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## Wild edible plants as potential antioxidant or nutritional supplements for beverages minimally processed

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### ABSTRACT

Wild edible plants (WEP) with high antioxidant capacity, *Rosa canina* fruits, *Quercus ballota* acorns and *Sanguisorba minor* (young leaves and stems), were added to orange and kiwifruit juices for increase the nutritional properties. The addition of WEP to orange juice (OJ) and kiwifruit cremogenate (KC) does not affect, or it can even improve, some parameters of consumer's acceptance, although the mixtures increasing sweetness and intensifying the matrix colour without changing the tone are the best valued. The beverages minimally processed with higher TSS were the matrices fortified with *Q. ballota*, the same that were considered sweetest by the judges. The addition of WEP to OJ did not significantly change the levels of vitamin C (ascorbic acid plus dehydroascorbic acid). The addition of *R. canina* and *S. minor* increased the content of phenolic compounds and antioxidant capacity in both matrices (OJ and KC) in more than 30%. Beverages with a high interest for consumers and with high antioxidant properties have been obtained.

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### 1. Introduction

During the last years, both food quality and its effects on human health has become a fundamental issue. As a result, many different research works about the functional properties of different foods, plant extracts and natural ingredients have been developed (Serrano et al., 2009). This new awareness have taken food industry to focus the attention on the development of functional or nutraceutical foods with beneficial properties for health (Lavecchia, Rea, Antonacci, & Giardi, 2013). Some years ago, the compounds with nutraceutical activity added to foods were usually chemically produced, but new demands for a sustainable life have made that the food industry is now using natural compounds, mainly those derived from plants. Therefore, we can now find in the market a great variety of functional products consisting of food matrices to which extracts of botanical species with known functional and nutraceutical properties have been added (González-Molina, Moreno, & García-Viguera, 2008).

In this regard, there is a great variety of wild non-cultivated plants that have been employed in the diet and the traditional

medicine of the Mediterranean region (Pardo de Santayana, Tardío, & Morales, 2005), but their use has now decreased, an even disappeared, as a consequence of the industrial revolution. Among them, *Rosa canina* L., *Quercus ballota* Desf. and *Sanguisorba minor* Scop., are good candidates for their study as possible functional ingredients due to the nutraceutical and therapeutic properties that many recent studies have found in them. *R. canina* is a significant source of tocopherols, flavonoids, carotenoids and ascorbic acid (Barros, Carvalho, & Ferreira, 2011) and this composition is probably the reason for its high antioxidant capacity (Egea, Sánchez-Bel, Romojaro, & Pretel, 2010). *R. canina* also presents anti-inflammatory (Lattanzio et al., 2011) and antibacterial activities (Yilmaz & Ercisli, 2011), anticholinesterase capacity (Boğa, Hacibekiroğlu, & Kolak, 2011) and antidiarrhoeal properties (Mandade, Choudhury, Harsulkar, & Wakade, 2011). On the other side, the fruits of *Q. ballota* are rich in  $\alpha$ - and  $\gamma$ -tocopherol and phenolic compounds, especially hydrolysable tannins (Tejerina, García-Torres, Cabeza de Vaca, Vázquez, & Cava, 2011). *Q. ballota* also presents a high antioxidant capacity (Romojaro, Botella, Obón, & Pretel, 2013; Tejerina et al., 2011), and the antioxidant activity of the acorn powder has been even characterised in order to be used as a new natural antioxidant for fats and oils (Lee, Jeong, & Oh, 1992). Regarding *S. minor*, it has been already shown that its young leaves and stems have many and varied phenolic compounds

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that are derivatives of caffeic acid, apigenin, quercetins, kaempferol with a high antioxidant and antimycotic activities (Gatto et al., 2011; Romojaro et al., 2013).

Fruit juices are usually employed as food matrices for the production of functional foods. In fact, recent studies have shown that the beneficial properties of fruit juices can be increased by adding antioxidant sources (González-Molina et al., 2008). Orange (*Citrus cinensis*) is a common raw material for the world fruit juice industry and its juice has a high nutritional value due to bioactive compounds, as well as an appreciated flavour and aroma (Pala & Toklucu, 2013). The kiwifruit has also a great potential for industrial exploitation, mostly due to its composition rich in phytonutrients (Tavarini, Degl'Innocentia, Remorinib, Massaib, & Guidi, 2008). However, the obtaining of a good quality processed kiwifruit is not simple and the possibility to have an industrial valorisation of the fruit is yet away to be found. The main goal of the kiwifruit processing is to obtain safe and stable products able to retain as much as possible the peculiarity of fresh fruit, including green colour, aroma, nutritional value and structural characteristics. One of the main limitations for obtaining kiwifruit minimally processed is the difficulty for maintaining the original green colour which, due to oxidation, quickly acquires brownish tonalities (Benlloch-Tinoco, Igal, Rodrigo, & Martínez-Navarrete, 2013; Cassano & Drioli, 2007).

Food industry is currently focused on the development of novel and minimally processed products with improved quality. This type of products has been traditionally preserved by means of conventional thermal technologies. However, it usually requires the use of high temperatures combined with long process times which has been widely associated with a marked deterioration in food quality, especially with the development of cooked off-flavours, colour alteration and the loss of thermosensitive compounds (Benlloch-Tinoco et al., 2013). For this reason, alternatives to conventional processing technologies are being explored. The addition of extracts of wild plants with high antioxidant capacity could, apart from increasing the nutritional value of the juices, help to stabilize and even improve their valued organoleptic properties.

Considering all the previous background, the main aim is to study if the incorporation of *R. canina*, *Q. ballota* and *S. minor* to orange and kiwifruit juices minimally processed could increase the nutritional properties and stabilize or even improve their organoleptic characteristics.

