purée and juices samples, immediately after their preparation, were mixed in the proportions 40/60 and 60/40, respectively, to obtain 12 treatments/samples (Table 1). Then, the products were heated to 100 °C and pasteurized (10 min at 90 °C).

Table 1

Formulation of smoothies consisting of pomegranate juice and figs, jujubes, or quinces purées.

Nº	Code ^a	Formulation ^b
1	F1Mo	40% F + 60% Mo
2	F1W	40% F + 60% W
3	F2Mo	60% F + 40% Mo
4	F2W	60% F + 40% W
5	J1Mo	40% J + 60% Mo
6	J1W	40% J + 60% W
7	J2Mo	60% J + 40% Mo
8	J2W	60% J + 40% W
9	Q1Mo	40% Q + 60% Mo
10	Q1W	40% Q + 60% W
11	Q2Mo	60% Q + 40% Mo
12	Q2W	60% Q + 40% W

^a Mo, Mollar de Elche pomegranate juice; W, Wonderful pomegranate juice; F, fig purée; J, jujube purée; Q, quince purée.

^b The percentage of each component was expressed in weight:weight, w:w.

2.2. Color parameters

Color coordinates (L^* , a^* , and b^*) were determined by reflectance measurement with a Color Quest XE Hunter Lab colorimeter. The samples were filled in a 1 cm cell, and L^* , a^* , b^* values were determined using Illuminant D65 and 10° observer angle. Samples were measured against a white ceramic reference plate (L^* ¼ 93.92; a^* ¼ 1.03; b^* ¼ 0.52). Measurements were run in triplicate.

2.3. Extraction, LC-PDA/MS and UPLC-PDA-FL analysis of polyphenolic compounds

The extract of polyphenols were performed as previously described (Wojdyło, Oszmiański, & Bielicki, 2013). The compound identification was done using fast liquid chromatography quadrupole time-of-flight mass spectrometry (LC/MS QToF), and the compound quantification was done using ultra-performance liquid chromatography-photodiode array (UPLC-PDA). The analysis of polymeric procyanidins was done using UPLC-FL by phloroglucinol method was performed according to the protocol described previously by Kennedy and Jones (2001). All measurements were run triplicate, and results were expressed as mg/100 g of product.

2.4. Antioxidant capacity (ABTS⁺ and FRAP)

Each sample (~1 g) was mixed with 5 mL of extractant solution (MeOH/water 80:20 v/v, 1% HCl), sonicated at 20 °C for 10 min, and left for 24 h at 4 °C. Then, the extract was again sonicated for 15 min, and centrifuged until the separation of the supernatant. The antioxidant capacity was determined using ABTS⁺ and FRAP assays, previously described by Re et al. (1999) and Benzie and Strain (1996), respectively. Calibration curves within the range 0.50–5.00 mmol Trolox L⁻¹ were used for the quantification using all of the two methods; these calibration curves showed good linearity ($R^2 \geq 0.998$). Determinations were performed using a UV-2401 PC spectrophotometer (Shimadzu, Kyoto, Japan). Analyses were run in triplicate and results were expressed as mmol TE/100 g.

2.5. Statistics

A four-way ANOVA (factor 1: Ratio purée:juice, factor 2: pomegranate cultivar, factor 3: fruit purée, and factor 4: storage conditions) was performed using XLSTAT Premium 2016 (Microsoft Corporation, Redmond, WA, USA), and means were separated by Tukey's multiple range test.

3. Results and discussion

3.1. Polyphenolic composition

Fig. 1 shows the quantification of the polyphenols found in fig (A), jujube (B) and quince (C) pomegranate smoothies before (T0) and after 6 months of storage at 4 °C (T1) and 20 °C (T2). The values of total polyphenolic content (TPC) in fig, jujube, and quince pomegranate smoothies at the beginning of the storage period (T0) ranged between 247 and 314 mg/100 g fresh weight (fw), 2939–3920 mg/100 g fw, and 3809–5324 mg/100 g fw, respectively. During storage, changes on TPC were noticed. Regarding fig pomegranate smoothies, the best formulation was F2W, which only experienced a TPC decrease of 13.3% after T1 and 52.2% after T2. The reduction of TPC content the rest of fig pomegranate smoothies ranged between 30.2 and 34.3% after T1 and 64.2–64.7% after T2, respectively. On the other hand, jujube pomegranate smoothies stored at 4 °C, suffered a TPC reduction between 24.71 and 24.9%, except when *Wonderful* cultivar was used, with a drastically lower reduction of only 6.30% or even an increase of 11.8% in the treatment J2W. During storage at 20 °C, the TPC was reduced

