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Vitamin C, vitamin A, phenolic compounds and total antioxidant capacity of new fruit juice and skim milk mixture beverages marketed in Spain

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Abstract

The growing interest in new functional foods with special characteristics and health properties has led to the development of new beverages based on fruit juice–skim milk mixtures. The proliferation of ready-to-drink beverages has caused the market to focus its interest on these products. Commercial conventionally pasteurized or sterilized beverages based on a mixture of fruit juice and skim milk were evaluated nutritionally for their concentrations of vitamin C, vitamin A and phenolic compounds and their total antioxidant capacity, taking the influence of physicochemical parameters into account. The main contribution to the total antioxidant capacity (TEAC, trolox equivalent antioxidant capacity) was provided by vitamin C, followed by phenolic compounds, in accordance with a mathematical equation obtained from the data: TEAC = −0.184 + 0.009 * [vitamin A] + 0.011 * [phenolic compounds] + 0.058 * [vitamin C]. The *R*-squared value was 86.88%. Citrus fruits, such as lemons or oranges, were the fruits associated with the greatest antioxidant capacity in the samples analysed.

Keywords: Antioxidant capacity; Vitamin C; Phenolic compounds; Carotenoids; Vitamin A; Fruit juice and skim milk mixture beverages

1. Introduction

Reactive oxygen species (ROS) could be important causative agents of a great number of human diseases. Antioxidant components provide protection against harmful free radicals, which are produced by aerobic metabolism and have been strongly associated with reduced risk of chronic diseases, such as cardiovascular disease, cancer, diabetes, Alzheimer's disease, cataracts and age-related functional decline, in addition to other health benefits (Byers & Perry, 1992; Knekt et al., 2002; Liu et al., 2000; Martínez-González et al., 2002; Slaterry et al., 2000; Zhang, Taylor, Kramer, & Li, 1995).

Fruits and vegetables contain various bioactive compounds with antioxidant activities, such as vitamins A, C and E, which have a high antioxidant capacity (Hassimoto, Genovese, & Lajolo, 2005; Sánchez-Moreno, Plaza, de Ancos, & Cano, 2006), and phenolic compounds, which recent studies have shown to be good contributors to the total antioxidant capacity of the foods that contain them (Cano, Plaza, Sánchez-Moreno, & de Ancos, 2003; Chaovanalikit & Wrolstad, 2004; Dillard & German, 2000; Vinson, Su, Zubik, & Bose, 2001), although their nutritional relevance is uncertain because they may be poorly absorbed and rapidly metabolized and thus have limited antioxidant ability in vivo (Gardner, White, McPhail, & Duthie, 2000). It is important, therefore, to determine the total phenol content in order to evaluate the possible synergistic or antagonistic effect on their contribution to the total antioxidant capacity. Vitamin C is highly bioavailable and is therefore the most important water-soluble antioxidant in cells and an efficient scavenger of reactive oxygen species (Halliwell, 1996).

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Not only fruits and vegetables but also various dairy products, and fractions obtained from them, have been found to be antioxidative, e.g. milk, skim milk, whey, casein and lactoferrin (Calligaris, Manzocco, Anese, & Nicoli, 2004; Lo Scalzo, Iannoccari, Summa, Morelli, & Rapisarda, 2004; Steijns & van Hooijdonk, 2000; Tong, Sasaki, McClements, & Decker, 2000). Milk antioxidants have important roles in preventing lipid peroxidation and maintaining milk quality (Lindmark-Mansson & Akesson, 2000). It is important to determine the antioxidant activity of milk and to characterize the compounds responsible for that activity (Chen, Lindmark-Mansson, Gorton, & Akesson, 2003; Vanderjagt, Okolo, Costanza, Blackwell, & Glew, 2001) because milk is a basic food for human development. Depending on their nature, milk antioxidants are differentiated into protein and non-protein antioxidants. Noteworthy, in the non-protein group, are vitamins A, C and E, and the protein group includes various kinds of enzymes, and also a number of proteins and peptides (Cervato, Cazzola, & Cestaro, 1999; Lindmark-Mansson & Akesson, 2000; Satué-Gracia, Frankel, Rangavaijhyala, & German, 2000).

The growing interest in the study of natural antioxidant compounds has been accompanied by an increase in the market presence of what are known as functional foods or nutraceuticals or health foods (Andlauer & Fürst, 2002; Bello, 2001). In Spain, the annual per capita consumption of fruit juice is about 9.7 l (MAPA, 2004). The increase in the consumption of ready-to-drink beverages in recent years has been associated with a reduction in the consumption of fresh fruit and vegetables. At present, with a consumption of only 1.7 portions of fruit per day, the Spanish population does not consume the recommended quantity of fresh fruit (3 items per day). However, the recommended intake is attained if consumption of fresh or canned juice is taken into account (MAPA, 2004), and it is important, therefore, to achieve high quality in beverages of this kind and to ascertain their nutritional value. Beverages based on fruits and milk products are currently receiving considerable attention because their market potential is growing. Besides being delicious, these beverages are highly nutritious. They may be particularly useful in places where there is inadequate nutrition, which could lead to nutritional deficiency diseases.