## 2. Materials and methods

### 2.1. Wild edible plants (WEP)

The non-cultivated and traditionally collected wild edible plants were harvested in the South East (SE) of Spain. Plants were frozen in liquid nitrogen, lyophilized and powdered using a domestic mixer and, finally, stored at 20 °C until further analysis. Wild edible plants used in this study were *R. canina* (fruits), *Quercus ballota* (fruits) and *Sanguisorba minor* (young leaves and stems).

### 2.2. Fruit matrices

For the making of kiwifruit cremogenate (KC), kiwifruit of variety Hayward (*Actinidia deliciosa*) from Galicia (España) was employed. For the making of orange juice (OJ), oranges of variety Navel Lane-Late (*Citrus sinensis*) from Valencia (España) were employed. Both kiwi and orange fruits were purchased at commercial maturity state. The orange juice was obtained using a domestic squeezer (Princesa, Barcelona, Spain) and the kiwifruit cremogenate was obtained using a domestic blender (Osterizer,

Madrid, Spain). Both fruits (kiwi and orange) were processed to make juice at eating ripeness.

### 2.3. Preparation of enriched beverages minimally processed

The lyophilised WEP were added to the fruit matrices: natural orange juice (OJ) and kiwifruit cremogenate (KC) and they were homogenised in polytron (Kinematica AG, Lucerne, Switzerland). Previous experiments performed in our laboratory adding 0.1, 0.5, 1 and 2 g of WEP per 100 mL of matrix, showed that the best organoleptic results were obtained when proportion of 0.5 g 100 mL<sup>-1</sup> was used. Thus this was the proportion used in the subsequent experiments. Homogenised mixtures were filtered in a gauzed filter and stored at room temperature for 24 h. Samples were analysed after filtering (time 0) and after 24 h of storage.

### 2.4. Sensory determination

Sensory evaluation was conducted by a selected and trained panel comprising ten judges (seven women and three men between twenty and fifty years old) in accordance with ISO 8586-1:1993. The tasting room was kept at 20 °C and beverages served in tasting glasses certified and coded. The evaluation was done using 10-point structured scales, 10 being the best and 1 the worst quality (colour, acidity, sweetness and aroma). The general acceptance quality attribute was assessed as the measurement of the acceptability of the product by the consumer using a scale from “very unpleasant” (level 1) to “very pleasant” (level 10). The selection and training of tasters, as well as the fitting-out of the tasting room, were carried out according to Sanchez-Bel, Egea, Romojaro, and Martínez-Madrid (2008). The purchase intent was also evaluated. Results were expressed as percentage of acceptance being 100 the punctuation obtained by the unfortified juice matrix.

### 2.5. Quality parameters (TSS, pH, TA)

The total soluble solids content (TSS) was determined by measuring the refractive index of the juice from the fruit with a digital refractometer (Atago, Tokyo, Japan). TSS content was expressed as percentage. pH was measured in a pHmeter (Crison, Barcelona, Spain). Titratable acidity (TA) was determined by potentiometric titration with 0.1 mol equi L<sup>-1</sup> NaOH to pH 8.1. The results were expressed as grams of malic acid per 100 g of fresh weight.

### 2.6. Colour quality

Colour was measured with a CR-400 colorimeter (Minolta, Paris, France) and results were expressed in CIELAB units of  $L^*$ ,  $a^*$  and  $b^*$ . Hue angle ( $H$ ) and chroma ( $C$ ) were computed from  $L^*$ ,  $a^*$  and  $b^*$  as  $H = \text{tg}^{-1}(b^*/a^*)$  and  $C = \sqrt{(a^*)^2 + (b^*)^2}$ .

### 2.7. Determination of vitamin C (ascorbic and dehydroascorbic acid)

Fruit ascorbate (ASC) content was determined according to Egea et al. (2010) with some modifications; 3 mL of samples homogenised in 10 mL of 5 g 100 mL<sup>-1</sup> cold metaphosphoric acid and stirred continuously for 30 min at 4 °C in darkness, and then centrifuged at 20,000 g for 25 min. The supernatant was passed through a C18 column (Sep-pack plus, Waters, Barcelona, Spain) and a 0.2 µm filter. The filtered supernatant was used for ascorbic acid determination using HPLC (LC-10Atpv, Shimadzu, Barcelona, Spain) with a thermostated ion-exchange column (ION-300) at 30 °C and isocratic elution. The absorbance was recorded with a

UV/vis detector at 245 nm. A standard curve in the range 10–100 mg kg<sup>-1</sup> ascorbic acid was used. The results were expressed as grams of ascorbic acid per 100 g of fresh weight.

Total ascorbate content was determined after reduction of dehydroascorbate (DHA) to ASC with DTT in a final concentration of 30 mmol L<sup>-1</sup> for 8 h in the dark, and then measured as ASC following re-chromatography. Concentration of DHA was estimated from the difference between total ascorbate pool (ASC plus DHA) and ASC.

### 2.8. Determination of total phenolic compounds

Total phenolic compounds were determined according to Serrano et al. (2009) using (3 g) homogenized in 10 mL of 100 mmol L<sup>-1</sup> sodium phosphate buffer containing 150 mmol L<sup>-1</sup> NaCl, pH 7.4 on ice. The concentration of the total phenolic compounds in fruit extracts was determined by comparison with the absorbance of analytical grade phenol standard, gallic acid, at different concentrations. The results were expressed as grams of galic acid per 100 g of fresh weight (FW).

