Colour, bioactive compounds and antioxidant capacity of mixed beverages based on fruit juices with milk or soya

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Summary

Fruit beverages are a source of phytochemicals, vitamins and minerals, with a high hydration capacity. The aim of this study was to evaluate mixed beverages based on fruit juices with milk or soya regarding their colour (L^*, a^*, b^*), antioxidant compounds (carotenoids, ascorbic acid and polyphenols) and antioxidant activity. The b^* parameter, which is associated with a yellow-orange colour, presented a positive correlation with β-carotene concentration ($r^2 = 0.917$). β-Carotene was the main carotenoid (58–3547 mg·l^{-1}), followed by zeaxanthin (37–97 μg·l^{-1}) and lutein (5–15 μg·l^{-1}). The concentration of deliberately added ascorbic acid ranged between 78 mg·l^{-1} and 993 mg·l^{-1}. The HPLC methods used proved to be suitable for the analysis of carotenoids and vitamin C in these mixed beverages. Polyphenols are naturally present in these mixed beverages (151–503 mg·l^{-1}). The antioxidant capacity of each mixed beverage was assessed in vitro by the ferric reducing antioxidant power (FRAP) method and ranged from 65 mmol·l^{-1} to 376 mmol·l^{-1} (expressed as Trolox equivalents). The antioxidant capacity of these beverages was mainly due to the vitamin C (97.7%) and, in a minor proportion, due to polyphenols (2.3%). Discriminant analysis was applied to these samples of mixed beverages and samples from 'tropical' and 'soyjuice' groups were found to be the best classified into their commercial denominations.

Keywords

fruit juice; milk; soya; mixed beverage; colour; carotenoids; ascorbic acid; antioxidant activity

Diet is one of the factors with the greatest effect on the health of the population and in the prevention of chronic diseases. Social concerns about healthy food have led to a profound change in our consumption patterns and to permanent innovation by the food industry to satisfy this demand. The industry tends to diversify its production lines and develop new functional, appealing and easy-to-eat products to improve the consumers' choices. The market for fruit juices and their derivatives is one of the most dynamic, and has seen considerable growth in recent decades, due to the widely demonstrated health benefits of these products [1, 2].

The total consumption of fruit juices in the European Union was 10387 million litres in 2012 [3]. This is a sector that rapidly responds to the consumer demand with new presentations and enhanced sensorial, nutritional and functional attributes. According to market data [3], thermal processing is the most widely used method for the industrial production of fruit juices, and is also applied to mixed milk-juice beverages to ensure microbiological safety as well as conservation without refrigeration. Heat treatments may induce a loss of labile nutritive and functional ingredients such as vitamin C and β-carotene [4] and these ingredients are therefore intentionally added by the manufacturers. Fruit juices are among the foods that are most stringently regulated by the European laws to control their authenticity and quality [5]. However the mixed beverages that are focused by our study lack a precise definition and legal recognition even in the most recent Directive [6], despite their significant growth in the market.

The beneficial effects associated with regular consumption of fruit or fruit-based products due to their bioactive compounds have been

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recognized. The health-promoting factors derived from the synergistic antioxidant action of carotenoids, polyphenols, vitamin C, tocopherols and certain minerals, such as Zn, highlight fruit juices and mixed beverages as particularly interesting. The mechanism of their protective effects is based on neutralization of the free radicals involved in oxidative damage, and this mechanism can thus be considered as preventive for cancer, cardiovascular and neurodegenerative diseases (carotenoids and flavonoids), cataracts and age-related macular degeneration (zeaxanthin, lutein, vitamins C and E, Zn) [7–9].

It is therefore necessary to characterize the functional compounds of mixed beverages (based on juice and milk or soy) in order to promote their consumption as a contribution to the healthy effects of the Mediterranean diet. The aim of this study was to evaluate commercial milk-juice and soya-juice beverages by determination of total soluble solids, pH and colour, and to analyse their bioactive compounds, namely, carotenoids, ascorbic acid and total polyphenols. The potential antioxidant capacity mediated by these components was also measured by the ferric reducing antioxidant power (FRAP) method.

MATERIAL AND METHODS

Samples

Twenty-four representative mixed juice-milk and juice-soya beverages were analysed. The samples were purchased in Madrid (Spain) and were grouped by commercial denomination (‘mediterranean’, ‘tropical’, ‘multifruit’, ‘caribbean’ and ‘soyjuice’). Tab. 1 presents the description of the samples as declared on the label. The beverages were kept under refrigeration (4 °C) in the laboratory until the analysis of total soluble solids, pH, colour, carotenoids, ascorbic acid and antioxidant capacity (FRAP). At the same time, 50 ml of these juices was freeze-dried in order to perform the extraction and quantification of total polyphenols, also in addition to FRAP carried out with these extracts.

Tab. 1. Ingredients of the beverages as indicated on the labels.