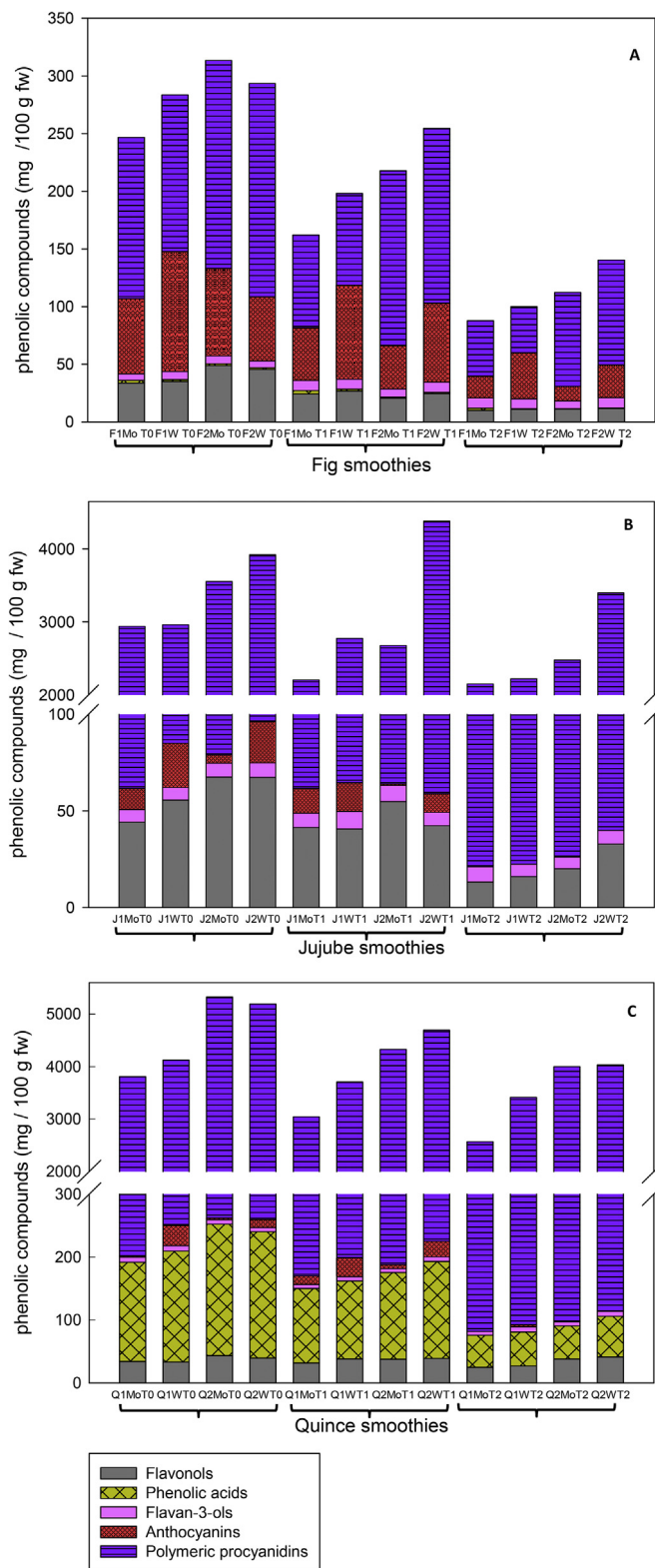


Fig. 1. Content of flavonols, phenolic acids, flavan-3-ols (as monomeric, dimeric and polymeric procyanidins), and anthocyanins, in mg/100 g fw (fresh weight) of fig (A), jujube (B), and quince (C) smoothies before (T0) and after 6 months of storage time at 4 °C (T1) and 20 °C (T2).