### 2.9. Determination of antiradical activity: Trolox equivalent antioxidant capacity (TEAC assay)

The ABTS<sup>•-</sup> radical anion solution was generated by incubating, at 60 °C for 6 min, a mixture of 2.5 mmol L<sup>-1</sup> 2,2'-azobis(2-amidinopropane) hydrochloride (ABAP) and 20 mmol L<sup>-1</sup> 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS<sup>2-</sup>) stock solution in 100 mL of phosphate buffer (100 mmol L<sup>-1</sup> phosphate and 150 mmol L<sup>-1</sup> NaCl, pH 7.4). Samples (3 g) were homogenized in 10 mL of 100 mmol L<sup>-1</sup> sodium phosphate buffer containing 150 mmol L<sup>-1</sup> NaCl, pH 7.4 on ice. The supernatant was collected as final extract. 40 µL of each extract were mixed with 1960 µL of the radical solution to measure the antioxidant activity at 734 nm after 6 min of incubation at 36 °C. A calibration curve was prepared with different concentrations of Trolox. The TEAC activity was calculated according to Egea et al. (2010) and represents the concentration of Trolox, in µmol L<sup>-1</sup>, that has the same antioxidant capacity as the analysed sample (Trolox equivalent).

### 2.10. Statistical analysis

Tests for significant differences were carried out using the general linear model of the SPSS (version 14.0, SPSS Inc., Chicago, USA) statistical package. Analysis of variance (ANOVA) was conducted using the treatments as a statistical parameter, the confidence level being 95%. Correlations between different parameters were computed as Pearson's correlation coefficient (*r*). When differences were significant, multiple comparisons test was made using least significant difference test (LSD). Values represent the

means ± SD of three replications for each of three sub-samples per sample.

## 3. Results and discussion

### 3.1. Consumers' acceptance

The sensory assessment is the main aspect for designing new foods because changes in aroma, texture or colour of the original matrices can significantly affect the consumer's acceptance. Although the overall impression is a function of many attributes and their interactions, it is interesting to identify the particular attributes that move acceptance. The complexity of food is such that it may well be impossible to truly discern the relative contributions of the different sensory inputs to food acceptance (Janzanti, Machado, & Monteiro, 2011). In this work, the attribute colour was assessed in different ways according to the matrix (OJ or KC) and the added WEP (*R. canina* -Rc-, *Quercus ballota* -Qb- or *Sanguisorba minor* -Sm-) (Table 1). The colour of the OJ fortified with Rc was significantly (*p* < 0.05) better valued than the control one (119.72 ± 5.31), while the addition of Qb did not present differences regarding control OJ (95.87 ± 3.73). The addition of Sm to OJ reduced the redness of the orange juice and this fact was negatively assessed by the consumer (39.14 ± 10.59). Regarding KC, consumers positively assessed the increase of green colour intensity provided by Sm (124.01 ± 8.73). On the contrary, the addition of Rc gave KC a brownish aspect that was significantly worse valued (61.86 ± 9.42) than that of control KC. All matrices were considered less acid than control, regardless of the added WEP. The WEP addition increased the sweetness between 35 and 85% regarding the unfortified matrices. The addition of WEP to OJ and KC decreased the aroma assessment in most mixtures, although not significantly. In our work, and in keeping with other authors (Tepper, 1993) regarding overall impression, colour was the most significant value because the worst assessed mixtures were OJ with Sm and KC with Rc, a fact coincident to the worst assessment of colour. The best valued were OJ with Rc and KC with Sm. Consumers showed a better purchase intent for OJ with Rc and KC with Sm and Qb.

It is worth mentioning the high purchase intent of KC with Qb because it would be expected that this enriched beverage show an increase of an astringent and bitter flavour due to the high content of tannins. However, matrices with Qb obtained a better overall impression and purchase intent than KC. Some authors have stated that the astringent flavour can be modified by the addition of other compounds, and that the sweetness can reduce the astringent sensation. In this study, matrices with Qb were those obtaining the highest punctuation in sweetness, a fact stating that, despite the high content in tannins of Qb, the extra supply of sweetness can avoid the astringent and bitter flavour of the tannins. In general, it

**Table 1**

Consumer acceptance of orange juice (OJ) and kiwifruit cremogenate (KC) after the addition of wild edible plants (WEP). Results were expressed as percentage of acceptance being 100 the punctuation obtained by the unfortified juice matrix.

Matrix with WEP <sup>a</sup>	Colour <sup>b</sup>	Acidity <sup>b</sup>	Sweetness <sup>b</sup>	Aroma <sup>b</sup>	Overall impression <sup>b</sup>	Purchase intent <sup>b</sup>
OJ with Rc	119.72 ± 5.31	87.36 ± 5.81	153.57 ± 17.45	84.76 ± 12.42	103.45 ± 8.35	117.62 ± 9.79
OJ with Qb	95.87 ± 3.73	92.08 ± 7.04	184.72 ± 31.93	83.57 ± 6.08	100.87 ± 9.40	95.24 ± 5.53
OJ with Sm	39.14 ± 10.59	85.92 ± 7.95	155.56 ± 35.44	80.95 ± 11.28	80.00 ± 24.34	89.76 ± 18.6
KC with Rc	61.87 ± 9.42	63.45 ± 10.67	135.75 ± 14.75	95.04 ± 5.72	84.01 ± 8.70	65.43 ± 15
KC with Qb	114.34 ± 6.97	84.64 ± 10.76	157.01 ± 30.06	82.54 ± 10.78	125.1 ± 15.5	115.00 ± 14
KC with Sm	124.01 ± 8.73	75.00 ± 6.96	152.38 ± 35.62	105.79 ± 23.85	129.6 ± 11.8	123.00 ± 17
LSD ( <i>p</i> < 0.05) <sup>c</sup>	11.67	10.67	40.44	17.31	29.09	15.17

<sup>a</sup> Matrix: Orange juice (OJ), kiwifruit cremogenate (KC) fortified with WEP (*Rosa canina* -Rc-, *Quercus ballota* -Qb- or *Sanguisorba minor* -Sm-).