From the determination of total antioxidant power it is possible to evaluate the antioxidant capacity of a product, irrespective of its particular composition. This parameter can be used for characterization of the raw material and its evolution in relation to processing or storage conditions (Arnao, Cano, & Acosta, 1998). In particular, thermal treatments are generally believed to be the main cause of the depletion of natural antioxidants (Anese, Manzocco, Nicoli, & Lerici, 1999).

In the literature available at present, there is a lack of information about the activity of antioxidants in commercial processed fruit juice–milk beverages. The present work studies the physicochemical composition of fruit juice–milk mixture beverages marketed in Spain, and also their nutritional value, providing a more extensive examination of their antioxidant characteristics (analysis of concentration of vitamins A and C and phenolic compounds) and therefore of their ability to prevent certain diseases, which might suggest their inclusion in the functional foods group.

2. Materials and methods

2.1. Samples

Three units from each of two batches of 17 different juice–milk beverages marketed in Spain were analysed. The samples were purchased from a local supermarket. Eleven of them (5A, 6A, 7A, 8A, 11C, 12C, 13D, 14D, 15D, 16D, 17E) were kept at room temperature (20 ± 2 °C), because they had been sterilized (UHT), and six (1A, 2A, 3A, 4A, 9B, 10B) were kept under refrigeration (4 ± 2 °C), because they had been pasteurized (the same letter indicates the same manufacturer). Table 1 gives details (as indicated on the label) of each of the samples analysed. The experiments were performed in triplicate.

2.2. Chemicals and reagents

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), as a standard substance (2 mM) to measure TEAC, metmyoglobin from horse, ABTS (2,2′-azinobis(3-ethylbenzothiazoline 6-sulphonate)), Folin–Ciocalteau reagent, β-carotene and *tert*-butyl hydroxytoluene (BHT) (special grade) were purchased from Sigma (Steinheim, Germany). Gallic acid in distilled water, as a standard (10 mg/ml) for phenolic compounds, was purchased from UCB (Brussels, Belgium). Lutein and zeaxanthin were provided free as standard substances by Roche (Basel, Switzerland). Ammonium acetate (LC grade), petroleum ether, hexane (LC grade), potassium hydroxide (85%), *tert*-butyl methyl ether (TBME) (LC grade) and hydrogen peroxide, 35% reagent grade, were purchased from Scharlau (Barcelona, Spain). Sodium and disodium phosphate, L(+)-ascorbic acid, acetonitrile (special grade) and magnesium hydroxide carbonate (40–45%) were purchased from Panreac (Barcelona, Spain), and ethanol, diethyl ether, methanol and sodium chloride (special grade) from Baker (Deventer, The Netherlands). Chloroform was obtained from Merck (Darmstadt, Germany).

2.3. Instrumentation

For polarographic analysis, a Metrohm 746 VA Trace Analyzer (Herisau, Switzerland) equipped with a Metrohm 747 VA stand was used. The working electrode was a Metrohm multi-mode electrode operated in the dropping mercury mode. A platinum wire counter electrode and a saturated calomel reference electrode were used.

The LC system consisted of a series 1050 chromatograph with a quaternary pump system, a diode array detector (Hewlett–Packard, 1100 series), a column thermostat (Agilent, 1100 series), an on-line degassing system and a

Table 1
Compositions of the juice–milk beverages analysed as indicated on the labels

Sample	Composition	Juice type
1A	Juice 25%, skim milk 20% Water, sugar, calcium salts, stabilizer (pectin), acidulant (citric acid, vitamin C, aromas and vitamin A)	Orange and pineapple
2A	Juice 25%, skim milk 20% Water, sugar, calcium salts, stabilizer (pectin), acidulant (citric acid, vitamin C, aromas and vitamin A)	Orange and mango
3A	Juice 25%, skim milk 20% Water, sugar, calcium salts, stabilizer (pectin), acidulant (citric acid, vitamin C, aromas and vitamin A), colorant (allura red)	Strawberry, banana and orange
4A	Juice 25%, skim milk 20% Water, sugar, calcium salts, stabilizer (pectin), acidulant (citric acid, vitamin C, aromas and vitamin A)	Peach and apricot
5A	Juice, skim milk Sugar, fibre, pectin, acidulant (citric acid), vitamins A, C and E and aromas	Orange, apple, pineapple and lemon
6A	Juice, skim milk Sugar, fibre, pectin, acidulant (citric acid), vitamins A, C and E and aromas	Peach, apricot, apple and lemon
7A	Juice, skim milk Sugar, fibre, pectin, acidulant (citric acid), vitamins A, C and E and aromas	Orange, mango, pineapple and lemon
8A	Juice, skim milk Sugar, fibre, pectin, acidulant (citric acid), vitamins A, C and E and aromas	Orange, carrot, pineapple, passion fruit, mango, guava, apricot and papaya
9B	Juice 25.6%, skim milk 16.5% Water, sugar, calcium salts, pectin, acidifier/citric acid, vitamin C, aroma and colorant (β-carotene)	Peach, apricot
10B	Juice 25.6%, skim milk 16.5% Water, sugar, calcium salts, pectin, acidifier/citric acid, vitamin C, aroma and colorant (β-carotene)	Orange, pineapple
11C	Juice 20%, milk 3–5%	Apple
12C	Juice 20%, milk 3–5%	Mango and peach
13D	Juice 33.7%, skim milk Saccharose, dextrose, fibre (pea, soy, orange, apple), pectin, natural aromas and aromas identical to natural ones, vitamins A, C and E	Orange, carrot, lemon, apricot, passion fruit, pineapple and haw
14D	Juice 42.5%, skim milk Saccharose, dextrose, pectin, natural aromas and aromas identical to natural ones, vitamins A, C and E, colorants (extracts of chlorophyll and turmeric)	Apple, kiwi, orange and lime
15D	Juice 7%, skim milk Saccharose, dextrose, pectin, natural aromas and aromas identical to natural ones, vitamins A, C and E, natural colorant (E-160a)	Mango and pineapple
16D	Juice 35%, skim milk Saccharose, dextrose, pectin, natural aromas and aromas identical to natural ones, vitamins A, C and E, colorant (E-100)	Apple (20%), orange (10%), banana (5%)
17E	Juice, skim milk Vegetable oil (rich in conjugated linoleic acid, 0.6%), citric acid, stabilizers (E-440, E-471), sweeteners (E-950, E-951, E-959) and natural aroma	Orange