Table 2

Flavonols, phenolic acid, flavan-3-ols and antioxidant capacity (ABTS⁺ and FRAP) in pomegranate smoothies during storage as affected by: (i) ratio purée:juice (40:60 and 60:40), (ii) pomegranate cultivar (W, Wonderful, and Mo, Mollar de Elche), (iii) fruit purée (fig, jujube, and quince), and, (iv) storage temperature during 6 months (4 and 20 °C).

Parameter	ANOVA [†]	Smoothies [‡]											
		F1Mo	F1W	F2Mo	F2W	J1Mo	J1W	J2Mo	J2W	Q1Mo	Q1W	Q2Mo	Q2W
Flavonols [‡]	***	22.7	24.3c	26.9bc	27.3bc	32.9b	37.3ab	47.4a	47.5a	30.2b	32.8b	39.8ab	40.1ab
Phenolic acids [‡]	***	2.32c	1.15d	0.84d	0.96d	nd	nd	nd	nd	109b	118b	133a	140a
Flavan-3-ols [‡]	***	7.82	8.20a	7.00b	7.91ab	7.32b	7.39b	7.18b	7.17b	6.45c	8.07a	6.54c	7.36b
PP [‡]	***	89.5e	85.2e	138d	143d	2383c	2593bc	2846b	3835ab	2989b	3566ab	4368a	4440a
FRAP [±]	***	0.30e	0.64cde	0.45de	0.45de	1.85b	2.52a	2.24ab	1.89b	0.76cd	1.01c	0.68cde	0.76cd
ABTS [±]	***	0.68c	1.01bc	0.72c	0.66c	1.34bc	2.88a	3.03a	3.09a	1.82b	1.07bc	1.31bc	1.12bc

Parameter	ANOVA [†]	Ratio purée:juice		ANOVA [†]	Pomegranate cultivar		ANOVA	Fruit purée			ANOVA	Storage temperature		
		40:60	60:40		Mo	W		Fig	Jujube	Quince		T0	T1	T2
Flavonols [‡]	**	30.0b	38.2a	NS	33.3	34.9	***	25.3c	41.3a	35.7b	***	45.7a	35.2b	21.4c
Phenolic ac. [‡]	**	38.4b	45.8a	NS	40.9	43.3	***	1.32b	nd	125a	***	62.5a	35.2b	18.7c
Flavan-3-ols [‡]	NS	7.64	7.19	NS	7.15	7.68	NS	7.76	7.26	7.23	NS	6.99	7.73	7.53
PP [‡]	NS	1951	2628	NS	2135	2443	***	114c	2914b	3840a	NS	2597	2270	2002
FRAP [±]	NS	1.18	1.04	NS	0.97	1.25	***	0.46b	2.14a	0.46b	***	1.19a	1.25a	0.88b
ABTS [±]	NS	1.47	1.64	NS	1.42	1.67	***	0.77c	2.59a	1.36b	***	1.85a	1.69a	1.07b

[‡]expressed in followed units: mg/100 g fw smoothie.

[±] expressed in followed units: mmol Trolox/100 g fw smoothie.

[†]NS = not significant F ratio ($p < 0.05$); *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively.

[‡]Values followed by the same letter, within the same factor (ratio purée:juice, pomegranate cultivar, fruit purée and storage time), were not significant different ($p > 0.05$), Tukey's multiple-range test.

[†]T0: freshly made; T1: storage 6 months 4 °C; and, T2: storage 6 months 20 °C.

between 25.0 and 30.3%, with the exemption of the J2W samples, which reduction was significantly lower, only 13.3%. Finally, the TPC reduction of quince pomegranate smoothies ranged between 9.5 and 20.1% after storage at 4 °C, and between 17.2 and 32.6% at 20 °C. The fact that the higher the storage temperature, the higher the loss of TPC in smoothies was previously reported in sour cherry smoothies (Nowicka et al., 2016). These authors indicated that after storing samples 6 months samples at 4 °C maintained more TPC, especially anthocyanins, than at 30 °C. It is worth mentioning that the ratio purée:juice 60:40 and *Wonderful* cultivar positively influenced the maintenance of TPC during storage in all types of smoothies.