<sup>b</sup> Colour, acidity, sweetness, aroma, overall impression and purchase intent. (100 the punctuation obtained by the unfortified matrix).

<sup>c</sup> LSD (*p* < 0.05), least significant difference, was used for significant difference comparison among means of various samples.

could be concluded that the addition of WEP to OJ and KC does not affect, or it can even improve, some parameters of consumer's acceptance, although the mixtures increasing sweetness and intensifying the matrix colour without changing the tone are the best valued.

### 3.2. Changes in quality parameters

The addition of WEP to matrices (OJ and KC) did not significantly change ( $p < 0.05$ ) the pH or the TA of the mixtures (Table 2). The TA of the new beverages ranged between  $1.66 \pm 0.04$  of OJ with *Sm* and  $1.34 \pm 0.04$  of KC with *Sm*. The pH of the mixtures remained between 3.5 and 4.25, within the levels found by other authors (Barros et al., 2011). However, the TSS with WEP was higher in all cases than in control matrices, and they coincide with the sweetness value given by judges. Therefore, while the TSS of the control OJ did not exceed  $10.67$  °Brix, when the juice was fortified with WEP this parameter was higher, ranging between  $11.11 \pm 0.15$  in freshly made OJ with *Sm* and  $11.83 \pm 0.11$  in OJ with *Qb* after 24 h of storage at  $20$  °C. The TSS of KC was also higher in matrices enriched with WEP than in control KC. While TSS of control KC did not exceed  $13.93$  °Brix, the TSS of the fortified mixtures of kiwifruit ranged between  $14.30 \pm 0.15$  °Brix of freshly made KC with *Sm* and  $14.94 \pm 0.24$  of KC with *Qb* after 24 h of storage at  $20$  °C. In general, the beverages minimally processed with higher TSS were the matrices fortified with *Qb*, the same that were considered sweetest by the judges (Table 2). This increase of TSS is an interesting characteristic because it can increase the sweetness of the beverages without adding sugars and, therefore, avoiding the increase of empty calories (Guenther, Reedy, & Krebs-Smith, 2008), a more and more demanded issue by consumers.

### 3.3. Changes in colour quality

The enrichment of OJ and KC with WEP with high antioxidant capacity can change colour, although it can also contribute to its

**Table 2**  
Acidity, pH and soluble solids of orange juice (OJ) and kiwifruit cremogenate (KC) after addition of wild edible plants (WEP).

Matrix with WEP <sup>a</sup>	Time (h) <sup>b</sup>	TA (g 100 g <sup>-1</sup> )	pH	TSS (Brix)
OJ Control	0	$1.61 \pm 0.02$	$3.61 \pm 0.20$	$10.63 \pm 0.11$
OJ with <i>Rc</i>		$1.54 \pm 0.03$	$3.73 \pm 0.03$	$11.10 \pm 0.12$
OJ with <i>Qb</i>		$1.58 \pm 0.05$	$3.74 \pm 0.07$	$11.43 \pm 0.07$
OJ with <i>Sm</i>		$1.66 \pm 0.04$	$3.45 \pm 0.30$	$11.01 \pm 0.15$
OJ Control	24	$1.65 \pm 0.01$	$3.64 \pm 0.14$	$10.67 \pm 0.22$
OJ with <i>Rc</i>		$1.57 \pm 0.03$	$3.43 \pm 0.02$	$11.30 \pm 0.10$
OJ with <i>Qb</i>		$1.60 \pm 0.04$	$3.39 \pm 0.02$	$10.50 \pm 0.06$
OJ with <i>Sm</i>		$1.63 \pm 0.08$	$3.57 \pm 0.06$	$10.83 \pm 0.09$
LSD ( $p < 0.05$ ) <sup>c</sup>		0.06	0.20	0.18
KC Control	0	$1.40 \pm 0.01$	$3.67 \pm 0.42$	$13.53 \pm 0.12$
KC with <i>Rc</i>		$1.41 \pm 0.06$	$4.07 \pm 0.13$	$14.78 \pm 0.38$
KC with <i>Qb</i>		$1.34 \pm 0.05$	$3.61 \pm 0.26$	$14.38 \pm 0.23$
KC with <i>Sm</i>		$1.34 \pm 0.04$	$4.25 \pm 0.26$	$14.33 \pm 0.07$
KC Control	24	$1.39 \pm 0.02$	$3.6 \pm 0.23$	$13.93 \pm 0.18$
KC with <i>Rc</i>		$1.44 \pm 0.03$	$3.74 \pm 0.29$	$14.87 \pm 0.30$
KC with <i>Qb</i>		$1.37 \pm 0.04$	$3.70 \pm 0.29$	$14.43 \pm 0.24$
KC with <i>Sm</i>		$1.35 \pm 0.03$	$3.64 \pm 0.25$	$14.30 \pm 0.15$
LSD ( $p < 0.05$ ) <sup>c</sup>		0.05	0.39	2.23

Values represent the means  $\pm$  SD of three replications for each of three sub-samples per sample.

<sup>a</sup> Matrix: Orange juice (OJ), kiwifruit cremogenate (KC) fortified with WEP (*Rosa canina* -*Rc*-, *Quercus ballota* -*Qb*- or *Sanguisorba minor* -*Sm*-).

