PAPER

BEVERAGES BASED ON RICOTTA CHEESE WHEY AND FRUIT JUICES

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ABSTRACT

For studying antioxidants, sugars and organic acids compositions and their impact on Sourness Index (SI) and Total Sweetness Index (TSI), beverages were produced by blending Ricotta-cheese whey (RCW), by-product of Ricotta cheese production, with fruit juices. Pear-RCW had higher malic acid and sorbitol, blueberry-RCW higher citric acid, total phenolics and anthocyanin pigments, apple-RCW higher sucrose and fructose, and strawberry-RCW higher glucose. Blueberry-RCW had the highest SI and the lowest TSI, while apple-RCW had the lowest SI and the highest TSI. Higher quality beverages may be obtained by using apple juice ('Yellow' type) and the apple:blueberry (50:50) blend ('Red' type).

Keywords: monomeric anthocyanin pigment, percent polymeric color, sourness index, total phenolic compounds, total sweetness index

1. INTRODUCTION

The whey-based fruit juice drinks could be considered novel functional beverages in which the nutraceutical components coming from fruit are combined with those of whey, so strengthening the functional value of the resulting product (HÖZER and KIRMACI, 2010). By changing the type of fruit juice used in the formulation, the functional properties of the beverage can be modulated. The presence of anthocyanins, flavonols, catechins and phenolic acids in blueberries and strawberries has been related to the prevention of oxidative stress by scavenging reactive oxygen species and free radicals (BRAMBILLA *et al*., 2008; ZAFRA-STONE *et al*., 2007; GRANATO *et al*., 2016), and risks reduction of several diseases, such as cardiovascular diseases and cancer (NAVINDRA, 2010). Pear juice, being rich in hydroxycinnamic acids, glycosides of the flavonols quercetin and isorhamnetin, catechins and procyanidin polymeric units with chain lengths of up to 25 units, has a potent antioxidant power, and its singlet oxygen quenching abilities have been linked to its protective activities against both human erythrocyte lyses and protein oxidation (FERREIRA WESSEL *et al*., 2016). Apple juice, containing polyphenols, including flavonoids (quercetin glycosides, procyanidins, epicatechins, chlorogenic acids, and phloretin glycosides) and phenolic acids, and apple fruit may reduce the risk of chronic disease by various mechanisms, including antioxidant, antiproliferative, and cell signaling effects (HYSON, 2011).

Several researches have focused on the development of whey-based fruit blends with various formulations exploiting acid whey and sweet rennet-based whey in order to select the optimal mixture based on sensory perception (DJURIĆ *et al*., 2004; GOUDARZI *et al*., 2015; SAKHALE *et al*., 2012), as the poor sensory profile of whey protein beverages still remains a challenge to consumer acceptance.

RIZZOLO and CORTELLINO (2017) replaced whey with Ricotta-cheese whey (RCW, *scotta*), the main by-product of Ricotta cheese production obtained after flocculation of whey proteins induced by thermal treatment of cheese whey at 85-90°C for about 20 min, and produced 'yellow' and 'red' fruit-RCW-based beverages (juice/RCW ratio: 80/20, 14% soluble solids content), using apple, pear, blueberry and strawberry pectin-free juices. Furthermore, CORTELLINO *et al*. (2015) showed that the type of fruit juice used in the RCW-based beverage impacted on sensory perception, and suggested blending the blueberry juice with the apple ones in order to buffer the high sourness of the blueberry-based drink, which on the other hand was preferred for color, and at the same time to enrich the apple-based drink, which was preferred for taste, with anthocyanin compounds from blueberry.

In this study, we investigated how antioxidants, sugars and organic acids compositions of the RCW-fruit juice beverages could be modulated by changing the type of fruit juice used in the formulation, considering pear-RCW and apple-RCW beverages for the 'yellow' type and blueberry-RCW, strawberry-RCW, apple:blueberry 50:50-RCW; apple:blueberry 75:25-RCW beverages for the 'red' ones.

2. MATERIALS AND METHODS

2.1. Preparation of RCW-fruit juice beverages

The RCW-fruit juice beverages have been prepared from a frozen cow RCW (dairy industry of Lombardy region) and four types of frozen concentrated clear fruit juices without any preservers and colorants addition (G. Mariani & C. S.p.A., Brescia, Italy) according to the process flow diagram described by RIZZOLO and CORTELLINO (2017).