Sample	Fruit concentrate [%]	Fruit ingredients	Milk or soymilk [%]
1M	33.7	Orange, lemon, carrot, peach, passion fruit, acerola, pineapple	10
2M	15	Orange, carrot, peach	10
3M	30	Orange, apple, grape, lemon peach	10
4M	20	Orange, pineapple, apple, carrot, mango, guava, papaya	10
5M	25	Orange, apple, pineapple, lemon	10
6T	7	Pineapple, mango	10
7T	15	Pineapple, mango, apple	10
8T	10	Orange, mango, pineapple	10
9T	7.2	Pineapple, grape, mango, apple	10
10T	15	Apple, mango, pineapple	10
11F	15	Orange, carrot, pineapple, passion fruit, mango, guava, apricot, papaya	10
12F	30	Apple, orange, grape, peach	10
13F	25	Orange, carrot, pineapple, passion fruit, mango, guava, apricot, papaya	10
14F	15	Pineapple, carrot, grape, mango	10
15C	15	Orange, apple, pineapple, lemon	10
16C	42.5	Apple, kiwi, orange, lime	10
17C	15	Grape, apple, orange, pineapple	10
18C	25	Orange, carrot, pineapple, passion fruit, mango, guava, apricot, papaya	10
19C	25	Orange, apple, pineapple, lemon	10
20S	11	Papaya, mango	2.7
21S	11	Orange	2.7
22S	11	Peach	2.7
23S	50	Apple, grape, banana, strawberry, blueberry	3
24S	50	Apple, pineapple, orange, grape, coconut milk	3

Commercial denomination: M – ‘mediterranean’, T – ‘tropical’, F – ‘multifruit’, C – ‘caribbean’, S – ‘soyjuice’.

Total soluble solids, pH and colour

The total soluble solids in the mixed beverages were measured using a digital refractometer (Atago RX-1000, Tokyo, Japan) at 20 °C and expressed in degrees Brix. pH was analysed with a pH-meter (Microph 2000, Crison, Barcelona, Spain). Colour analysis was performed using a Minolta CR-200 Chroma Meter (Minolta, Osaka, Japan) as L^*, a^*, b^* values. L^* is an estimation of the relative luminosity, where 0 is black and 100 is white; a^* takes positive values for reddish colours and negative values for greenish colours; and b^* takes positive values for yellowish colours and negative values for bluish colours. The values provided for each beverage were the average of six measurements. The equipment was previously calibrated against a standard white reference.

Determination of carotenoids

Carotenoids were analysed by the HPLC method following the procedure described by Tenorio et al. [10], with some modifications to be applied for the analysis of mixed beverages. The samples were treated with magnesium carbonate and ascorbic acid to prevent oxidation. Pigment extraction was performed with hexane:methanol (3:4). Sample 16 was green in colour and was extracted with acetone, followed by petroleum ether to extract chlorophyllins. The carotenoid or chlorophyll extracts were evaporated to dryness under N_2 stream and re-dissolved in the mobile phase A. Chromatographic separations were performed on a Phenomenex Luna RP-C18 column 250 mm × 4.60 mm; particle size 5 μm (Torrance, California, USA) in Agilent 1100 Series chromatograph (Agilent Technology, Palo Alto, California, USA) at 25 °C, and the flow rate was set at 2 ml·min^{-1}. The mobile phases used were acetonitrile:dichloromethane: methanol (96.5:1.75:1.75) (A) and acetonitrile: ethyl acetate (50:50) (B). The gradient profile for the separation was set as follows 0–6 min: 100% A eluent, 6–13 min: 100% B eluent. The eluted compounds were monitored by a diode array detector (DAD) at 440 nm (coloured carotenoids), 290 nm (colourless carotenoids: phytoene) and 660 nm (chlorophyllin). Calibration curves were established from patterns of different concentrations of β-carotene and lutein for the identification and quantification of carotenes and xanthophylls, respectively. The correlation coefficients were with both standards $r^2 = 0.999$. The accuracy showed recoveries between 95% and 103%, and the precision was less than 5%.

Determination of ascorbic acid

The determination was carried out by HPLC (Agilent 1100 Series). The samples were diluted with 4.5% metaphosphoric acid and the identification and quantification was performed with a Luna RP-C18 column (250 mm × 4.60 mm, particle size 5 μm) at an oven temperature of 25 °C. The mobile phase used was Milli-Q water (Millipore, Billerica, Massachusetts, USA) acidified with 12 mol·l^{-1} H_2SO_4 (pH 2.55 ± 0.05) at a flow rate of 0.9 ml·min^{-1}. Ascorbic acid was identified using DAD at 245 nm. The standard curve was linear ($r^2 = 0.999$) and covered the concentrations of all samples. The accuracy showed recoveries between 97% and 101% and the precision was less than 3.4%.

Determination of polyphenols

Polyphenols were extracted from the lyophilized samples with methanol:water (50:50 v/v) acidified to pH 2 with 2 mol·l^{-1} HCl and acetone:water (70:30 v/v), following the procedure of Bravo and Saura-Calixto [11]. The Folin–Ciocalteu procedure was used to quantify the extractable polyphenols by spectrophotometry at 750 nm (UV/Vis Lambda EZ210; Perkin–Elmer, Jügesheim, Germany). The regression equation for polyphenols (expressed as gallic acid) was linear ($r^2 = 0.996$).