<sup>b</sup> Time (0 h and after 24 h at  $20$  °C).

<sup>c</sup> LSD ( $p < 0.05$ ), least significant difference, was used for significant difference comparison among means of various samples.

stability against oxidation. In OJ, the addition of *Rc* significantly increased ( $p < 0.05$ ) the orange colour of the juice by increasing the redness (CIEa\* value) and the yellowness (CIEb\* value) (Table 3). On the contrary, the addition of *Qb* did not change the CIAa\*, although it increased the lightness (CIEl\* value) and decreased the intensity of the orange colour. The addition of *Sm* significantly changed ( $p < 0.05$ ) colour (CIEa\* more negative than that of control) due to the chlorophyll of leaves and young shoots of this plant that gives the juice a colour less valued by consumers (Table 2). It is worth mentioning that the addition of *Rc* to KC gave a brownish colour to the mixture (CIEa\*  $-4.53 \pm 0.04$  and Hue angle  $105.36 \pm 0.09$ ), coincident with a low value given by consumers (61.87% regarding control). However, the resulting colour of the mixture of *Qb* or *Sm* with KC, with lower CIEa\* and CIEb\* values, was better valued by consumers than that of control (Table 2). The mixtures of different matrices change the profile of the pigments (Lemmens, Van Buggenhout, Van Loey, & Hendrickx, 2010). So WEP with high contents of chlorophyll and brown pigments are more suitable for KC, while those rich in carotenoids (orange and red pigments) are more suitable for OJ in order to obtain a product well valued by consumers.

After 24 h at  $20$  °C, the orange colour of OJ was significantly ( $p < 0.05$ ) less intense, as it can be shown by the decrease of CIEa\* and CIEb\* (Table 3) probably caused by the degradation of  $\beta$ -cryptoxanthin, antheraxanthin and violaxanthin, the main responsible carotenoids for the colour of the orange juice (Carmona, Zacarias, & Rodrigo, 2012). Although the addition of *Rc* to OJ did not avoid the degradation of carotenoids of the juice, the OJ with *Rc* preserved an intense red colour due to the increase in the mixture of  $\beta$ -carotene found in *R. canina* ( $97.77$  mg  $100$  g<sup>-1</sup> DW) (Barros et al., 2011). It is already known that the stability of red colour is a big problem in food processing (Rein & Heinonen, 2004) and, therefore, the addition of *Rc* as colouring agent to certain matrices could compensate for the loss of pigments during the processing and could enrich the juices with other bioactive compounds found in *R. canina* (Barros et al., 2011; Egea et al., 2010). On the other side, the addition of *Qb* to the OJ implied a quicker loss of red colour of the OJ regarding control, while the OJ with *Sm* practically reached the same colour than the control one, it won redness (less negative CIEa\* value). Therefore, it seems that *Qb* potentiates the degradation of the characteristic carotenoids of the orange juice and the OJ potentiates the degradation of the chlorophyll of *Sm*.

The colour of KC changed after 24 h at  $20$  °C, with a significant decrease of the Hue angle (more than  $70^\circ$  of difference between initial and final values), a fact showing a high percentage of darkening (González-Molina et al., 2008). The addition of WEP to KC affects in different ways according to the species. While the addition of *Sm* does not reduce darkening (Hue angle value similar to the control), the addition of *Rc* and *Qb* significantly reduce darkening of KC and, particularly, the addition of *Rc* reduce darkening to half. Therefore, *Qb* and *Rc* could be used as an alternative to synthetic compounds for minimizing the darkening in other products that are minimally processed like vegetables cremogenate. However, the use of these species as anti-darkening agents would need a deeper study because the colour stabilization depends upon the structure and concentration of the pigment, pH, temperature, presence of co-pigments, ascorbic acid, metallic ions, etc (Wicklund et al., 2005).

### 3.4. Changes in vitamin C and polyphenols contents

The addition of WEP to OJ did not significantly change ( $p < 0.05$ ) the levels of vitamin C (ascorbic acid plus dehydroascorbic acid) regarding the non fortified OJ and KC matrices (Fig. 1a, c). The initial levels of vitamin C in OJ were  $124.75 \pm 3.35$  mg  $100$  g<sup>-1</sup> FW, similar

**Table 3**

Colour of orange juice (OJ) and kiwifruit cremogenate (KC) after enrichment with wild edible plants (WEP).