A–E: the same letter indicates the same manufacturer.
Batches/units per batch: 2/3.
The experiments were performed in triplicate.

ChemStation (series A.06.03) data system (Hewlett–Packard, Waldbronn, Germany).

To determine total antioxidant capacity (TEAC) and total phenolic compounds (TPC), a Perkin–Elmer Lambda 2 UV/Vis spectrophotometer (Überlingen, Germany) was used.

2.4. Methods

2.4.1. Chromatographic determination of carotenes

The method applied was a modification of the method of Cortés, Esteve, Frígola, and Torregrosa (2004). Carotenoids were extracted from 30 g of juice–milk beverage. The best extraction corresponded to a time of 45 min, with N_2 atmosphere and in darkness. The identification and quantification of carotenes (including geometrical isomers) were done by means of liquid chromatography with an ultraviolet–diode array detector, using a Vydac 201TP C_{18} column. The mobile phase used was the ternary methanol mixture (0.1 M ammonium acetate), *tert*-butyl methyl ether and water, in a concentration gradient, and a temperature gradient was applied.

2.4.2. Determination of vitamin A

Vitamin A was expressed as retinol equivalents (RE), using the following conversion: RE = (μg of β-carotene)/12 + (μg β-cryptoxanthin + α-carotene)/24.

2.4.3. Polarographic determination of ascorbic acid

Juice (5 ml) was diluted to 25 ml with the extraction solution (oxalic acid 1%, w/v, trichloroacetic acid 2%, w/v, sodium sulphate 1%, w/v). After vigorous shaking, the solution was filtered through a folded filter (Whatman no. 1). Oxalic acid (9.5 ml) 1% (w/v) and 2 ml of acetic acid/sodium acetate 2 M buffer (pH = 4.8) were added to an aliquot of 0.5 ml of filtrate and the solution was transferred to the polarographic cell. The following instrumental conditions were applied: DP_{50}, mode DME, drop size 2, drop time 1 s, scan rate 10 mV/s, initial potential −0.10 V. Determinations were carried out by using the peak heights and standard additions method (Aparicio, Farré, & Frígola, 1992).

2.4.4. Total phenolic compounds

The total phenol contents of the samples were determined using the Folin–Ciocalteu method (Singleton & Rossi, 1965), reading samples on a Perkin–Elmer Lambda 2 UV/Vis spectrophotometer at 750 nm. Results were expressed as gallic acid equivalents (mg/100 ml).

2.4.5. Total antioxidant capacity

Adapted from the method of Rice-Evans and Miller (1994), this method is based on the inhibition, by antioxidants, of the absorbance of the radical cation of 2,2′-azino-bis(3-ethylbenzothiazoline 6-sulphonate), which has a characteristic long-wavelength absorption spectrum showing maxima at 734 nm. The ABTS radical cation is formed by the interaction of ABTS (150 μM) with the ferrylmyoglobin radical species, generated by the activation of metmyoglobin (2.5 μM) with H_2O_2 (75 μM). Antioxidant compounds suppress the absorbance of the ABTS radical cation to an extent and on a time scale dependent on the antioxidant capacity of the substance under investigation.

This inhibition assay has a fixed time point of 3 min. ABTS, myoglobin and a sample are mixed, and the reaction is initiated by the addition of hydrogen peroxide. After a fixed time the absorbance of the solution is read, along with a buffer blank (which has a greater absorbance value). The results are obtained from the difference in absorption before and after adding the oxidant (H_2O_2), interpolating the value obtained in a calibration curve prepared daily with a trolox standard in the range 0.5–2 mM.

2.4.6. Physiochemical parameters

°Brix was determined by measurement of the refraction index with an Atago model RX-1000 digital refractometer.

Density was measured at 20 °C with a pycnometer (FIPJF, 1968).

The pH was determined with a Crison model 2001 micro pH meter.