Chemical composition, nitrogen distribution and mineral composition of RCW were reported in Table 1 (MONTI *et al*., 2015).
Before blending, RCW was thawed at 2°C for 24 h and filtered in order to remove the majority of fat, whereas the concentrated clear fruit juices (65 % pectin-free strawberry, 65 % pectin-free blueberry, 70 % pear and 70 % apple) were thawed and diluted to 16.6 % soluble solids content for the preparation of the following beverages: apple-RCW, pear-RCW, strawberry-RCW, blueberry-RCW, apple:blueberry (50:50, v/v)-RCW and apple:blueberry (75:25, v/v)-RCW drinks. Hereafter the two formulations containing a blend of apple and blueberry juices are referred to as RCW-Mix50:50 for apple:blueberry (50:50, v/v)-RCW drink and RCW-Mix75:25 for apple:blueberry (75:25, v/v)-RCW beverage.

Table 1. Chemical composition, nitrogen distribution and mineral composition of RCW. Adapted from MONTI *et al*. 2015.

Chemical composition (g/100 g)				Mineral composition (mg/kg)			
pH	6.06	Lactose	2.72	K	1023	P	218.00
Total solids	4.16	Glucose	0.01	Ca	267.00	Mg	158.00
Ash	0.45	Galactose	0.10	Fe	0.48	Cu	0.07
Fat	0.05	Citric acid	0.14	Mn	< 0.01	Se	0.008
Total proteins	0.39	Lactic acid	0.13	Na	881.00	Zn	0.40
Nitrogen distribution							
%	g/100 g		g/100 g		%		
NPN/NT	True protein (NT-NPN)*6.38	Denatured protein (NT-NCN)*6.38	α-la	β-lg	α-la+β-lg	CMP	Peptides
45.9	0.21	0.05	0.02	0.046	37.7	24.0	38.2

NT = total nitrogen; NPN = non-protein-nitrogen; NCN = non-casein-nitrogen; α-la = α-lactalbumin;
β-lg = β-lactoglobulin; CMP = casein macropeptide.

Beverages were packed in 125 mL uncolored glass bottles capped with twist-off lid. In order to obtain beverages standardized at about 14 % of soluble solids content, 16.6 % fruit juices and RCW were blended in 80:20 (v/v) ratios separately for every bottle. Six bottles per juice type were divided into two lots and each lot (3 bottles/lot) was separately pasteurized by autoclaving (Ghizzoni, Parma, Italy). Pasteurization temperatures were detected in the autoclave and at the core of the bottle by means of flexible thermocouple probes and monitored with E-Val 2.10 software system; for strawberry, apple and blueberry based beverages, characterized by pH<4.2, the lethal rate F_100^10 value was 11 while for pear beverage, having pH>4.2, an F_100^10=16 value was adopted. After pasteurization bottles were kept for 15 days on open shelves at ambient temperature (20°C); then 50 mL aliquots from every bottle were frozen at −20°C till analysis.
For each beverage, 3 bottles per pasteurization lot (6 replicates/beverage) were analyzed for physical-chemical parameters, (pH, titratable acidity, soluble solids content, color) soon after the 15 days of shelf life at 20°C, and for soluble sugar and organic acid compositions, phenolics, anthocyanins, polymeric color and total antioxidant capacity after overnight thawing at 2°C of the frozen samples. Each bottle was analyzed separately (1 bottle=1 replicate).

2.2. Physical-chemical analyses

The pH values and titratable acidity (TA) were determined with a titroprocessor (model 682, Metrohm AG, Switzerland) by titrating with 0.1 mol/L NaOH to pH=8.2. Color parameters were measured by means of a Spectrophotometer CM-2600 (Minolta, Japan) equipped with a sample holder for 10 mm-plastic cells suited for liquid analysis, using the primary illuminant D65 (CIE, 2006) and 2° observer in the L^*, a^*, b^* color space. Specular component included (SCI) mode was selected and a white calibration tile was used as background. From L^*, a^*, b^* values, chroma (C^*) and hue ($h°$) were computed according to:

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (1)$$

$$h° = \text{arctangent}\ (b^*/a^*) \times 360/\ (2\times3.14) \quad (2)$$

with $h°$=90° corresponding to the yellow color and $h°$= 360° to the violet one.