Total antioxidant capacity by FRAP

The ferric reducing antioxidant power (FRAP) according to Pulido et al. [12] was used to evaluate the reducing power of the mixed beverages and polyphenol extracts described in the previous section. Increase in absorbance due to the formation of a coloured 2,4,6-tri(2-pyridyl)-1,3,5-triazine-Fe^{2+} (TPTZ-Fe^{2+}) complex was monitored at 595 nm in a UV/Vis Perkin–Elmer Lambda EZ210 spectrophotometer. A Trolox reference curve (0.1–1 mmol·l^{-1}) was used ($r^2 = 0.997$).

Statistical analysis

In all cases, analyses were performed at least in triplicate. Analysis of variance (ANOVA) was performed for comparison between different commercial groups. The post-hoc Duncan's test was used to evaluate significant differences in the parameters studied. Pearson's correlation test was conducted to determine the linear correlations among variables. Differences were considered significant when $p < 0.05$. Linear discriminant analyses were used for the purpose of multiple group classification according to the parameters studied. The statistical software used was Statgraphics version 5.1 (StatPoints Technologies, Warrenton, Virginia, USA).

RESULTS AND DISCUSSION

The total soluble solids, pH and colour (L^*, a^* and b^*) of the mixed beverages based on fruit juices with milk or soya are shown in Tab. 2. Total soluble solids of mixed beverages ranged from 10.4 °Brix to 13.8 °Brix (samples 4M and 24S, respectively) except for sample 12F, which showed a much lower value (5.4 °Brix) as it has no added sugar. The average total soluble solids for 'multifruit' beverages was significantly lower ($p < 0.05$) than for the other commercial denominations ('mediterranean', 'tropical', 'caribbean' and 'soyjuice'). Total soluble solids were slightly higher than the values reported on the label, but they were found to be strongly correlated ($r^2 = 0.884$). pH ranged between 3.1 (sample 18C) and 4.0 (sample 24S), with significantly higher ($p < 0.05$) values for 'tropical' and 'soyjuice' groups.

Colour is one of the most important attributes of juices and is associated by consumers with the quality and appearance of these beverages. The lightness values (L^*) did not show any significant differences between the commercial groups of milk-juices, although it was significantly higher ($p < 0.05$) in 'soyjuice'. The differences found between skim-milk and soymilk mixed beverages revealed that soymilk gave greater intensity, although it was added in smaller amounts (2.7–3%) than skim milk (10% in all samples). Sample 23S showed the highest positive a*, with the red colour due to the presence of strawberry and cochineal or carminic acid as additives. Sample 16C had the lowest negative value due to its green colour, which may be due to the kiwi content and the deliberate addition of chlorophyllin. Significant differences ($p < 0.05$) were found for a^* value in 'soyjuice', and no differences were detected between milk-juices groups. The highest b^* value corresponded to sample 22S, which contained β-carotene and curcumin to imitate a peach colour, and the lowest result (sample 15C), with no orange colouring, did not include the addition of β-carotene. The statistical comparison between groups showed that the 'caribbean' group had the

Tab. 2. Total soluble solids, pH and colour of the beverages.