2.5. Statistical analysis

Significant differences between the results were calculated by analysis of variance (ANOVA). One-way ANOVA was calculated on three triplicate measurements. Differences at $p < 0.05$ were considered to be significant. Where there were differences, an LSD test was applied to indicate the samples between which there were differences. A multiple regression analysis was performed to study the influence of different factors on a given parameter. Finally, we studied whether there were correlations between a pair of variables.

All statistical analyses were performed using Statgraphics Plus 5.0 (Statistical Graphics Corporation, Inc., Rockville, MD, USA).

3. Results and discussion

3.1. Samples analysed

As Table 1 shows, the samples analysed (mixtures of fruit juice and milk) contain various kinds of fruits. A multiple regression analysis was therefore applied in order to determine the influence of each fruit on the parameters analysed.

3.2. Physicochemical characterization

The physicochemical parameters of the commercial beverages studied in this work are shown in Table 2. These quality parameters are related to the stability of bioactive compounds in plant-derived products. Sample 11C, an apple juice, had the lowest pH (2.96 ± 0.012), and it also had the lowest °Brix (4.00 ± 0.001) and the lowest density (1.017 ± 0.005).

Table 2
Physicochemical parameters of the juice–milk beverages analysed

Sample	°Brix	pH	Density
1A	14.13 ± 0.06	4.11 ± 0.01	1.059 ± 0.005
2A	14.43 ± 0.06	3.91 ± 0.01	1.062 ± 0.012
3A	14.00 ± 0.01	3.99 ± 0.01	1.060 ± 0.012
4A	14.20 ± 0.06	3.85 ± 0.01	1.056 ± 0.005
5A	12.98 ± 0.01	3.65 ± 0.01	1.055 ± 0.005
6A	12.67 ± 0.06	3.71 ± 0.02	1.054 ± 0.003
7A	12.70 ± 0.01	3.83 ± 0.01	1.055 ± 0.009
8A	13.03 ± 0.06	3.57 ± 0.01	1.056 ± 0.006
9B	13.50 ± 0.06	3.84 ± 0.01	1.056 ± 0.004
10B	13.70 ± 0.06	3.80 ± 0.01	1.054 ± 0.002
11C	4.00 ± 0.01	2.96 ± 0.01	1.017 ± 0.005
12C	4.70 ± 0.06	3.27 ± 0.01	1.019 ± 0.005
13D	14.30 ± 0.01	3.32 ± 0.01	1.059 ± 0.003
14D	14.03 ± 0.06	3.82 ± 0.006	1.058 ± 0.008
15D	14.16 ± 0.06	3.79 ± 0.006	1.060 ± 0.007
16D	13.33 ± 0.06	3.90 ± 0.007	1.055 ± 0.006
17E	7.47 ± 0.01	3.63 ± 0.008	1.026 ± 0.003

Values are means ± SD, $n = 17$.
A–E: the same letter indicates the same manufacturer.

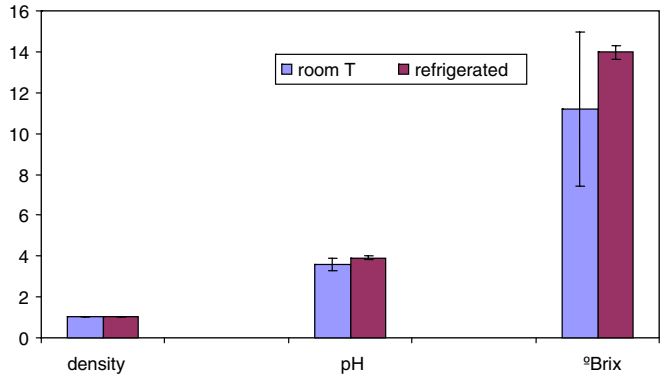

Fig. 1. Physicochemical parameters of samples stored under refrigeration and at room temperature.

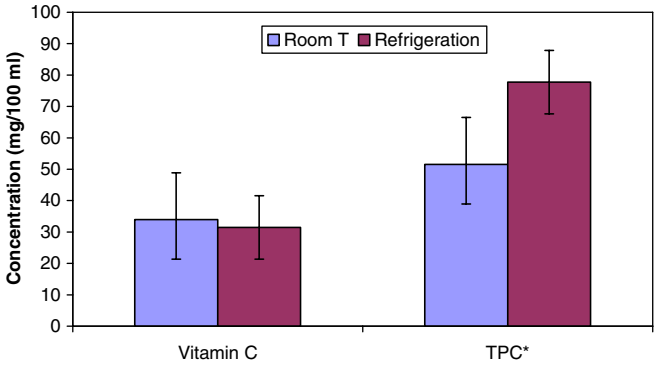

Fig. 2. Vitamin C and total phenolic compounds in samples stored under refrigeration and at room temperature. (*) TPC (total phenolic compounds) as mg gallic acid equivalents/100 ml.