Table 2 shows the contents of flavonols, phenolic acids, flavan-3-ols, and polymeric procyanidins as affected by ratio purée:juice, pomegranate cultivar, type of fruit purée, and storage conditions on smoothies. Although a positive effect was observed on flavonols and phenolic acids at the ratio purée:juice 60–40, each purée had a different behavior. Regarding flavonols, jujube pomegranate smoothies presented the highest content, followed by the quince ones. However, no phenolic acids were detected on jujube pomegranate smoothies, whilst quince pomegranate smoothies presented the highest content of these compounds. Previous studies indicated that jujubes and quinces are rich sources of flavonols and phenolic acids, respectively (Wojdyło et al., 2013, 2016). Finally, the content of the monomer and dimeric forms of the flavan-3-ols was not significant affected by any of the studied factors.

As to polymeric procyanidin (PP), a huge difference among all smoothies was found. The values of PP in fig, jujube and quince pomegranate smoothies in T0 ranged between 136 and 185, 2875–3823, and 3607–5062 mg/100 g fw, respectively. The PP content was only affected by the fruit purée. It is very important to know which treatment was the best in keeping high PP content due to their healthy properties; although the PP has been reported that is not absorbed, unabsorbed PP are directly linked with colon cancer (Gossé et al., 2005). Fig. 1 clearly shows how a reduction of PP after storage was noticed. Among fig pomegranate smoothies, a reduction of 41.9% was found after 6 months at 4 °C, except smoothies based on the 60:40 purée:juice ratio, which only decreased 17.0%. Moreover, after

6 months at 20 °C a mean reduction of 44.1% was observed. Among jujube pomegranate smoothies, the most relevant factors were pomegranate cultivar and storage conditions. The combination *Wonderful* cultivar and storage at 4 °C maintained the PP content, or even increased its content by 13.1% (J2W); while after storage at 20 °C, *Mollar de Elche* smoothies had a better maintenance (mean reduction of 3.5%) than *Wonderful* smoothies (mean reduction of 20.6%). Finally, pomegranate cultivar was the factor which affected the reduction of PP content on quinces pomegranate smoothies, with reduction values being 9.1% and 14.4% in *Wonderful* and *Mollar de Elche* smoothies, respectively.

Table 3 shows significant effects of (i) ratio purée:juice, (ii) pomegranate cultivar, (iii) fruit purée, and, (iv) storage time, on the contents of individual and total anthocyanins. Specifically, the anthocyanins (ANCs) profile was significantly affected by fruit purée and storage time, while the factors ratio purée:juice and pomegranate cultivar only affected the contents of A1 and A2 out of 5 ANCs. As expected, the highest ANCs were observed in smoothies with a ratio purée:juice of 40:60, with ANCs mostly coming from the pomegranate juice. Moreover, it was noticed that depending on the pomegranate cultivar, ANC profile and Σ ANCs content were different presenting higher values those smoothies based on *Wonderful* fruits as compared to those of *Mollar de Elche*; the differences were mainly due to the contents of delphinidin-3,5-di-O-glucoside (A1) and cyanidin-3,5-di-O-glucoside (A2). These results agreed with previous studies showing that the *Wonderful* fruits had higher ANCs content than the *Mollar de Elche* ones (Mena, Martí, & García-Viguera, 2014). According to previous studies (Mena et al., 2014), the major component in all smoothies was cyanidin-3,5-di-O-glucoside (A2) coming from pomegranate. On the other hand, cyanidin-3-O-glucoside (A4) and pelargonidin-3-O-glucoside (A5) were mostly detected when fig purée was included in the formulation (Wojdyło, Nowicka, Carbonell-Barrachina, & Hernández, 2016). Moreover, as expected fig smoothies presented 6.5 and 5.0 higher times of ANCs than jujube and quinces smoothies, respectively due to the presence of ANCs in figs.

Although further research is needed to identify the mechanisms responsible for anthocyanin degradation during storage, several studies

Table 3

Anthocyanins contents (ACNs) in pomegranate smoothies during storage as affected by: (i) ratio purée:juice (40:60 and 60:40), (ii) pomegranate cultivar (W, Wonderful, and Mo, Mollar de Elche), (iii) fruit purée (fig, jujube, and quince), and, (iv) storage temperature during 6 months (4 and 20 °C).