Samples	Fruit concentrate [%]	TSS [°Brix]	pH	L^*	a^*	b^*
1M	33.7	10.90 ± 0.00	3.35 ± 0.01	45.50 ± 0.14	–0.21 ± 0.01	15.04 ± 0.21
2M	15	12.97 ± 0.06	3.32 ± 0.02	40.43 ± 0.14	0.07 ± 0.01	10.30 ± 0.50
3M	30	13.50 ± 0.00	3.68 ± 0.01	43.63 ± 0.18	3.91 ± 0.04	18.57 ± 0.30
4M	20	10.47 ± 0.06	3.25 ± 0.02	41.07 ± 0.06	1.55 ± 0.02	11.86 ± 0.12
5M	25	12.30 ± 0.00	3.55 ± 0.01	41.53 ± 0.06	–2.29 ± 0.02	3.68 ± 0.03
6T	7	11.13 ± 0.12	3.50 ± 0.01	44.35 ± 0.08	–2.98 ± 0.03	6.77 ± 0.03
7T	15	13.27 ± 0.06	3.67 ± 0.01	44.22 ± 0.05	–2.75 ± 0.02	7.87 ± 0.17
8T	10	13.73 ± 0.06	3.94 ± 0.02	42.45 ± 0.12	–2.15 ± 0.03	16.88 ± 0.25
9T	7.2	11.20 ± 0.00	3.22 ± 0.01	44.20 ± 0.13	–3.07 ± 0.04	8.63 ± 0.33
10T	15	13.60 ± 0.10	3.70 ± 0.01	43.03 ± 0.09	–2.76 ± 0.03	7.26 ± 0.35
11F	15	12.07 ± 0.15	3.31 ± 0.01	45.71 ± 0.11	1.87 ± 0.02	17.46 ± 0.83
12F	30	5.47 ± 0.06	3.40 ± 0.01	41.28 ± 0.05	0.50 ± 0.03	11.31 ± 0.68
13F	25	13.17 ± 0.06	3.46 ± 0.01	44.08 ± 0.11	3.87 ± 0.04	19.91 ± 0.91
14F	15	12.80 ± 0.00	3.20 ± 0.01	42.08 ± 0.09	0.55 ± 0.02	10.98 ± 0.43
15C	15	12.53 ± 0.06	3.58 ± 0.03	47.79 ± 0.13	–2.49 ± 0.02	3.18 ± 0.03
16C	42.5	11.60 ± 0.10	3.50 ± 0.01	40.03 ± 0.06	–6.09 ± 0.05	6.24 ± 0.04
17C	15	13.67 ± 0.06	3.50 ± 0.02	42.26 ± 0.08	–2.39 ± 0.02	4.78 ± 0.03
18C	25	12.50 ± 0.10	3.20 ± 0.01	34.72 ± 0.12	0.98 ± 0.04	17.10 ± 0.89
19C	25	13.50 ± 0.00	3.44 ± 0.01	45.13 ± 0.12	–3.43 ± 0.04	3.87 ± 0.02
20S	11	11.83 ± 0.15	3.61 ± 0.06	52.87 ± 0.13	5.11 ± 0.05	11.91 ± 0.12
21S	11	11.07 ± 0.06	3.72 ± 0.02	56.45 ± 0.14	0.90 ± 0.01	10.59 ± 0.10
22S	11	11.27 ± 0.06	3.81 ± 0.02	55.69 ± 0.12	3.92 ± 0.04	20.40 ± 0.86
23S	50	13.57 ± 0.06	3.81 ± 0.01	51.45 ± 0.11	6.59 ± 0.05	7.98 ± 0.08
24S	50	13.87 ± 0.25	4.01 ± 0.03	62.64 ± 0.16	–2.30 ± 0.02	12.81 ± 0.09

Data are expressed as mean ± standard deviation ($n = 4$).
TSS – Total soluble solids. Samples: M – 'mediterranean', T – 'tropical', F – 'multifruit', C – 'caribbean', S – 'soyjuice'.

lowest b^* values ($p < 0.05$) in comparison to other four commercial denominations. Samples from different groups (5M, 15C and 19C) were made from the same fruit concentrate and had similar b^* values (3.1–3.8), as well as samples 11F, 13F and 18C (b^*: 17.1–19.9). The commercial denomination did not therefore provide clear information for milk-juices with regard to their fruit profile and to sensorial characteristics, such as colour, suggesting that the manufacturers did not follow uniform criteria for this classification. ZULUETA et al. [13] showed different correlations between colour parameters and the profile of fruit concentrate in mixed beverages, and concluded that positive a^* values were associated with carrots, apricots and passion fruit, and negative ones with apple, kiwi and lime; whereas apricot and peach correlated with positive b^* values. 'Mediterranean' (samples 1M to 5M) and 'multifruit' (samples 11F to 14F) gave a good corelation with yellow-orange colours associated with the presence of orange, carrot, peach and mango, although sample 5M ('mediterranean') was white in colour, probably due to the significant presence of apple, pineapple and lemon, and the lack of β-carotene. The mixed beverages included in this study were heat-treated, and a^* and b^* were probably affected as described by SAMPEDRO [14]. Those authors studied milk-based orange juice beverages with or without thermal treatment, and found no differences in luminosity and saturation, but a significant decrease in a^* and b^* after heat treatment (less bright and less yellowish).

The HPLC method was suitable for determination of carotenoids in mixed beverages due to its satisfactory accuracy and precision, and also for the easy preparation of samples and a short run time (13 min). The spectral and chromatographic characteristics of carotenoids are shown in Tab. 3. The elution order was: lutein, valenciaxanthin, antheraxanthin, *cis*-lutein-epoxide, auroxanthin, mutatoxanthin, β-cryptoxanthin, α-carotene, zeaxanthin, ε-carotene, β-carotene and phytoene. The presence of the green colouring agent chlorophyllin was detected in sample 16C. The pigment was identified by means of three characteristic maximum absorption wavelengths, the shape of the spectrum of each carotenoid and the III/II ratio that indicates the fine structure of the compound.