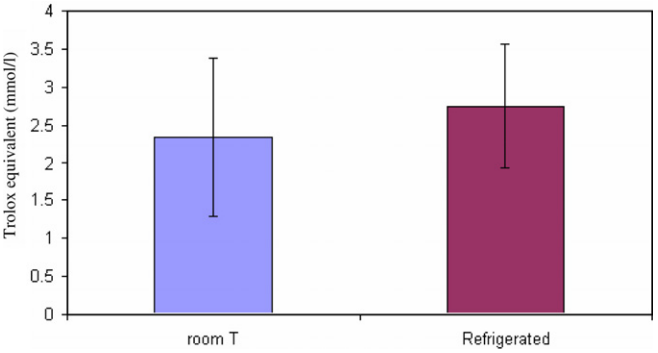

Fig. 3. Total antioxidant capacity of samples stored under refrigeration and at room temperature.

With regard to the influence that the thermal treatment carried out by the manufacturers had on pH, °Brix and density (Fig. 1), the UHT samples (stored at room temperature) had lower °Brix and density as a result of the destruction of soluble compounds in the juice by the temperature (≅120 °C).

The values for °Brix ranged from 4 to 14.43. Manufacturers add sugar to offset the acid taste of some fruits, such as lemon and apple, and seek to achieve low acidity, which has better consumer acceptance (Sánchez-Moreno et al., 2006). °Brix correlated with the presence in the fruit juice composition of orange ($r = 0.379$, $p < 0.01$) or pineapple ($r = 0.357$, $p < 0.01$), while lemon correlated negatively with this parameter ($r = -0.414$, $p < 0.05$).

The pH value ranged from 2.96 to 4.10. The presence of lemon or haw correlated negatively with this parameter ($r = -0.519$, $p < 0.01$; $r = -0.343$, $p < 0.01$, respectively), while orange correlated positively with pH ($r = 0.352$, $p < 0.05$). Thus lemon contributes significantly to a decrease in pH.

The density ranged from 1.017 to 1.062 and was the parameter with the least variable values. It was significantly positively correlated ($p < 0.05$) with the presence of pineapple or orange in the fruit juice composition ($r = 0.374$; $r = 0.362$, respectively), while lemon was negatively correlated ($r = -0.375$, $p < 0.01$).

All the parameters were higher for the refrigerated products, showing better quality and characteristics in accordance with consumer acceptance.

3.3. Vitamin C content

It is known that packaging material influences the quality of liquid foods during storage, due to the absorption of flavour compounds by packaging materials or permeation through them and degradation of flavour, colour, and nutrients by oxygen transmission through packages (Ayhan, Yeom, Zhang, & Min, 2001). All the samples were marketed in Tetra Brik cartons, so that this factor would have no influence. In the commercial fruit juice–skim milk beverages tested, the ascorbic acid content ranged from 9.32 to 53.9 mg/100 ml. The average of the samples stored at room temperature (34.0 ± 10.1 mg/100 ml) was higher than that of the refrigerated samples (31.5 ± 16.1 mg/100 ml), but the differences were not statistically significant (Fig. 2). We determined whether the addition of vitamin C declared on the label influenced the final concentration of the vitamin. Depending on the manufacturer, there were three different concentrations of addition of ascorbic acid declared on the label: 30 mg (manufacturer A), 9 mg (manufacturers B and D) and no addition (manufacturers C and E). As one might expect, a correlation was found between the different quantities added and the concentration of vitamin C in the sample ($r = 0.631$, $p < 0.01$).

For the statistical analysis of the quantity of vitamin C contributed by the type of fruit, the values were corrected by subtracting the addition declared on the label from the total vitamin found. The concentration of ascorbic acid correlated significantly with the presence of lemon in the composition of the beverage ($r = 0.383$, $p < 0.01$), with significantly higher mean contents ($p < 0.05$) (43.3 ± 9.87 mg/100 ml), results that agree with those obtained by Gorinstein et al. (2001) when they compared vitamin C concentrations in various types of citric fruits. The presence of lemon also caused a decrease in the pH of the samples con-

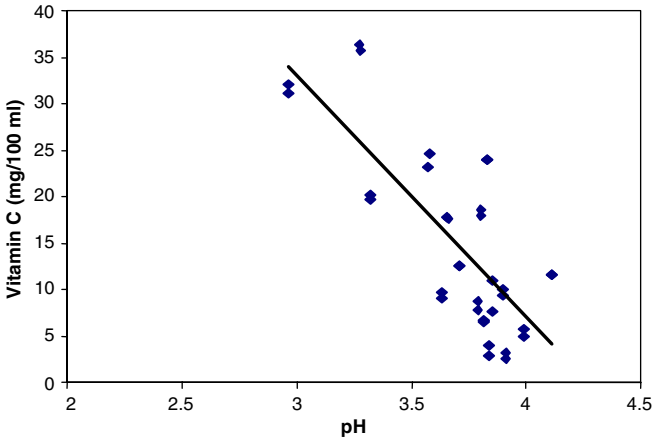

Fig. 4. Correlation between pH and concentration of vitamin C present in samples analysed.

taining it (3.30 ± 0.39), a consequence that helps to stabilize the ascorbic acid, as the results showed.