2.3. Determination of total phenolic content (TPC) and total anthocyanin pigments (MAP)

Total phenolic content (TPC) was measured spectrophotometrically by the Folin-Ciocalteau assay (SINGLETON and ROSSI, 1965) and was expressed as gallic acid equivalents (mg GAE/100 mL). Total monomeric anthocyanin pigment (MAP) was estimated spectrophotometrically by the pH-differential method (GIUSTI and WROLSTAD, 2001) and was expressed as cyanidin 3-glucoside equivalents (mg C3GE/100 mL). Color density (CD) and percent polymeric color (PPC) were measured according the bisulphite bleaching method described by GIUSTI and WROLSTAD (2001).

2.4. Determination of antioxidant capacity

The antioxidant activity (AntOx) was measured spectrophotometrically by using the stabilized artificial free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the method described by LO SCALZO *et al*. (2004) and was expressed as gallic acid equivalents (mg GAE/100 mL).

2.5. Soluble sugar and organic acid compositions

Soluble sugars were quantified by HPLC (FORNI *et al*., 1992) using a Rezex™ RPM-monosaccharide Pb^{+2} (8%) (300x7.8 mm, Phenomenex) column.
Organic acids were analyzed on a Hypersil Gold (5 μm, 4.6x250 mm, Thermo Scientific) column kept at 30°C with a variable wavelength detector (UV-1575) set at 210 nm, using as mobile phase 0.02 M orthophosphoric acid at the flow rate of 0.7 mL/min.
Data of sugar composition were expressed as g/100 mL of beverage, whereas those of organic acids as mg/100 mL of beverage.

2.6. Sourness Index and Total Sweetness Index

The sour taste of each beverage was estimated by means of Sourness index, taking into account the fact that, within the same pH level, sourness increases with increasing acid concentration (SOWALSKI and NOBLE, 1998).

Hence, Sourness Index was computed according to the following equation:

$$Sourness\ Index = C_{mal} \times a + C_{cit} \times b + C_{fum} \times c + C_{lac} \times d \quad (3)$$

where: C_{mal}, C_{cit}, C_{fum} and C_{lac} are the amounts of malic, citric, fumaric and lactic acids, respectively, expressed in g/100 mL, whereas a, b, c and d are the estimated sourness units (Table 2) of malic, citric, fumaric and lactic acids, respectively, at the pH value of each beverage.

Table 2. Estimated sourness units of malic acid (a), citric acid (b), fumaric acid (c) and lactic acid (d) at the pH value of each beverage used for computation of Sourness Index. The values are taken from the plot estimated sourness in units from ratio scales of MOSKOWITS (1971) vs pH in the 3.0-4.4 range reported by SORTWELL (2005).

	a	**b**	**c**	**d**
Apple	3.2	3.3	1.8	2.5
Pear	2	2	1	1
Strawberry	4.1	3.6	2.5	4
Blueberry	7	5.3	4	8
Mix 50:50	5.3	4.7	3	6
Mix 75:25	5	4.1	2.8	5

Total Sweetness Index (TSI) was estimated by multiplying the amounts of each sugar by its relative sweetness respect to sucrose according to the following equation:

$$TSI = C_{suc} \times 1.00 + C_{glu} \times 0.45 + C_{fru} \times 0.91 + C_{sorb} \times 0.37 + C_{lact} \times 0.22 \quad (4)$$

where: C_{suc}, C_{glu}, C_{fru}, C_{sorb} and C_{lact} are the average amounts of sucrose, glucose, fructose, sorbitol and lactose, respectively, expressed in g/100 mL, multiplied for the relative sweetness values respect to sucrose as determined by MOSKOWITZ (1970) from sweetness power functions related to the weight of solute in solution with the exponent fixed at 1.3.

2.7. Statistical analysis

The Statgraphics v.5.2 (Manugistic Inc., Rockville, MD, USA) software package was used. Data were submitted to one-way analysis of variance (ANOVA) and means were compared by Tukey's test ($P<0.05$).