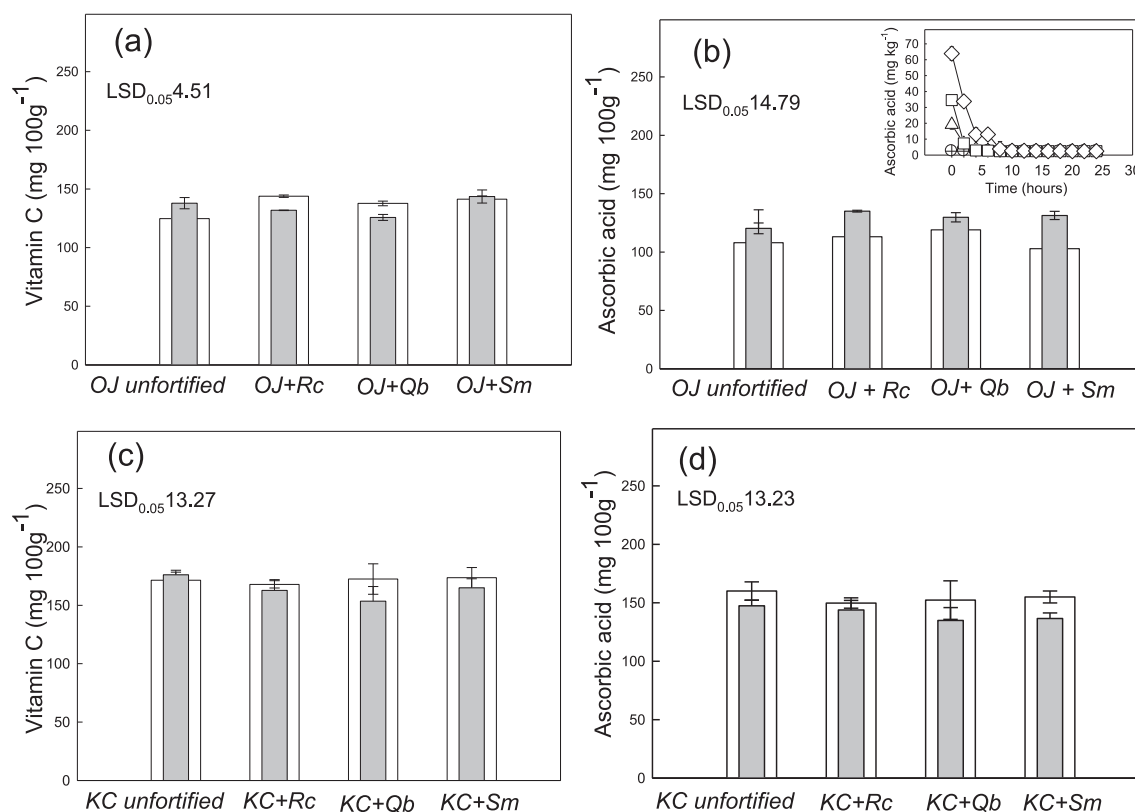
Matrix with WEP <sup>a</sup>	Time (h) <sup>b</sup>	CIE L*	CIE a*	CIE b*	Croma	Hue
OJ Control	0	38.08 ± 0.28	-5.58 ± 0.04	18.47 ± 0.45	19.30 ± 0.44	106.83 ± 0.27
OJ with Rc		39.82 ± 0.19	-2.78 ± 0.01	20.26 ± 0.09	20.45 ± 0.08	97.81 ± 0.02
OJ with Qb		43.41 ± 0.27	-5.38 ± 0.03	22.37 ± 0.31	23.01 ± 0.31	103.53 ± 0.15
OJ with Sm		38.22 ± 0.18	-7.55 ± 0.14	17.24 ± 0.44	18.83 ± 0.45	113.66 ± 0.18
OJ Control	24	38.82 ± 0.12	-5.92 ± 0.12	17.92 ± 0.37	18.87 ± 0.39	108.29 ± 0.21
OJ with Rc		39.58 ± 0.33	-2.96 ± 0.08	19.04 ± 0.18	19.27 ± 0.19	98.82 ± 0.15
OJ with Qb		42.85 ± 0.12	-6.15 ± 0.19	20.80 ± 0.53	21.70 ± 0.53	106.49 ± 0.47
OJ with Sm		38.74 ± 0.78	-5.41 ± 0.24	17.36 ± 0.89	18.18 ± 0.92	107.32 ± 0.18
LSD ( $p < 0.05$ ) <sup>c</sup>		0.49	0.18	0.65	0.66	0.33
KC Control	0	43.75 ± 0.70	-7.41 ± 0.08	16.12 ± 0.22	17.74 ± 0.22	114.69 ± 0.32
KC with Rc		42.70 ± 0.03	-4.53 ± 0.04	16.48 ± 0.21	17.09 ± 0.22	105.36 ± 0.09
KC with Qb		47.08 ± 0.12	-7.76 ± 0.05	18.06 ± 0.20	19.60 ± 0.21	113.32 ± 0.11
KC with Sm		42.41 ± 0.50	-9.14 ± 0.17	18.75 ± 0.12	20.86 ± 0.15	115.98 ± 0.38
KC Control	24	38.96 ± 0.27	-2.77 ± 1.23	11.39 ± 0.38	19.25 ± 1.00	44.64 ± 1.82
KC with Rc		39.00 ± 0.36	-2.27 ± 0.27	13.24 ± 0.26	32.34 ± 7.32	71.84 ± 12.81
KC with Qb		43.42 ± 0.41	-4.42 ± 0.10	13.26 ± 0.62	34.29 ± 7.61	62.81 ± 16.06
KC with Sm		39.00 ± 0.36	-2.27 ± 0.27	13.24 ± 0.26	18.26 ± 1.31	48.49 ± 11.03
LSD ( $p < 0.05$ ) <sup>c</sup>		0.55	0.65	0.44	5.28	11.55

Values represent the means ± SD of three replications for each of three sub-samples per sample.

<sup>a</sup> Matrix: Orange juice (OJ), Kiwifruit cremogenate (KC) fortified with WEP (*Rosa canina* -Rc-, *Quercus ballota* -Qb- o *Sanguisorba minor* -Sm-).<sup>b</sup> Time (0 h and after 24 h at 20 °C).<sup>c</sup> LSD ( $p < 0.05$ ), least significant difference, was used for significant difference comparison among means of various samples.

to those found by other authors (Tarrago-Trani, Philips, & Cotty, 2012). Although the addition of WEP increases vitamin C between 10 and 15%, it was not a significant increase. Vitamin C in KC did not change regarding control when WEP were added; the levels

ranged around 170 mg 100 g<sup>-1</sup> FW, similar to those found by other authors (Celik, Sezai, & Turgut, 2007). The stability of vitamin C in matrices (OJ and KC) at 20 °C during 24 h did not significantly change ( $p < 0.05$ ). So, in general, the addition of WEP to OJ and KC