Tab. 4 shows the concentrations of main pigments, namely, β-carotene, zeaxanthin and lutein. Other carotenoids were detected only in traces and simply identified. The 'multifruit' samples presented significantly higher concentrations ($p < 0.05$) of β-carotene than the other commercial groups. Our results agree with those reported by ZULUETA et al. [13] for mixed milk – fruit juice beverages with levels ranging between 16 $\mu g \cdot kg^{-1}$ and 2990 $\mu g \cdot kg^{-1}$, considering the sum of *trans* and *cis* β-carotene. MORALES DE LA PEŃA et al. [1] reported a concentration of 430 $\mu g \cdot l^{-1}$ of β-carotene in soymilk – fruit juice beverages made from orange, kiwi and pineapple. In contrast, MENDIOLA et al. [15] did not detect β-carotene above the detection limit of their analytical method in different functional drinks. In the present study, the inclusion of orange as the first ingredient in beverages, in addition to carrot and mango, may be associated with a high concentration of β-carotene (samples 3M, 8T, 11F, 13F, 18C). However, there was no correlation between levels of carotene and the percentage and profile of fruits in the concentrate, suggesting that the principal source of this compound is the deliberate addition by the manufacturers to obtain the appropriate colour attributes. The β-carotene concentration was highly correlated with b^* values associated with a yellow-orange colour ($r^2 = 0.917$; Tab. 6). The addition of β-carotene was indicated in 75% of the samples, but only labels of samples 4M, 5M and 9T specified the amount added (120 μg). This nutritional information on the label,

Tab. 3. Chromatographic and spectral characteristics of carotenoids separated and identified in the beverages.

Carotenoid	t_R [min]	λ_1 [nm]	λ_2 [nm]	λ_3 [nm]	III/II [%]
Lutein	4.1	424	448	476	54.5
Valenciaxanthin	4.6	352	370	390	65.4
Anteraxanthin	4.8	(424)	448	474	59.1
Cis lutein epoxide	5.1	416	442	470	42.9
Chlorophyllin 1	5.9	(388)	402	616	–
Auroxanthin	8.2	380	402	428	90.5
Mutatoxanthin	8.6	406	428	454	–
β-cryptoxanthin	8.7	(429)	452	478	24
Na-Cu chlorophyllin	8.8	(392)	406	626	–
Clorophyllin 2	8.8	(408)	426	612	–
α-carotene	9.4	424	445	473	37.5
Zeaxanthin	9.5		454	480	23.8
ε-carotene	10.0	380	402	426	91.2
β-carotene	10.5		454	480	18.2
Phytoene	11.2	277	287	298	8.3

t_R – time of retention, λ_1, λ_2, λ_3 – three specific maximum absorption wavelengths. Parentheses in the $\lambda 1$ column indicate a shoulder.
III/II is the ratio of the height of the longest-wavelength absorption peak (designated III) to that of the middle absorption peak (designated II), taking the minimum between the two peaks as the baseline, multiplied by 100.

Tab. 4. Concentration of carotenoids in the beverages.

Samples	Lutein [$\mu g \cdot l^{-1}$]	Zeaxanthin [$\mu g \cdot l^{-1}$]	β-carotene [$\mu g \cdot l^{-1}$]	Vitamin A [$\mu g \cdot l^{-1}$]	Traces
1M	12.3 ± 0.6	53.5 ± 5.1	1 822.9 ± 63.7	152.0 ± 7.0	valenciaxanthin, anteraxanthin, ε-carotene, α-carotene, β-cryptoxanthin
2M	–	73.0 ± 0.7	1 082.1 ± 15.5	90.3 ± 0.4	α- carotene, ε-carotene
3M	7.0 ± 0.2	97.5 ± 0.6	2 551.8 ± 33.9	212.6 ± 0.9	
4M	–	47.0 ± 0.2	1 195.6 ± 12.8	99.6 ± 0.2	
5M	–	–	67.9 ± 0.2	5.7 ± 0.2	ε-carotene, valenciaxanthin
6T	–	–	25.1.7 ± 65	21.0 ± 0.6	
7T	–	37.0 ± 0.2	694.1 ± 15.8	57.9 ± 0.1	auroxanthin
8T	–	89.5 ± 0.1	2 401.8 ± 48.6	200.2 ± 0.9	auroxanthin
9T	–	–	237.6 ± 1.5	19.8 ± 0.2	
10T	–	–	435.5 ± 0.4	36.3 ± 0.1	
11F	–	74.0 ± 1.0	3 097.4 ± 44.5	258.3 ± 1.2	ε-carotene, mutatoxanthin isomer
12F	5.0 ± 0.2	41.0 ± 0.2	1 986.7 ± 5.1	165.6 ± 0.6	ε-carotene, phytoene
13F	–	54.2 ± 0.4	2 772.5 ± 28.3	231.0 ± 1.3	β-cryptoxantine, α-carotene
14F	–	38.0 ± 0.2	1 505.7 ± 22.7	15.6 ± 0.2	
15C	–	–	58.0 ± 0.4	4.9 ± 0.1	
16C	5.0 ± 0.1	–	215.6 ± 1.5	17.9 ± 0.2	chlorophyllins, anteraxanthin
17C	–	–	187.4 ± 1.1	15.6 ± 0.1	
18C	–	76.2 ± 0.04	3047 ± 31.9	254.0 ± 1.5	β-cryptoxanthin, ε-carotene
19C	–	–	67.6 ± 0.4	5.7 ± 0.1	
20S	–	–	358.4 ± 01.3	29.9 ± 0.2	
21S	–	–	917.2 ± 3.3	76.4 ± 0.2	
22S	–	–	2 595.8 ± 20.8	216.3 ± 1.4	
23S	7.5 ± 0.1	–	350.1 ± 04.0	29.2 ± 0.2	*cis* lutein epoxide
24S	15.0 ± 0.2	–	720.5 ± 7.8	60.0 ± 0.3	