3. RESULTS AND DISCUSSION

3.1. Physical-chemical analyses

Table 3 shows the physical-chemical parameters of the six types of RCW-fruit juice beverages. As expected, a clear influence of the type of fruit juice used as ingredient in the beverage formulation was found for pH, TA and color parameters. The two 'Yellow' formulations had higher pH and lower TA than the four 'Red' drinks, with the apple-RCW drink having lower pH and higher TA than the pear-RCW ones. Strawberry-RCW beverage had higher pH than all the three formulations based on blueberry juice, and a TA amount intermediate between that of blueberry-RCW drink and the two apple:blueberry-based beverages. Considering the formulations based on blueberry juice, pH increased and TA decreased as the proportion of apple juice added increased (Table 3). Color parameters indicated that 'Yellow' beverages had lighter and more vivid color (higher L^* and C^*) than the four 'Red' beverages. The pear-RCW drink had a yellower (hue value more close to 90°) and lighter (higher L^*) color than apple-RCW drink, while all the 'Red' beverages had a very dark and grayish color ($L^*<25$ and $C^*<2.5$), with blueberry-RCW and RCW-Mix50:50 drinks having lower L^* and C^* than RCW-Mix75:25 beverage. The 'Red' beverages, in addition, differed for hue, which indicated a violet-red color for strawberry-RCW drink ($h \approx +15°$) and a red-violet one for blueberry-RCW beverage ($h \approx -9°$). The blending of blueberry juice with apple juice provoked a shift of hue towards the violet-red color, with the higher change at the higher proportion of apple juice (apple:blueberry 50:50, $h \approx -2°$; apple:blueberry 75:25: $h \approx +5°$). A similar scenario on the relationship between proportion of apple juice added to a fruit juice containing anthocyanins and shift of hue from red violet to violet red has already been reported also for blends of clear apple juice with clear sour cherry juice (NANI *et al.*, 1991) and blackcurrant juice (NANI *et al.*, 1993).

Table 3. Physical-chemical parameters (TA, titratable acidity; L^* lightness; C^* chroma; $h°$, hue angle) of RCW-fruit juices beverages. Data are mean±standard error (n=6). Means within columns having different letters are significantly different (Tukey's test); *P*-value, significance of *F* ratio.

	pH	TA (meq/100 mL)	L^*	C^*	$h°$
Apple	3.80±0.01 b	6.77±0.03 e	44.4±0.07 b	25.1±0.05 b	76.6±0.1 e
Pear	4.49±0.05 a	3.34±0.05 f	52.2±0.08 a	25.8±0.05 a	85.2±0.0 f
Strawberry	3.61±0 c	15.80±0.14 b	25.2±0.03 c	2.2±0.05 c	15.6±0.4 d
Blueberry	3.19±0.04 e	19.23±0.10 a	24.6±0.05 e	1.4±0.05 d	351.3±1.4 c
Mix 50:50	3.35±0.01 d	13.28±0.04 c	24.5±0.07 e	1.4±0.02 d	357.9±2.3 b
Mix 75:25	3.45±0.02 d	11.02±0.04 d	24.8±0.01 d	2.4±0.18 c	364.7±0.3 a
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

3.2. Sugar and organic acid compositions

Both sugar and organic acid compositions (Tables 4 and 5) were clearly influenced by the ingredients. The sugar and organic acid compositions of the RCW were analyzed according to the methods described in section 2.5 suitable to quantify components from fruit juices. The following data were obtained (mean±standard error, n=3): lactose, 2.08±0.07 g/100 mL; lactic acid, 0.41±0.03 g/100 mL; acetic acid, 0.13±0.002 g/100 mL; citric acid, 0.11±0.005 g/100 mL; fumaric acid, 0.53±0.02 mg/100 mL, whereas glucose and galactose were present in trace levels, below the limit of quantification of the method. In agreement with these data, and taking into account the proportion of RCW used in all the formulations (20 %), in all the beverages it was not possible to quantify galactose and acetic acid from RCW, and, as expected, the amounts of lactose (approx. 416.7±26.4 mg/100 mL) and lactic acid (approx. 66.8±2.9 mg/100 mL) did not differ among formulations (Tables 4 and 5).

Table 4. Amounts of organic acids (mg/100 mL) of RCW-fruit juices beverages. Data are mean±standard error (n=6). Means within columns having different letters are significantly different (Tukey's test); *P*-value, significance of F ratio.