**Fig. 1.** Changes in levels of total ascorbate (Vitamin C) and ascorbic acid content of orange juice (OJ), (figures a and b) and Kiwifruit cremogenate (KC) (figures c and d) fortified with WEP (*Rosa canina* -Rc-, *Quercus ballota* -Qb- o *Sanguisorba minor* -Sm-) after filtering, time 0 (□) or after 24 h (■) at 20 °C. Figure 1b embedded shows the degradation rate of ascorbic acid in aqueous solution, control (○), 20 (△), 40 (◻) and 60 (◇) mg kg<sup>-1</sup> ascorbic acid under the same tested conditions as OJ and KC (24 h at 20 °C). LSD ( $p < 0.05$ ), least significant difference, was used for significant difference comparison among means of samples. Values represent the means ± SD of three replications for each of three sub-samples per sample.

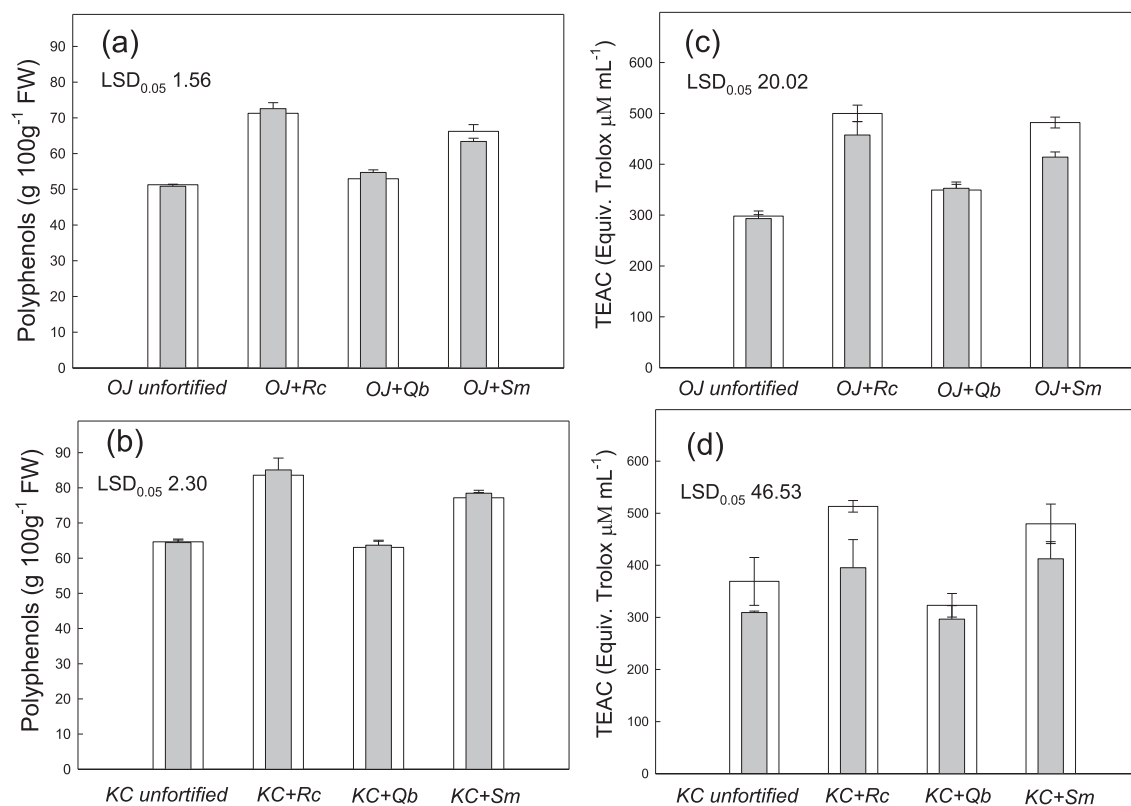
did not significantly change the content of vitamin C or increase its stability under tested conditions. For nutritional purposes, vitamin C is the sum of AA and dehydroascorbic acid (DHAA); DHAA absorbed from the intestinal tract undergoes intracellular reduction (via NADPH- and glutathione-dependent reductases), rendering it biologically active (González-Molina et al., 2008). It must be mentioned that when the matrix is OJ, the proportion of AA (Fig. 1b) after 24 h at 20 °C significantly increased ( $p < 0.05$ ). AA is susceptible to oxidation in aqueous solution; therefore, temperature, light, pH, the presence of oxygen, metal catalysts (iron, copper) and enzymes such as ascorbate oxidase affect the rate of AA degradation, at a variable rate in different foods (Johnston, Steinberg, & Rucker, 2007). It was verified (Fig. 1b embedded) that, in aqueous solution and under tested conditions (24 h at 20 °C), the speed of degradation of the ascorbic acid is very high during the first hours, regardless the concentration of AA of the initial solution.

The ascorbic acid in OJ or KC (Fig. 1b, d) did not significantly degrade ( $p < 0.05$ ) after 24 h at 20 °C. The TA and the pH of the matrices contribute to the AA stability because at low pH AA is fully protonated and relatively stable, with maximal stability at 4–6; at  $pH > 6$ , AA is reversibly oxidized to DHAA, and DHAA may be further irreversibly oxidized to 2,3-diketo-l-gluconic acid, followed by degradation to other by-products (Johnston et al., 2007). On the other side, OJ is a rich source of flavanols, including hesperidin and narirutin, which are also main phenolic compounds of OJ (Proteggente et al., 2002). Moreover, kiwifruit is rich in polyphenols compared with other fruits (Park et al., 2006). The addition of Rc and Sm increased the content of phenolic compounds in both matrices (OJ and KC) in more than 30% due to the fact that both Rc and Sm have a high concentration of these compounds (Barros

et al., 2011; Egea et al., 2010). However, the addition of Qb did not significantly change the levels of polyphenols (Fig. 2a, b). The high concentration of tannins found in Qb could cause the precipitation of tannin acids or tannin-protein complexes responsible for the astringent flavour of the tannins, a flavour that is disgusting to the consumer (Tejerina et al., 2011). The phenolic compounds remained stable during 24 h at room temperature in all matrices.