With regard to the other physicochemical parameters studied, only pH had a significant influence ($p = 0.016$), and it correlated negatively with the vitamin C concentrations contributed by the fruit ($r = -0.738$, $p < 0.01$) (Fig. 4), as acid media contribute to the stability of the vitamin. This explains why samples 11C and 12C – to which the manufacturer had added only 9 mg of vitamin C and which were composed of fruits that were not rich in vitamin C – had high values, because of their low pHs. Summing up, pH values greater than 3.59 corresponded to a mean vitamin C concentration of 30.3 ± 3.45 mg/100 ml, whereas pH values less than 3.59 corresponded to a mean concentration of 42.1 ± 4.23 mg/100 ml, results that agree with those obtained by Tannebaum, Archer, and Young (1985) and Bull et al. (2004).

3.4. Soluble phenolic compounds

The concentration of total phenolic compounds found in the samples analysed varied widely between samples. The values ranged from 26.5 ± 0.02 to 99.8 ± 0.04 mg/100 ml of gallic acid equivalents (GAE).

The total concentrations of phenolic compounds were significantly different ($p < 0.05$) in different storage conditions (Fig. 2). Greater concentrations were obtained for samples stored under refrigeration (77.8 ± 13.7 mg GAE/100 ml) than for samples stored at room temperature (52.5 ± 22.2 mg GAE/100 ml), with significant statistical differences between groups ($p = 0.001$). The values of pH, °Brix and density did not correlate with the contents of total phenolic compounds.

Statistically, beverages which contained apple or mango in their composition showed lower concentrations of phenolic compounds (50.3 ± 11.5 and 48.8 ± 10.1 mg GAE/100 ml, respectively), although sample 11C, which only contained apple juice, had good levels of phenolic compounds (Table 3).

3.5. Vitamin A

Vitamin A contents, expressed as retinol equivalents (RE), ranged from 0.44 ± 0.08 to 34.0 ± 5.98 RE, as

Table 3
Values of various antioxidant compounds in the samples analysed

Sample	Vitamin C (mg/100 ml)	Vitamin A* (RE)	Total vitamin A** (RE)	Phenolic compounds (mg/100 ml gallic acid equivalents)	TEAC (mmol trolox/l)
1A	41.6 ± 0.02	5.54 ± 0.37^{a}	76.2 ± 18.8^{ab}	66.9 ± 0.04	3.36 ± 0.07
2A	32.9 ± 0.39	14.7 ± 0.25^{bc}	136 ± 48.5^{abc}	75.5 ± 0.09	2.99 ± 0.11
3A	35.4 ± 0.56	0.44 ± 0.08^{a}	0.44 ± 0.08^{d}	84.7 ± 0.03	3.41 ± 0.05
4A	39.3 ± 2.39	25.5 ± 0.37^{de}	261 ± 20.1	99.9 ± 0.04	3.31 ± 0.01
5A	47.7 ± 0.18	1.40 ± 0.16^{a}	1.39 ± 0.16^{ad}	44.5 ± 0.13	3.60 ± 0.03
6A	42.6 ± 0.05	15.4 ± 1.18^{bc}	15.4 ± 1.18^{ad}	56.6 ± 0.12	3.53 ± 0.11
7A	53.9 ± 0.01	5.23 ± 0.72^{a}	5.23 ± 0.72^{ad}	56.3 ± 0.15	3.41 ± 0.06
8A	53.9 ± 0.99	34.0 ± 5.98^{e}	178 ± 42.5^{c}	44.1 ± 0.01	3.51 ± 0.17
9B	12.4 ± 0.69	26.1 ± 2.91^{de}	26.1 ± 2.91^{ad}	69.0 ± 0.07	1.31 ± 0.13
10B	27.2 ± 0.41	9.03 ± 1.24^{ab}	9.03 ± 1.24^{ad}	71.0 ± 0.05	2.12 ± 0.23
11C	40.6 ± 0.57	0.09 ± 0.01^{a}	0.09 ± 0.01^{d}	61.2 ± 0.05	1.77 ± 0.28
12C	45.0 ± 0.41	3.56 ± 1.12^{a}	3.56 ± 1.12^{ad}	49.2 ± 0.14	2.35 ± 0.09
13D	28.9 ± 0.34	22.3 ± 5.89^{cd}	22.3 ± 2.89^{ad}	73.2 ± 0.01	2.44 ± 0.16
14D	15.6 ± 0.04	0.45 ± 0.08^{a}	164 ± 6.71^{c}	40.2 ± 0.01	0.61 ± 0.21
15D	17.3 ± 0.63	28.8 ± 2.14^{de}	28.8 ± 2.14^{ad}	26.5 ± 0.02	1.48 ± 0.13
16D	18.7 ± 0.42	0.31 ± 0.01^{a}	67.8 ± 12.0^{abd}	41.6 ± 0.01	2.08 ± 0.14
17E	9.3 ± 0.48	0.64 ± 0.02^{a}	0.65 ± 0.02^{ad}	74.1 ± 0.03	1.02 ± 0.02

Values are means ± SD, $n = 17$.
A–E: the same letter indicates the same manufacturer.
[a–e] The same letter indicates that there are no significant differences between the samples.
[*] Vitamin A calculated with the formula: RE = (μg of β-carotene)/12 + (μg β-cryptoxanthin + α-carotene)/24.
[**] Total vitamin A calculated as RE = vitamin A (RE) + retinol (μg/ml).