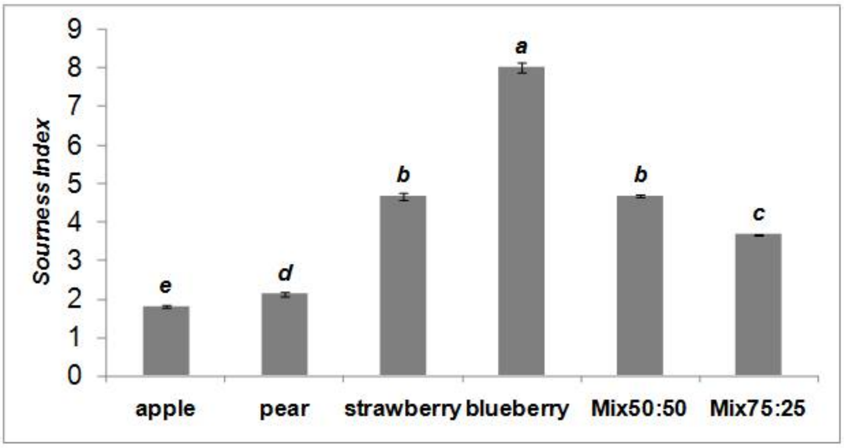
	Malic acid	Lactic acid	Citric acid	Fumaric acid
Apple	483.5±6.8 b	69.0±2.9 a	29.5±0.4 e	0.34±0.01 b
Pear	1,004.0±24.5 a	63.8±2.4 a	28.3±1.7 e	0.41±0.05 b
Strawberry	375.9±9.6 de	66.3±2.2 a	792.6±16.8 b	1.62±0.18 a
Blueberry	351.4±8.3 e	69.9±1.8 a	938.8±9.3 a	1.60±0.36 a
Mix 50:50	425.5±0.8 cd	69.0±3.2 a	424.1±4.5 c	0.41±0.01 b
Mix 75:25	442.3±3.1 bc	63.0±3.4 a	278.4±4.9 d	0.23±0.01 b
P-value	< 0.001	ns	< 0.001	< 0.001

Table 5. Amounts of sugars (mg/100 mL) of RCW-fruit juices beverages. Data are mean±standard error (n=6). Means within columns having different letters are significantly different (Tukey's test); *P*-value, significance of F ratio.

	Sucrose	Glucose	Fructose	Sorbitol	Lactose
Apple	1,219±10 a	3,362±40 c	7,206±76 a	362±8 b	392±15 a
Pear	1,039±12 b	2,941±30 d	5,880±65 d	3,286±40 a	433±12 a
Strawberry	235±11 e	4,484±116 a	6,581±176 bc	247±10 c	402±16 a
Blueberry	4±2 f	4,505±119 a	6,022±168 cd	37±2 d	396±13 a
Mix 50:50	489±16 d	4,184±99 ab	7,016±150 ab	209±4 c	461±15 a
Mix 75:25	717±11 c	3,928±72 b	7,172±127 b	266±8 c	416±13 a
P-value	< 0.001	< 0.001	< 0.001	< 0.001	ns

As for the other organic acids (Table 4) and sugars (Table 5), the amounts and proportions were in agreement with those from the literature for the four fruit species (EISELE and DRAKE, 2005 for apple; SHA *et al*., 2011 and VIERA ARROYO *et al*., 2013 for pear; KALLIO *et al*. 2000 for strawberry; RIZZOLO *et al*., 2002 and BETT GARBER *et al*., 2005 for blueberry) and, hence, mirrored the characteristic of the type of fruit juice used in the formulation. Our results on organic acid compositions showed that apple-RCW and pear-RCW beverages had higher malic acid content than strawberry-RCW and blueberry-RCW ones, with pear-RCW showing the highest amount (approx. 1 g/100 mL). Blueberry-RCW beverage had the highest amount, and strawberry-RCW one the second highest amount of citric acid and both these red beverages were characterized by the highest fumaric acid contents (approx. 1.6 mg/100 mL). The blending of blueberry juice with apple juice induced an increase in malic acid and a decrease in citric acid contents, with the major changes observed in the RCW-Mix75:25 drink, but not significant changes in fumaric acid amounts. Considering the sugar composition (Table 5), apple-RCW beverage had the highest amounts of sucrose and fructose, while pear-RCW drink the second highest amount of sucrose, about a tenfold quantity of sorbitol than all the other formulations, and the lowest amounts of glucose and fructose; strawberry-RCW drink was characterized by the highest amount of glucose and about one fifth of sucrose concentration of apple-RCW and pear-RCW formulations. In contrast, blueberry-RCW beverage had very low amounts of sucrose (<10 mg/100 mL) and sorbitol (<40 mg/100 mL) and glucose as much as strawberry-RCW drink. The blending of blueberry juice with apple juice induced an increase of sucrose, fructose and sorbitol and a decrease in glucose, with the major changes observed in the RCW-Mix75:25 drink.