Data are expressed as mean ± standard deviation (n = 4). Concentration of vitamin A is expressed in retinol equivalents in micrograms per liter.
Samples: M – 'mediterranean', T – 'tropical', F – 'multifruit', C – 'caribbean', S – 'soyjuice'.

although is not mandatory, would be helpful for ascertaining the intake from these mixed beverages. Zeaxanthin was quantified in 'mediterranean' samples except for sample 5M, 'multifruit', and in individual samples from other groups such as 'tropical' and 'caribbean', and was not detected in 'soyjuice'. This carotenoid was found mainly in several fruits such as orange, mango, carrot and pineapple. Lutein was detected in samples 1M, 3M, 12F, 16C, 23S and 24S with a high concentration of fruit concentrate (30% to 50%), and with orange as a fruit component. Partial degradation of lutein to its *cis*-epoxide was detected in sample 23S. Other carotenoids such as α-carotene, ε-carotene and β-cryptoxanthin, which are formed by hydroxylation of β-carotene, were identified in some mixed beverages due to the presence of orange as the main component (Tab. 4). According to several authors [9, 13], β-cryptoxanthin is present in the pulp of citrus fruits and also in peach, papaya and apricot. β-cryptoxanthin is hydroxylated to zeaxanthin, which is epoxidized to antheraxanthin. Moreover, mutatoxanthin and auroxanthin are synthesized by epoxy-furanoside-type reactions [9]. This explains why some of these molecules may also be present in beverages with zeaxanthin. Morales de la Peña et al. [1] quantified mainly lutein and zeaxanthin in freshly prepared beverages with soymilk and orange, kiwi and pineapple juices. . In our soymilk – fruit juice samples, only lutein was detected, together with its *cis*-epoxide (23S). Vitamin A activity from β-carotene, shown in Tab. 4, was in a wide range. The consumption of a carton of 330 ml per day could contribute up to a maximum of 15% of the recommended dietary allowances [16].

Chlorophyllins were identified in sample 16, and were responsible for the green colour of the beverage. According to Mortensen and Geppel [17], chlorophyllins derive from chlorophyll hydrolysis, resulting in a mixture of a wide variety of compounds generically known as chlorines,

with a carboxylic acid structure. The sodium salts of these substances are water-soluble and allow the use of sodium copper chlorophyllin as a food additive.

A simple, rapid, accurate and precise HPLC method was performed to quantify vitamin C. The results on its concentrations in the mixed beverages fell in a range with the highest value for sample 16C (Tab. 5). The vitamin C concentration in the 'caribbean' group differed significantly ($p < 0.05$) from the other commercial denominations. There was no clear correlation between the concentration of ascorbic acid and the percentage of the fruit concentrate. This suggests that the main source of this vitamin was its inclusion as an additive, similar to the case of β-carotene. This can be explained by the fact that labile components, such as ascorbic acid, can easily degrade [18] and the manufacturers therefore often add higher quantities than indicated on the label in order to ensure that the beverage never provides less total ascorbic acid than the amount on the label, during the storage time. Thus, the determined concentrations of vitamin C did not correlate well with the indications on the label. In general, the amounts determined by HPLC were much higher than those shown on the labels.

There was a great variability in the polyphenol concentrations in the analysed beverages (Tab. 5). Our results were lower than those published by Zulueta et al. [2] and Gardner et al. [19]. Morales de la Peña et al. [20] determined polyphenol concentrations that were higher than our data in soymilk – fruit juice beverage. This could be related to the proportion of soymilk in the formulation, considering that the samples analysed in our study present smaller percentages. Commercial 'mediterranean', 'multifruit' and 'soyjuice' groups presented similar values of polyphenols and did not differ significantly. The highest levels

Tab. 5. Vitamin C and polyphenol concentrations and antioxidant capacity (FRAP) developed by the beverages and by polyphenol extracts.