### 3.5. Changes in Trolox equivalent antioxidant capacity (TEAC) and relationship with phytonutrient levels

One of the main quality attributes of juices is their antioxidant capacity and, during the last years, this has been a very solid argument for the professionals of the food industry. Fig. 2 (c and d) shows the antioxidant capacity (TEAC) of OJ and KC, respectively, after their enrichment with different WEP. The initial antioxidant capacity of KC was higher than that of OJ ( $359.04 \pm 46$  and  $298.07 \pm 10.2$  equi Trolox  $\mu\text{M L}^{-1}$ , respectively). There is a controversial debate in the literature about the influence of vitamin C on the antioxidant capacity of fruits or vegetables (Guo et al., 2003; Tavarini et al., 2008). It is also known that fruits with high antioxidant capacity generally contain more antioxidants and most of these antioxidants has been showed to be phenolic compounds and, in particular, flavonoids (Guo et al., 2003). According to some authors (Proteggente et al., 2002; Tavarini et al., 2008), the antioxidant capacity of OJ mainly results from its ascorbic acid content, while flavanon glycosides have a limited contribution to its antioxidant capacity. In our work, the addition of Qb did not significantly increase the antioxidant capacity of the matrices (OJ and KC), while the addition of Rc increased in 67 and 39% the antioxidant capacity of OJ and KC, respectively. The antioxidant capacity of OJ



**Fig. 2.** Changes in levels of total polyphenol content (figures a and b) and Trolox Equivalent Antioxidant Capacity (TEAC) (figures c and d) of orange juice (OJ), (figures a and c) and Kiwifruit cremogenate (KC) (figures b and d) fortified with WEP (*Rosa canina* -Rc-, *Quercus ballota* -Qb- o *Sanguisorba minor* -Sm-) after filtering, time 0 (a) or after 24 h (■) at 20 °C. LSD ( $p < 0.05$ ), least significant difference, was used for significant difference comparison among means of samples. Values represent the means  $\pm$  SD of three replications for each of three sub-samples per sample.

**Table 4**

Analysis of correlations between total antioxidant activity (TEAC), total polyphenols content and vitamin C in the prepared mixtures.

	Antioxidant capacity (TEAC)			
	OJ <sup>0 h</sup>	KC <sup>0 h</sup>	OJ <sup>24 h</sup>	KC <sup>24 h</sup>
Phenolics	0.9620 <sup>MS</sup>	0.9669 <sup>MS</sup>	0.9500 <sup>MS</sup>	0.9280 <sup>MS</sup>
Vitamin C	0.3095	0.5220	0.1988	0.2091

<sup>MS</sup> Marginally significant ( $P > 0.05$ ).

and KC with *Sm* also increased in 60 and 30%, respectively. Coincident to our results, some studies have shown that the activity in the TEAC assay increases with the number of hydroxyl groups of the antioxidant compound, so the antioxidant activity is higher for phenolic substances than for ascorbic acid, in which only one hydroxyl group reacts under experimental conditions (Hodisan, Socaciu, Ropan, & Neamtu, 1997). This relation between total phenols and the antioxidant activity has been found in many fruits and all their derived products (Egea et al., 2010), a fact suggesting that the phenolic compounds could be responsible for the antioxidant properties of the new beverages fortified with *Rc* and *Sm* because there is a good correlation (Table 4) between the content in polyphenols of the new beverages and the antioxidant capacity and, however, there is a very low correlation between the content in vitamin C and the antioxidant capacity of the mixtures.

However, when the new beverages were stored during 24 h at 20 °C the antioxidant capacity of the beverages fortified with *Rc* and *Sm* significantly decreased, between 10 and 15% in OJ and between 15 and 25% in KC. This decrease could be due to reactions of polymerization, condensation or complexation that can cause the formation of new compounds with a lower antioxidant capacity (González-Molina et al., 2008). Moreover, the presence of other antioxidant compounds from *Rc* and *Sm*, such as proteins, which we have not analysed, could react in the mixtures decreasing the in vitro antioxidant values (González-Molina et al., 2008). The antioxidant properties of food matrices are due to the presence in complex mixtures of compounds of varying polarity, such as vitamin C, vitamin E, carotenoids and polyphenols. Due to the potential synergistic action of all these bioactive compounds present in food (Egea et al., 2010) the total antioxidant capacity of some foods has increasingly become a sales argument for professionals in the food industry.

#### 4. Conclusion

The beverages that result from the addition of wild edible plants with high antioxidant capacity to orange juice or kiwifruit cremogenate are well accepted by consumers, even improving the acceptance of the fresh product, and they improve the content in phenolic compounds and the antioxidant capacity without changing the physico-chemical properties of the matrices. This better acceptance of enriched beverages minimally processed by the consumer was due, to a great extent, to the intensification of the natural colour (in the case of addition of *Rc* to OJ and *Sm* to KC) and to the extra sweetness provided mainly by *Qb* and, to a lesser extent, by *Rc* because the addition of wild edible plants to the matrices increased their content in soluble solids. Beverages minimally processed with a high interest for consumers and with high antioxidant properties have been obtained.

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