The different organic acid composition coupled to a different pH value and the different sugar composition exerted an influence on sour taste intensity and total sweetness of beverages. In fact it has been reported that, within the same concentration level, sourness intensity increases with decreasing pH, as well as that, within the same pH level, sourness increases with increasing acid concentration (SOWALSKY and NOBLE, 1998); furthermore, DA CONCEICAO NETA *et al*. (2007) found that sour taste intensity was linearly related to the molar concentration of hydrogen ions and to the molar concentration of all organic acid species that have at least one protonated carboxyl group, and, hence, that weaker organic acids were more sour at a given pH than a stronger organic acid because a larger fraction of the carboxyl groups were protonated. On these bases, in order to have a rough estimation of sourness for each RCW-fruit beverage, the Sourness Index, computed according to the Eq. (3) reported in section 2.6, was used (Fig. 1, top). Basing on this index RCW-fruit beverages can be ordered from the most to the less sour as following: blueberry > strawberry = apple:blueberry 50:50 > apple:blueberry 75:25 > pear >apple. As for sugars, in order to have an estimate of the sweetness for each RCW-fruit formulation, the Total Sweetness Index, calculated according to the Eq. (4) reported in section 2.6, was used (Fig. 1, bottom).



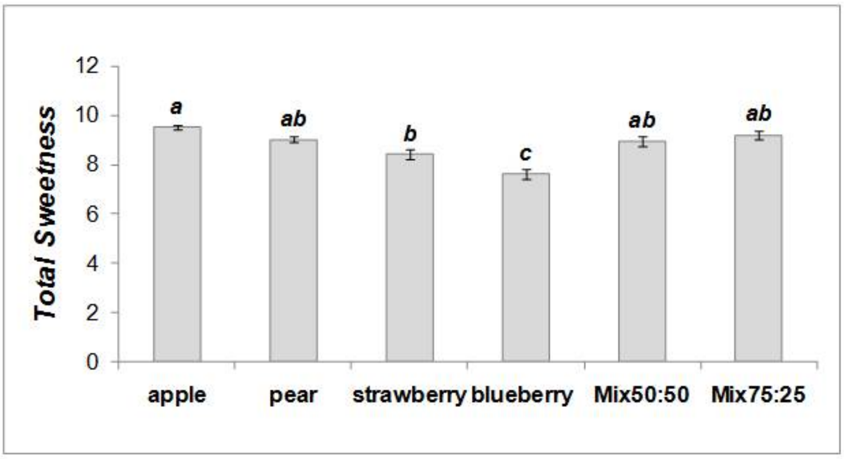


Figure 1. Sourness Index (top) and Total Sweetness Index (bottom) of RCW-fruit juices beverages. Bars refer to standard error (n=6). Sample abbreviations: Mix 50:50, RCW-apple:blueberry (50:50 v/v); Mix 75:25, RCW-apple:blueberry (75:25 v/v). Means having different letters are significantly different (Tukey's test).

Considering the TSI, apple-RCW beverage was characterized by the highest total sweetness, and the blueberry-RCW drink by the lowest; strawberry-RCW had lower TSI than apple-RCW, but higher than blueberry-RCW, while pear-RCW, RCW-Mix50:50 and RCW-Mix75:25 drinks had TSI values not different from apple-RCW and strawberry-RCW ones.

Even if Sourness Index and TSI are an approximation of the more complex scenario which actually occurs, as they do not consider the suppression effect of sugars on sourness intensity (SAVANT and MCDANIEL, 2004), they could have a practical use in evaluating the sensory impact of a product starting from the compositions in the principal organic acids and sugars. In fact, Sourness Index and TSI results are in agreement with the scores of sensory taste pleasantness reported by CORTELLINO *et al*. (2015), which gave to blueberry-RCW beverage the lowest score, being judged too sour and astringent, and to apple-RCW drink the highest.