Samples	Vitamin C [mg·l^{-1}]	Polyphenols [mg·l^{-1}]	Antioxidant capacity	
			Mixed beverages [mmol·l^{-1}]	Polyphenol extracts [mmol·l^{-1}]
1M	93.9 ± 2.9	297.2 ± 18.9	78.7 ± 2.8	1.5 ± 0.05
2M	158.3 ± 3.5	295.1 ± 15.0	82.3 ± 1.8	2.4 ± 0.3
3M	236.6 ± 4.3	339.3 ± 7.0	76.6 ± 2.2	3.0 ± 0.2
4M	393.8 ± 5.3	220.3 ± 7.4	135.0 ± 7.3	0.9 ± 0.09
5M	395.7 ± 5.2	450.6 ± 26.1	165.5 ± 7.6	3.1 ± 0.2
6T	170.6 ± 3.4	235.1 ± 15.9	70.8 ± 2.4	1.4 ± 0.1
7T	91.9 ± 3.7	176.3 ± 16.8	105.8 ± 0.9	1.3 ± 0.1
8T	339.5 ± 6.9	322.0 ± 12.0	98.5 ± 2.5	2.9 ± 0.3
9T	221.5 ± 5.0	151.6 ± 8.4	70.7 ± 2.6	1.3 ± 0.04
10T	78.8 ± 2.9	249.9 ± 5.8	89.2 ± 2.3	1.4 ± 0.01
11F	88.0 ± 3.1	269.8 ± 5.8	104.9 ± 1.6	1.8 ± 0.08
12F	275.7 ± 4.7	300.5 ± 5.5	143.5 ± 1.4	2.8 ± 0.02
13F	465.7 ± 6.2	503.9 ± 22.9	182.0 ± 1.6	5.5 ± 0.05
14F	190.0 ± 1.2	230.6 ± 13.3	68.4 ± 2.1	1.6 ± 0.02
15C	97.9 ± 2.0	276.7 ± 31.5	65.9 ± 1.8	1.7 ± 0.07
16C	993.3 ± 9.1	790.9 ± 9.2	376.7 ± 4.0	10.2 ± 0.04
17C	405.2 ± 3.9	423.8 ± 24.2	161.0 ± 4.4	3.0 ± 0.02
18C	384.3 ± 3.6	406.3 ± 19.0	114.3 ± 3.9	3.5 ± 0.01
19C	494.0 ± 4.7	426.9 ± 8.5	169.2 ± 2.6	4.1 ± 0.03
20S	259.3 ± 3.3	318.0 ± 7.8	102.7 ± 2.7	2.5 ± 0.05
21S	275.9 ± 3.2	353.6 ± 4.0	74.9 ± 8.9	2.3 ± 0.09
22S	145.1 ± 5.1	299.2 ± 4.5	77.2 ± 2.4	2.4 ± 0.1
23S	167.4 ± 1.8	442.7 ± 28.6	120.9 ± 1.9	3.1 ± 0.02
24S	221.2 ± 1.2	474.3 ± 37.1	140.3 ± 3.6	3.3 ± 0.03

Data are expressed as mean ± standard deviation ($n = 4$). Antioxidant capacity is expressed as equivalents of Trolox.
Samples: M – 'mediterranean', T – 'tropical', F – 'multifruit', C – 'caribbean', S – 'soyjuice'.

($p < 0.05$) of polyphenols were found in the 'caribbean' beverages, and the lowest ($p < 0.05$) in the 'tropical' ones. Moreover, 'tropical' had the lowest percentages of the fruit concentrate (7–15%), while 'caribbean' was among the beverages with the highest percentages of the fruit concentrate (15–42.5%). Thus, concentration of polyphenols and percentage of fruit concentrate showed a correlation ($r^2 = 0.425$), suggesting that polyphenols were related mainly to the amount used and profile of fruits in the concentrate. The highest concentration of polyphenols was found in the mixed beverages with orange, apple, pineapple and/or grape as the main fruits. These fruits appear to contribute to the total polyphenol concentration in drinks [21]. Conventional pasteurization (90 °C for 30 s) of orange juice has negligible effects on its phenolic compounds [22]. Time and storage conditions do not appear to significantly influence the polyphenol profile of the samples due to the enzyme inactivation of polyphenol oxidase that takes place during thermal processing [1].

Total antioxidant activity of the mixed beverages measured as FRAP expressed as equivalents of Trolox was mainly related to ascorbic acid and polyphenols (Tab. 5). The highest value of antioxidant capacity was found in sample 16C, due to the levels of vitamin C and total polyphenols. This antioxidant capacity was positively correlated with ascorbic acid concentration ($r^2 = 0.893$) and also with polyphenol concentration ($r^2 = 0.896$; Tab. 6). These data are consistent with the findings of GARDNER et al. [19]. THAIPONG et al. [23] reported a high correlation between FRAP and both ascorbic acid and total phenolic compounds in guava fruit extracts. XIAOWEI et al. [24] showed a strong correlation ($r^2 = 0.99$) between FRAP and total polyphenol concentrations in mango samples.

In order to determine the contribution of phenolic compounds to the total antioxidant capacity of the mixed beverages, another FRAP assay of polyphenol extracts was conducted. Extracts that were found to have a greater antioxidant activity corresponded to those with a higher polyphenol content. Thus, a strong correlation ($r^2 = 0.952$) was found between the FRAP results of the extracts and the polyphenol concentration (Tab. 6). These results agree with data obtained by other authors such as MENDIOLA et al. [15] or GORINSTEIN et al. [25]. The statistical comparison of FRAP results of polyphenol extracts showed that values in the 'caribbean' samples were higher ($p < 0.05$) than in other commercial groups. The results suggest that the presence of orange, apple, pineapple and carrot provided high levels of polyphenols, and developed the highest FRAP in the beverages. These data are consistent with those obtained by GARDNER et al. [19]. ZULUETA

Tab. 6. Correlation coefficients and *p*-values describing relation between the analysed variables.