Table 4
Concentrations of carotenoids with vitamin A activity in the samples

Sample	β-Cryptoxanthin (μg/100 ml)	α-Carotene (μg/100 ml)	β-Carotene (μg/100 ml)	Retinol (μg/100 ml)
1A	12.3 ± 1.00^{a}	3.84 ± 0.27^{a}	25.3 ± 3.17^{ab}	70.7 ± 19.2^{ab}
2A	–	9.18 ± 0.20^{a}	83.6 ± 1.58^{de}	122 ± 48.7^{bc}
3A	–	–	2.61 ± 0.48^{a}	–
4A	146 ± 4.22	6.24 ± 0.40^{a}	75.6 ± 5.48^{de}	235 ± 19.8^{d}
5A	12.9 ± 2.12^{a}	0.31 ± 0.13^{a}	1.64 ± 0.30^{a}	–
6A	71.5 ± 5.35	6.34 ± 1.05^{a}	53.4 ± 10.23^{bcd}	–
7A	–	4.62 ± 0.28^{a}	29.0 ± 4.47^{abc}	–
8A	–	74.3 ± 8.58	159 ± 30.42^{f}	144 ± 46.6^{c}
9B	–	9.09 ± 0.06^{a}	140 ± 0.02^{f}	–
10B	20.9 ± 8.20^{a}	2.46 ± 0.35^{a}	42.4 ± 3.23^{abcd}	–
11C	–	–	0.50 ± 0.03^{a}	–
12C	–	1.28 ± 0.45^{a}	20.7 ± 6.47^{ab}	–
13D	–	41.0 ± 14.31	114 ± 37.87^{ef}	–
14D	–	–	2.28 ± 0.06^{a}	163 ± 6.79^{cd}
15D	–	1.91 ± 0.83^{a}	160 ± 3.89^{f}	–
16D	–	0.19 ± 0.15^{a}	1.21 ± 0.66^{a}	67.5 ± 12.0^{ab}
17E	–	7.75 ± 0.29^{a}	–	–

Values are means ± SD, $n = 17$.
[a–f]Means with different letters within a column are significantly different at $p < 0.05$.

shown in Table 3, while the various concentrations of carotenoids with vitamin A activity – α-carotene, β-carotene, β-cryptoxanthin and retinol palmitate – appear in Table 4.

Neither total vitamin A nor carotenoids were statistically different under different storage conditions. This was due to the great variety of concentrations found in the samples, resulting from the fruit composition, fortification of the product by the manufacturer, or their introduction as antioxidants. Legal guidelines for vitamin fortification in foods in Spain were established by the government and are included in BOE, 2003. Vitamin A can be added as retinol, retinyl acetate, retinol palmitate or β-carotene (Annex II, BOE, 2003). The quantities added should provide not less than 15% and not more than 100% of the RDI. β-Carotene is approved as a food colour in the EU (E160a) (European Parliament & Council, 1994).

Statistically, the total antioxidant capacity of carotenoids showed significant positive differences ($p < 0.05$) in β-cryptoxanthin concentrations. These results agree with those obtained by Miller, Sampson, Candeias, Bramley, and Rice-Evans (1996), who showed that the antioxidant capacity of carotenoids with vitamin A capacity was greatest for β-cryptoxanthin, followed by β-carotene and α-carotene, because the relative abilities of carotenoids to scavenge the ABTS radical cation are influenced by the presence of functional groups with increasing polarities, such as carbonyl and hydroxyl groups, in the terminal rings, and by the number of conjugated double bonds.

The pH values did not show correlations with carotenoids or total vitamin A, whereas °Brix and density each showed a positive correlation for β-carotene ($r = 0.381$, $p < 0.01$; $r = 0.396$, $p < 0.01$, respectively), which indicates that these two physicochemical parameters affect its stability.

3.6. Total antioxidant capacity

The antioxidant capacity (TEAC) of the various commercial beverages ranged from 3.60 to 0.61 mmol trolox/l. The lowest value corresponded to sample 14D (Table 3), which also had a low vitamin C concentration (15.6 mg/100 ml) and a low phenolic compounds content (40.2 mg GAE/100 ml).

The total antioxidant capacity of the (pasteurized) samples stored under refrigeration (2.74 ± 0.82 mmol trolox/l) was slightly higher (Fig. 3) – although the difference was not statistically significant – than that of the (UHT) samples stored at room temperature (2.34 ± 1.04 mmol trolox/l). This links up with the results obtained by Anese et al. (1999) and Sánchez-Moreno et al. (2006), who, when studying the antioxidant properties of tomato juice and their alteration with temperature, found that the high temperatures (~120 °C) to which the samples kept at room temperature had been subjected were responsible for the destruction of vitamins and other antioxidant compounds, so that the total antioxidant capacity of those samples was lower.

With regard to the composition of the samples, we investigated whether the differences in the percentage of fruit juice influenced the antioxidant capacity of the beverage. The differences were not significant ($p > 0.05$), but the fruit from which the juice was obtained did have a significant influence on the total antioxidant capacity. It was greater in samples that contained lemon ($p = 0.031$), followed by orange ($p = 0.043$), results that agree with those obtained by Arnao et al. (1998) when they compared the antioxidant power of lemon, orange and grapefruit juices, observing that the TEAC of lemon was approximately 33% higher than that of orange or grapefruit. Similarly, by means of a TRAP analysis (total radical trapping antioxidant potential), Gorin-

stein et al. (2001) found that lemon had a greater antioxidant capacity than had other citric fruits.