ACNs (mg/100 g fw)	ANOVA [†]	Smoothies [‡]											
		F1Mo	F1W	F2Mo	F2W	J1Mo	J1W	J2Mo	J2W	Q1Mo	Q1W	Q2Mo	Q2W
A1 ±	***	0.90b	9.73a	0.00b	8.87a	0.00b	1.64b	0.00b	1.98 b	0.00b	4.50ab	0.000 b	2.50b
A2	***	14.9abc	31.9a	10.8bc	21.2ab	5.47bc	9.31bc	0.00d	6.32bc	3.25bc	14.0abc	1.46c	7.68bc
A3	NS	3.28a	3.55a	0.74a	1.48	1.17	0.75	0.23	0.35	0.82	1.32	0.59	1.15
A4	***	13.9a	12.5a	11.8ab	15.0ab	1.21a	0.88b	1.39b	1.67b	0.73b	1.93b	0.89b	1.08b
A5	***	10.2b	17.4a	18.6a	4.37c	0.00d	0.00d	0.00d	0.00d	0.38d	0.00d	0.00d	0.00d
ΣA	***	43.1ab	75.0a	41.9ab	50.8ab	7.85b	12.6b	1.63b	10.3b	5.18b	21.8b	2.93b	12.4b

ACNs(mg/100 g fw)	ANOVA [†]	Ratio purée:juice		ANOVA [†]	Pomegranate cultivar		ANOVA	Fruit purée			ANOVA	Storage temperature		
		40:60	60:40		Mo	Wond		Fig	Jujube	Quince		T0	T1	T2
		A1 ±	NS		2.79	2.22		***	0.15b	4.87a		***	4.87a	0.90b
A2	**	13.1a	7.91b	**	5.98b	15.1a	***	19.7a	5.27b	6.61b	***	12.5ab	14.5a	4.56b
A3	NS	1.82	0.76	NS	1.14	1.43	**	2.26a	0.63b	0.97ab	***	0.55b	2.96a	0.36b
A4	NS	5.19	5.30	NS	4.98	5.51	***	13.3a	1.29b	1.16b	***	5.32b	7.98a	2.43c
A5	NS	4.65	3.82	NS	4.85	3.63	**	12.6a	0.00a	0.09a	***	12.0a	0.71a	0.00a
ΣA	**	27.6a	20.0b	***	17.1b	30.5a	***	52.7a	8.09b	10.6b	***	34.1a	28.7b	8.58c

± A1: Delphinidin-3,5-di-O-glucoside; A2: cyanidin-3,5-di-O-glucoside; A3: Delphinidin-3-O-glucoside; A4: Cyanidin-3-O-glucoside; A5: Pelargonidin-3-O-glucoside.

† NS = not significant F ratio ($p < 0.05$); *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively.

‡ Values followed by the same letter, within the same factor (ratio purée:juice, pomegranate cultivar, fruit purée and storage time), were not significant different ($p > 0.05$), Tukey's multiple-range test.

¶ T0: freshly made; T1: storage 6 months 4 °C; and, T2: storage 6 months 20 °C.

reported that is due to the polymerization of the monomeric compounds, leading to an undesirable brown color (Sinela et al., 2017). The temperature is a key factor during storage to avoid the loss of ANCs and their linked color. The higher the temperature, the higher the ANCs degradation. Regarding ANCs stability in the samples under study, after 6 months of storage the total content of ANCs was reduced only 15.8% when smoothies were stored at 4 °C (T1), but this content was reduced by 74.8% when stored at 20 °C; these results agreed with those from the literature (Sinela et al., 2017). Besides, results also agreed with an almost complete reduction, 94% and 98% for delphinidin-3,5-di-O-glucoside and cyanidin-3,5-di-O-glucoside, when samples were stored at a higher temperature, 37 °C. It was observed that figs smoothies maintained more the ANCs content than jujube and quinces ones at the end of the storage period (Fig. 1). Besides the initial highest content of ΣACN, the high pectin content found in previous studies in fig fruits might be the reason of the anthocyanin pigment stability and extension of pigment shelf-life (Wojdyło et al., 2016). An anthocyanin and pectin interaction might influence the above mentioned trend (Fernandes, Brás, Mateus, & de Freitas, 2014). Recently, two mechanisms for the binding of pectin fractions and ANCs were described in blueberry: i) ionic interaction between anthocyanin flavylium cations and free pectic carboxyl groups, and/or, ii) anthocyanin aromatic stacking on bound anthocyanins (Lin, Fischer, & Wicker, 2016).