3.3. Total monomeric anthocyanins and color indices

The mean concentration of total monomeric anthocyanins was highest in blueberry-RCW drink, decreased with blending of blueberry juice with apple juice (approx. −53 % for RCW-Mix50:50; approx. −73 % for RCW-Mix75:25) and was lowest in strawberry-RCW beverage (Table 5). Our results for blueberry-RCW drink are in line with previous reports on anthocyanins content of blueberry juices, which varied according to the cultivar and juice production conditions from 8 to 130 mg/100 g (LEE *et al*., 2002; MÜLLER *et al*. 2012; RIZZOLO *et al*., 2002; ROSSI *et al*., 2003), whereas the MAP content of strawberry-RCW drink was low if compared to the 36.2-38 mg/100 mL range found by ODRIOZOLA-SERRANO *et al*. (2008) for a 7 % clear strawberry juice after heat treatments at 90°C.

The color indices were measured exploiting the ability of bisulphite to form colorless adducts with monomeric anthocyanin compounds but not with the polymeric pigments (GIUSTI and WROLSTAD, 2001) which are formed by polymerization reaction mostly occurring by binding monomeric anthocyanins with other phenolics compounds, such as phenolic acid and condensed tannins (TÜRKYILMAZ and ÖZKAN, 2012). CD (monomeric anthocyanin compounds plus polymeric pigments) of blueberry-RCW drink was twice as much than CD of the other three red formulations, while PC (polymeric pigments) was highest in blueberry-RCW drink and with blending of blueberry juice with apple juice it decreased approx. by 49 % for RCW-Mix50:50 and approx. 61 % for RCW-Mix75:25 beverages. As a consequence, the three formulations based on blueberry juice had PPC values of approx. 50 % independently from the proportion of blueberry juice used in the beverage (Table 6). This value is in line with LEE *et al*. (2002) results on 14-15 % pasteurized blueberry juices, for which PPC ranged from 40.7 to 50.4 %. A different scenario was observed for strawberry-RCW drink; notwithstanding CD was not different from the value observed for the two beverages prepared using blueberry juice blended with apple juice, PPC was 30 % higher than the PPC values found for the three blueberry based formulations. As a matter of fact, RIZZOLO and CORTELLINO (2017) found for the strawberry-RCW beverage already before the pasteurization step an high PPC value (approx. 71 %) coupled to low MAP (approx. 46 mg C3GE/100 mL) and suggested that likely anthocynanins had undergone some degradation already at the factory during the production of the 65 % pectin-free strawberry juice used for the preparation of the drink.

Beverage contained 20% of RCW and consequently very small quantity of total protein (0.08 g/100g), which only partially can be considered as true protein (0.05 g/100g). RCW brought α-lactalbumin, β-lactoglobulin, casein macropeptide and peptides to the drink; these compounds may interact with anthocyanins increasing their stability as reported in the literature.

Anthocyanins are susceptible to chemical degradation and consequently to color fading in the presence of vitamin C. CHUNG *et al*. (2015) demonstrated that the addition of biopolymers significantly enhanced the color stability of purple carrot anthocyanin in model beverage during storage. The best stability was obtained by adding heat denatured whey protein isolate, which through complexation with anthocyanin reduced its degradation due to ascorbic acid. A fluorescence quenching study showed that the anthocyanin formed stronger interactions with the protein through hydrogen bonding than with the ascorbic acid. Moreover CHUNG *et al*. (2017) suggested that also the addition of three amino acid (L-phenylalanine, L-tyrosine and L-tryptophan) and a polypeptide (ε-poly-L-lysine) may prolong the color stability of the same beverage. The most significant improvement was observed for L-tryptophan, which interacted with anthocyanins mainly through hydrogen bonding but also by some hydrophobic interaction.

Table 6. Monomeric anthocyanin pigments (MAP), Color density (CD), polymeric color (PC) and percent polymeric color (PPC) of 'Red' RCW-fruit juice beverages. Data are mean±standard error (n=6). Means within columns having different letters are significantly different (Tukey's test); *P*-value, significance of F ratio.