Orange has a high concentration of carotenoids, especially β-cryptoxanthin, which has a significant influence on TEAC, and it is also rich in phenolic compounds (naringin, hesperetin), and consequently has a high antioxidant capacity. In order to evaluate the compounds that affect total antioxidant power, we educed a multivariant regression analysis (Eq. (1)), which showed that vitamin A, phenolic compounds and vitamin C had a positive influence on the total antioxidant capacity. The contribution of vitamin C was five times greater than were vitamin A or phenolic compounds.

$$\begin{aligned}\text{TEAC} = {} & -0.184 + 0.009 * [\text{vitamin A}] + 0.011 \\ & * [\text{phenolic compounds}] + 0.058 \\ & * [\text{vitamin C}] \ (R - \text{squared}, 86.88\%) \end{aligned} \quad (1)$$

This is similar to the result obtained by Gardner et al. (2000) and Sánchez-Moreno et al. (2003), who found that the compound with the greatest antioxidant capacity in different orange juices was vitamin C. Similarly, Rice-Evans and Miller (1996) observed that ascorbic acid was the chief contributor to antioxidant capacity in apple juice. However, when Rapisarda et al. (1999) studied different orange juices, they found that the antioxidant capacity of the juice could be attributed mainly to its phenolic content, whereas ascorbic acid played a lesser part.

As the results show, the best correlation was obtained between TEAC and vitamin C content ($r = 0.834$, $p < 0.01$) (Fig. 5), but we also found correlations between TEAC and vitamin additions ($r = 0.370$, $p < 0.05$), and between TEAC and β-cryptoxanthin ($r = 0.349$, $p < 0.01$). When a Student's t-test was performed, statistical differences were found for β-cryptoxanthin concentration ($p = 0.048$), retinol palmitate ($p = 0.027$), phenolic compound content ($p = 0.032$), vitamin additions ($p = 0.011$) and storage conditions ($p = 0.018$). The best antioxidant capacity was shown by the samples that were stored under refrigeration (2.74 ± 0.82 vs 2.34 ± 1.04 mmol trolox Eq. (1).

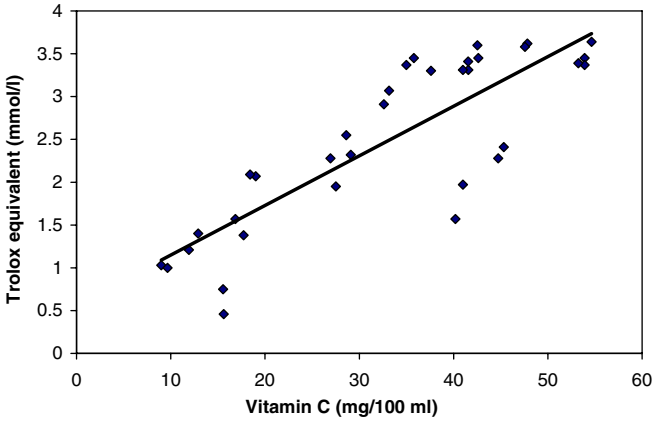

Fig. 5. Correlation between total antioxidant capacity (trolox mmol /l) and vitamin C (mg/100 ml).

3.7. Influence of physicochemical parameters on TEAC

°Brix and density showed statistical positive differences ($p = 0.031$), as the samples with °Brix >11.11 and density >1.05 had TEAC values of 2.65 mmol trolox eq./l, compared with a value of 1.71 mmol trolox eq./l for the samples with lower values for °Brix and density. The pH value also had an indirect influence, helping to stabilize vitamin C, which was the compound that contributed most to TEAC.

After obtaining all the results we verified whether the percentage of milk in the composition of the beverage might have had some influence on the parameters analysed. We found significant correlations ($p < 0.01$) for °Brix, density and pH ($r = 0.955$, $r = 0.953$, $r = 0.701$, respectively). The absence of correlations with antioxidant power, phenolic compounds and vitamins C or A was probably due to the use of skim milk, in which vitamin concentrations are significantly lower. The percentage of juice also did not interact significantly with the parameters analysed, as most manufacturers correct possible differences by the addition of vitamins, irrespective of the percentage of juice or milk that the mixtures contain. Consequently we can say that the differences between parameters were due to the composition of the juice (types of fruit) and the storage or processing conditions.

4. Conclusions

The total antioxidant capacity of the samples analysed was higher in the refrigerated samples than in the samples stored at room temperature. This might be the result of industrial thermal treatment and might also be related to storage conditions, favourable for antioxidants when storage temperature is in the range 4 ± 2 °C. The TEAC is mainly due to vitamin C, followed by phenolic compounds. Greater °Brix and density contribute to the stability of the antioxidants present in the samples. Lemon and orange are the fruits that provide the highest concentrations of vitamin C, resulting in a greater antioxidant capacity.

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