3.2. Color coordinates

Table 4 shows the values of instrumental color coordinates as affected by the 4 factors under study, ratio purée:juice, pomegranate cultivar, fruit purée, and, storage time. A bright and intense red color is one of the most valued quality attribute of pomegranate products, which influences consumer acceptance and commercial value. The green-red coordinate, a^* , was significantly affected by all factors described above. As expected, the highest a^* values (more intense red color) were found in smoothies with ratio 40:60 purée:juice, coming mostly from pomegranate juice. Moreover, the pomegranate cultivar selected to prepare the smoothies also affected the a^* values, having smoothies based on Wonderful pomegranate a more intense red color than those of the cultivar Mollar de Elche; these results agreed with previous studies dealing with fresh hydroSustainable pomegranate

fruits (Cano-Lamadrid et al., 2018). Adding the different types of fruit purée significantly affected L^* , a^* and b^* coordinates. The highest lightness (L^*) values were found in the quince- and jujube-pomegranate smoothies, followed by the fig ones. The values of a^* coordinate in quince-pomegranate smoothies were higher than those found in the fig and jujube samples. Finally, the highest b^* values (blue-yellow coordinate) were found in jujube-pomegranate smoothies, followed by those found in the quince and fig products. On the other hand, after 6 months of storage at two temperatures, the intensity of the red color (a^*) significantly decreased, being greater the final color more reddish in samples stored at 4 °C (T1) as compared to those stored at 20 °C. Results agreed with previous studies, which pointed that the enzymatic browning of phenolic compounds was the main reason of the decrease of red color during storage in smoothies based on sour cherry (Nowicka et al., 2016). In a similar trend, the original blue color notes (b^*) of the initial pomegranate smoothies experienced more intense changes when stored at 20 °C as compared to those at 4 °C. As a summary, it can be concluded that ratio purée:juice 40:60, Wonderful pomegranate juice, quince purée and storage 6 months at 4 °C of the assayed smoothies were the conditions leading to a more intense red smoothies.

Total anthocyanin content is well-known as the main parameter behind the red color of pomegranate products. Pearson's correlation coefficient showed that the total anthocyanin content was positively and significantly ($p < 0.05$) correlated in fig, jujube and quince based smoothies with the a^* coordinate values ($R^2 = 0.84$, 0.86, and 0.59 respectively). As seen, the type of smoothies with lower correlation was that of quinces, perhaps because of the pH of the smoothies (Cano-Lamadrid, Trigueros, Wojdyło, Carbonell-Barrachina, & Sendra, 2017); these authors concluded that the food matrix pH controlled the changes in the ANCs color. Besides, a low pH is a favorable environment for the formation of anthocyanin-procyanidin polymers which are more stable than their anthocyanins precursors, maintaining the color during storage (Li & Sun, 2017). For all mentioned above, although fig pomegranate smoothies were more enriched on ANCs after storage, a higher red color was found in quince-pomegranate smoothies.

3.3. Antioxidant capacity by ABTS and FRAP assay

Table 2 shows the results of antioxidant capacity (AC) measured by

Table 4

CIEL**a***b** coordinates in smoothies during storage time as affected by: (i) ratio purée:juice (40:60/60:40), (ii) pomegranate cultivar (W, Wonderful, and Mo, Mollar de Elche), (iii) fruit purée (fig, jujube and quinces), and, (iv) storage temperature during 6 months (4 and 20 °C).

Parameter	ANOVA [†]	Smoothies [‡]											
		F1Mo	F1W	F2Mo	F2W	J1Mo	J1W	J2Mo	J2W	Q1Mo	Q1W	Q2Mo	Q2W
<i>L</i> *	***	35.1d	35.5d	34.1d	34.9d	48.8bc	46.7c	53.5ab	49.4abc	50.8abc	47.7bc	54.8a	52.1abc
<i>a</i> *	***	7.45d	12.1b	7.01d	9.73cd	5.90e	12.1b	3.89f	9.19cd	10.4c	16.2a	9.10cd	13.2b
<i>b</i> *	***	4.61b	4.35b	4.98b	4.63b	13.1ab	11.0ab	17.6a	14.1ab	9.54ab	7.04ab	9.19ab	9.69ab

