Bioaccessibility of carotenoids, vitamin A and α-tocopherol, from commercial milk-fruit juice beverages: Contribution to the recommended daily intake

Highlights

- Carotenoids, retinol and α-tocopherol in commercial milk fruit are presented
- The composition shows a great variability depending on the type and amount of fruit
- The predominant carotenoid present in all the samples was β-carotene
- Xantophylls were the most bioaccessibility bioactive carotenoids
- Consumption provides up to 11% of the Recommended Dietary Allowance for Vitamin A

Abstract

Twenty-two commercial milk-fruit juice beverages (MFJBs) were analysed by high performance liquid chromatography with diode array detector for carotenoids, retinol and α-tocopherol content. Bioaccessibility was also investigated by *in vitro* enzymatic digestion. Total carotenoids content and vitamin A calculated as retinol plus pro-vitamin A carotenoids varied widely among samples as well as the bioaccessibility, depending on the formulations. The predominant carotenoid present in all the samples was β-carotene, followed by α-carotene, β-cryptoxanthin and zeaxanthin, which were not detected in all the samples. One daily consumption of MFJB (200 mL) provides on average from 5% to 11 % of the Recommended Dietary Allowance (RDA) for vitamin A, depending on the age group and a slightly higher value for α-tocopherol. The bioaccessibility of bioactive carotenoids in increasing order was the following: β-carotene < α-carotene <phytofluene =phytoene < lutein = β-cryptoxanthin < zeaxanthin. If we consider bioaccessibilities the contribution to the RDA is drastically reduced by 40% for vitamin A and about 13% for vitamin E.

KEYWORDS: bioaccessibility, carotenoids, milk-fruit juice beverage, retinol vitamin A, vitamin E

Abbreviations: Milk-fruit juice beverage (MFJB); retinol activity equivalent (RAE); retinol equivalent (RE).

1. Introduction

Changes in climate and in per capita income have brought about a change in beverage consumption habits. Beverages are no longer consider as simply thirst quenchers, consumers demand healthier refreshment, variety and convenience in their beverages with an increasing demand of ready-to-drink beverage products (Chambers IV, 2014). Among them are those containing milk and fruit juice beverage (MFJB). They are a useful commodity to reach healthier dietary habits, which is a growing concern in the prevention of chronic diseases. Consumption of fruits and vegetables has long been considered beneficial for health. A meta-analysis by Wang et. al. (2014) provided further evidence that a higher consumption of fruit and vegetables is associated with a lower risk of all-cause mortality, particularly cardiovascular mortality. Despite this fact, recent data on food consumption indicates that in half of developed countries the consumption is lower than 400 g per day of fruit and vegetables, and in one third of the countries the average intake is less than 300 g per day (Micha et al., 2015). In this sense, fruits and vegetables are known for their antioxidant activities due to the presence of compounds like flavonoids, phenolic acids, carotenoids, vitamins A, vitamins C and tocopherol. A moderate consumption of fruit juice beverages may be an important source of these compounds, while excessive fruit juice consumption has been associated with weight gain and development of dental caries (Ludwig et al., 2001; Wootton-Beard and Ryan, 2011). On the other hand, milk is an important source of vitamin D, calcium and bioactive peptides with health promoting effects (Marcone et al., 2017). Low dietary calcium intakes and poor vitamin D status are common findings in different population groups, mainly children and elderly (Durá-Travé et al., 2017; Gennari, 2001). Also in developed populations like the Americans some vitamins (A, C, D and E), fiber and calcium, among other minerals, are considered as “underconsumed nutrients” (USDA, 2015). To address nutritional deficiencies there is a growing demand of functional foods in place of dietary supplements and therefore MFJBs due to their composition represent a good option to obtain beverages with functional properties. However, an important factor to be taken into account is the bioavailability (Fernández-García et al., 2012). This is a mandatory aspect to consider when discussing the potential role as a functional food of any product. In a recent review by Kopec and Failla, (2017) different aspects related with the impact of lipid, food matrix and the effects of emulsifiers like pectin are discussed in relation to the bioavailability of carotenoids.