	Brix	pH	*L**	*a**	*b**	β-carotene	Ascorbic acid	Poly-phenol	FRAP1
pH	0.365 (<.001)								
*L**	0.133 (0.195)	0.608 (<.001)							
*a**	–0.102 (0.319)	0.021 (0.835)	0.289 (0.004)						
*b**	–0.066 (0.522)	0.023 (0.818)	0.077 (0.452)	0.562 (<.001)					
β-carotene	–0.152 (0.137)	–0.132 (0.199)	–0.187 (0.067)	0.432 (<.001)	0.917 (<.001)				
Ascorbic acid	0.051 (0.615)	–0.109 (0.286)	–0.308 (0.002)	–0.292 (0.003)	–0.137 (0.181)	–0.108 (0.291)			
Polyphenol	0.229 (0.024)	0.160 (0.118)	–0.032 (0.751)	–0.198 (0.052)	–0.103 (0.313)	–0.110 (0.282)	0.829 (<.001)		
FRAP1	0.069 (0.501)	–0.014 (0.885)	–0.218 (0.032)	–0.327 (0.001)	–0.218 (0.032)	–0.182 (0.074)	0.893 (<.001)	0.896 (<.001)	
FRAP2	0.149 (0.145)	0.066 (0.519)	–0.145 (0.156)	–0.240 (0.018)	–0.062 (0.543)	–0.059 (0.565)	0.868 (<.001)	0.952 (<.001)	0.912 (<.001)

p-values are given in brackets.
FRAP1 – total antioxidant capacity of the beverages, FRAP2 – antioxidant capacity of polyphenol extracts.

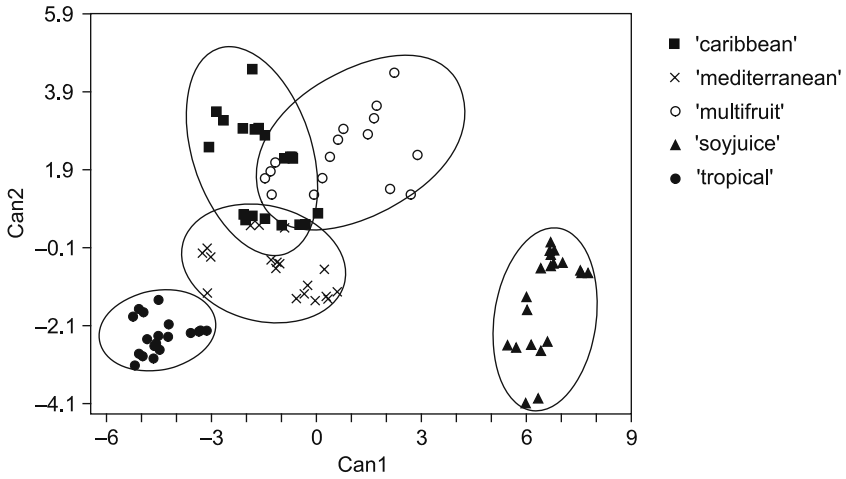

Fig. 1. Scatter diagram of the two principal canonical variables.

et al. [2] reported that citrus fruits were associated with the highest antioxidant capacity.

The difference between the FRAP results in the mixed beverages and in the polyphenolic extracts suggests that the total antioxidant capacity of these beverages came mainly from added vitamin C (97.7%) and, in a negligible proportion, from polyphenols (2.3%). GARDNER et al. [19] and XU et al. [26] reported contributions from ascorbic acid of between 65–100% and 48–76%, respectively, of the total antioxidant capacities of juices derived from citrus fruits. ZULUETA et al. [2] concluded that, in fruit juice and milk mixtures, the antioxidant activity from vitamin C is five times greater than that from phenolic compounds.

Linear discriminant analysis was applied to the sensorial attributes and bioactive compounds studied. The first and second canonical variables were the most satisfactory for discriminating among the samples grouped by commercial denominations. The highest coefficients for the first canonical variable were brightness (L^*, 0.8737), yellowness (b^*, 0.6229), redness (a^*, 0.8798) and pH (0.4995), while the parameters that contributed most to the second canonical variable were ascorbic acid (0.8026), polyphenols (0.7141) and antioxidant capacities derived from them (0.8479, 0.7883). Fig. 1 illustrates the distribution of all samples on a scatter chart whose axes are the two main canonical variables. Samples from 'soyjuice' and 'tropical' groups were correctly classified (100%) into their a priori established commercial denomination. However, the samples assigned to 'multifruit' 'caribbean' and 'mediterranean' groups provided 73%, 70% and 65% of correct classifications, respectively.

CONCLUSIONS

Milk- and soymilk-fruit juice mixed beverages were shown to be a good source of bioactive compounds such as carotenoids, vitamin C and polyphenols with potential antioxidant activity, thereby contributing to the promotion of good health. The HPLC methods described proved to be satisfactory for application to the analysis of these compounds in mixed beverages. Due to the existing legal gap in the definition of these products, we were unable to determine whether the results agreed with legal criteria. Thus, it seems reasonable that a regulatory framework for these products would have an advantage for consumers in order to provide clearer and complete information.

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