	MAP (mg C3GE/100 mL)	CD	PC	PPC
Strawberry	24.34±4.11 c	5.15±1.20 b	3.76±0.65 b	80.0±6.1 a
Blueberry	119.04±4.28 a	12.01±0.12 a	5.87±0.10 a	48.9±0.6 b
Mix 50:50	56.03±0.98 b	5.86±0.02 b	3.00±0.02 bc	51.2±0.4 b
Mix 75:25	33.22±0.30 c	4.11±0.11 b	2.29±0.05 c	55.7±0.5 b
P-value	< 0.001	< 0.001	< 0.001	< 0.001

3.4. Total phenolic content and antioxidant capacity

Total phenolic content and antioxidant capacity of RCW-fruit based beverages as expected greatly depended on the type of fruit juice used for the preparation of the beverage (Table 7). The mean amount of TPC was highest in blueberry-RCW drink and decreased with blending of blueberry juice with apple juice [−50 % for apple:blueberry (50:50); approx. −52 % for apple:blueberry (75:25)]. Strawberry-RCW beverage had the second highest TPC amounts, which was higher than the values found for both apple:blueberry blends. Strawberry-RCW drink had higher antioxidant capacity than blueberry-RCW beverage, even if the difference between the mean values was very low (approx. 1.8 mg GAE/100 mL). Similarly to what found for TPC, antioxidant capacity of apple:blueberry blends was approx. 50 % (RCW-Mix50:50) and 47 % (RCW-Mix75:25) of that found for blueberry-RCW drink. Apple-RCW and pear-RCW drinks were characterized by the lowest TPC amounts along with the lowest antioxidant capacity. The higher values of antioxidant capacity found for the four 'Red' beverages may be related to the presence of anthocyanins coupled with to higher TPC content compared to the two 'Yellow' drinks, compounds which have demonstrated powerful *in vitro* antioxidant capacity at various tests (TABART *et al*., 2009).
Our results on TPC agree well with those in previous studies on blueberry (CASATI *et al*, 2012; GRANATO *et al*., 2015; RIZZOLO *et al*., 2002), apple (GRANATO *et al*., 2015), pear (SAEEDUDDIN *et al*., 2015) and strawberry (CAO *et al*., 2012; HARTMANN *et al*., 2008) juices, while data of antioxidant capacity cannot be directly compared with those reported in the literature for apple (GRANATO *et al*., 2015; TABART *et al*., 2009), pear (SAEEDUDDIN *et al*., 2015), strawberry (CAO *et al*., 2012; HARTMANN *et al*., 2008;

ODRIOZOLA-SERRANO *et al*., 2008) and blueberry (GRANATO *et al*., 2015; CASATI *et al*., 2012) juices due to either differences in the methodology adopted for the DPPH reaction with the samples or to the use of a different type of test used to estimate the antioxidant capacity of juice samples. The fact that strawberry-RCW beverage showed similar antioxidant capacity to that of blueberry-RCW drink, notwithstanding the lower MAP and TPC amounts, could be due to the higher proportion of anthocyanin polymers (higher value of PPC), whose antioxidant capacity likely compensates for the loss of antioxidant capacity due to monomeric anthocyanin degradation, as well as to the formation of Maillard reaction products in response to thermal treatment which exert antioxidant capacity (YILMAZ and TOLEDO, 2005).

Table 7. Total phenolics content (TPC) and antioxidant capacity (AntOx) of RCW-fruit juices beverages. Data are mean±standard error (n=6). Means within columns having different letters are significantly different (Tukey's test); *P*-value, significance of F ratio.

	TPC (mg GAE/100 mL)	AntOx (mg GAE/100 mL)
Apple	47.3±0.5 d	3.26±0.02 d
Pear	38.5±1.0 d	1.86±0.03 d
Strawberry	216.9±2.5 b	34.03±0.78 a
Blueberry	325.4±3.3 a	32.22±0.42 b
Mix 50:50	162.7±1.8 c	16.20±0.38 c
Mix 75:25	156.0±2.3 c	15.41±0.252 c
P-value	< 0.001	< 0.001

4. CONCLUSIONS

Consumer interest in "healthy" foods and beverages has increased over the last decade, and RCW-fruit juice beverages may be considered novel functional drinks in which the functional status of the product could be modulated by changing the type of fruit juice used in the formulation. Considering the interplay among antioxidant capacity, phytonutrient content and beverage sourness and sweetness indices, it could be concluded that higher quality fruit juice-RCW beverages may be obtained by using apple juice for the 'Yellow' type and the apple:blueberry (50:50) blend for the 'Red' ones. Specifically, apple juice was preferred to the pear one as, being equal for Total Sweetness and Sourness Index, it was richer in total phenolic content and antioxidant capacity. As for the apple:blueberry (50:50) blend, it represented a good compromise having an acceptable Sourness Index along with a remarkable source of phenolic compounds and monomeric anthocyanin pigments.

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