Parameter	ANOVA [†]	Ratio purée:juice		ANOVA [†]	Pomegranate cultivar		ANOVA	Fruit purée			ANOVA	Storage temperature ^α		
		40:60	60:40		Mo	W		Fig	Jujube	Quince		T0	T1	T2
<i>L</i> *	NS	44.1	46.5	NS	46.2	44.4	***	34.9b	49.6a	51.3a	NS	44.5	46.1	45.3
<i>a</i> *	***	10.7a	8.69b	***	7.30b	12.08a	***	9.08b	7.76c	12.2a	***	14.1a	8.53b	6.45c
<i>b</i> *	***	8.26b	10.6a	NS	10.4	8.46	***	4.64c	13.9a	9.70b	***	6.4c	10.0b	11.8a

[†] NS = not significant F ratio ($p < 0.05$); *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively.

[‡] Values followed by the same letter, within the same factor (ratio purée:juice, pomegranate cultivar, fruit purée and storage time), were not significant different ($p > 0.05$), Tukey's multiple-range test.

^αT0: freshly made; T1: storage 6 months 4 °C; and, T2: storage 6 months 20 °C.

the ABTS⁺ and FRAP assays. The AC was significantly affected by fruit purée and storage time, while no significant effect of ratio purée:juice and pomegranate cultivar were found. There were no drastic changes in the values of the antioxidant capacity after 6 months of storage, with this trend not being correlated with those previously discussed for the main bioactive compounds. Similar results were found in smoothies based on other fruits as apple, quince, orange, and pear at 4 and 30 °C (Andrés, Villanueva, & Tenorio, 2016; Nowicka et al., 2016); these authors explained this trend by the fact that some degradation products also have antioxidant properties, such as Maillard compounds. On the other hand, a higher loss of antioxidant capacity was noticed in smoothies stored at 20 °C as compared to those stored at 4 °C. Recently, this trend was reported in the storage of red and green smoothies (Di Cagno, Minervini, Rizzello, De Angelis, & Gobbetti, 2011) and commercial ones (Nunes et al., 2016).

Pearson's correlation coefficient showed that PP were significantly and positively correlated with FRAP ($R = 0.40$; $p < 0.05$) and ABTS⁺ ($R = 0.44$; $p < 0.05$) data. Besides, anthocyanin content was significantly but negatively correlated with FRAP ($R = -0.47$; $p < 0.05$) and ABTS⁺ ($R = -0.40$; $p < 0.05$) data. No significant ($p < 0.05$) correlation was observed among flavonols and flavan-3-ols with FRAP and ABTS⁺ data.

4. Conclusions

At the beginning (T0), all developed smoothies presented high values of total polyphenolic content (TPC): 247–314 mg/100 g fresh weight (fw), 2939–3920 mg/100 g fw, and 3809–5324 mg/100 g fw, in fig, jujube and quinces pomegranate smoothies, respectively. Regarding higher content of bioactive compounds and attractive color, the best formulation and storage conditions for pomegranate smoothies were ratio purée:juice 40:60, Wonderful pomegranate juice, and storage at 4 °C, being only a reduction of 30.1%, 13.1% and 9.5% in fig, jujube and quinces smoothies, respectively. Moreover, regarding red color, a more intense red color was found in quince-pomegranate smoothies after storage. On the other hand, the type of smoothie which maintained the highest total polyphenolic content [TPC: sum of anthocyanin, flavanols, flavan-3-ols (as monomeric and dimeric), polymeric procyanidins and phenolic acid] was the quince ones. On the other hand, fig pomegranate smoothies had higher contents of anthocyanins, after storage TPC presented a high reduction of them. After checking the quality and functionality of the smoothies developed (blend of minor Mediterranean crops purées with pomegranate juice) and their behavior during storage, the next logical step will be to evaluate their sensory profile and consumer acceptance.

Acknowledgments

Author M.C-L. was funded by a FPU grant (Reference number: FPU15/02158) from the Spanish Ministry of Education. The authors are grateful to the project AGL2013-482-45922-C2-2-R (Ministerio de Economía y Competitividad, Spain). This work has been carried out thanks to a double co-tutelle PhD between WUELS (Poland) and UMH (Spain).

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