In the formulation of MFJB usually fruit juice from concentrate is present from 7% up to 41% and milk up to 30%, they also contain fiber, vitamins, sugar and emulsifier, besides the other bioactive compounds provided by the added fruit juice and milk. MFJBs are designed to meet technological, safety and sensory aspects rather than the nutritional ones. The impact of the new matrix of the blended beverage on the bioaccessibility of these compounds has been studied in model solution (He et al., 2015) or in laboratory designed products (Rodríguez-Roque et al., 2016, 2014; Shukla et al., 2003) but there is little information on commercial products. The presence of milk has shown to reduce the bioaccessibility of the hydrophilic compounds probably due to milk protein interaction (He et al., 2015; Rodríguez-Roque et al., 2015). However, in the case of carotenoids, due to their hydrophobic nature, their uptake is generally quite low from fruit and vegetables while the addition of digestible lipids has shown to increase their bioaccessibility (Lemmens et al., 2014) also in blended milk-fruit formulations (Granado-Lorencio et al., 2009; Rodríguez-Roque et al., 2014). This effect could be attributed to the increased solubilisation of these long and highly lipophilic molecules within the gastrointestinal intestinal track fluids in the presence of lipid digestion products, such as micelles and vesicles (Salvia-Trujillo et al., 2017).

The main objective of this paper was to characterize the carotenoid composition of commercial MFJB present in the Spanish market, to have an overview of the potential role as functional food of these products, considering the bioaccessibility of carotenoids, tocopherols, and vitamin A from the new matrix, and the contribution to the dietary intake of vitamins A and E.

2. Materials and Methods

2.1.Chemicals

Extraction solvents were analytical-grade hexane, acetone, and ethyl-acetate from Carlo-Erba (Milan, Italy). Analytic solvents were HPLC-grade methanol and methyl tert-butyl ether (MTBE) from Merck (Darmstadt, Germany). Purified water was obtained from a NANOpureDiamond system (Barnsted Inc.). Mineral salts (KCl, NaCl), sodium bicarbonate, monopotassium-phosphate, magnesium chloride hexahydrate, chlorhydric acid, pepsin (porcine gastric mucosa), pancreatin (porcine pancreas), bile salt, β-carotene, β-cryptoxanthin, zeaxanthin, retinol and α-tocopherol were purchased from Sigma-Aldrich (Steinheim, Germany). Other carotenoids standards were either isolated from appropriate sources or semisynthesized in accordance to standard procedures as explained elsewhere (Kimura and Rodriguez-Amaya, 2002; Rodriguez-Amaya, 2001).

2.2.Samples

22 different commercial milk-fruit juice beverages (MFJBs) of all the different brands available in the Spanish market were purchased from local supermarkets. **Table** 1 gives details (as indicated on the label) of each of the samples analysed. Most of them had been UHT processed and so with a shelf life of up to 12 months. They were kept at room temperature (20 ± 2 ◦C) until analysed. Five of them had shorter shelf life (2 months)

because they were pasteurized and so were kept under refrigeration (4 ± 2 ◦C). The analyses were performed in triplicate.

2.3. *In vitro digestion method*

The *in vitro* simulated gastrointestinal digestion protocol used in this study was based in the international consensus methodology explained elsewhere (Minekus et al., 2014), with some variations (Alminger et al., 2014). Briefly the method consisted of a first digestion using pepsin at pH=3 for 2 h at 37 ºC (to simulate gastric digestion) and a second digestion with bile salts and pancreatin at pH=7, for 2 h at 37°C (to simulate small intestine). Two electrolytic fluids, Simulated Gastric Fluid (SGF) and Simulated Duodenal Fluid (SDF) were used in the digestions. Both were prepared from the corresponding electrolyte stock solution, according to the consensus method proposed by Minekus et al. (2014) and recently adopted to evaluate the bioaccessibility of carotenoids (Estévez-Santiago et al., 2016). The simulated oral phase was not used, as the food matrix evaluated was liquid.

For the gastric digestion, 5 mL of MFJB was added to 13.5 ml of SGF, 0.15 μL of $CaCl_2$ $(H2O)_2$ (588 g/L, w/v), and the pH was adjusted to 3±0.1 by addition of HCl 1M. Then 450 μL of porcine pepsin (40 mg/mL in 0.1 M HCl) was added to each sample (Garrett et al., 1999; Hedrén et al., 2002). The mixtures were incubated in a shaker Max Q5000 (Thermofisher scientific Inc., Waltham, MA, USA) at 150 rpm and 37 °C for 2 h to complete the gastric phase. The samples were placed in an ice bath to stop the gastric digestion.

For the duodenal digestion, 4 ml of SDF and 1.05 μL of $CaCl_2$ $(H_2O)_2$ were added. The pH of the solution was adjusted to 7 with NaOH 1 M or HCl 1 M. Finally, 3 ml of the mixture of pancreatin and porcine bile extract solubilized in the SDF were added (Garrett et al., 1999). The samples were incubated in a shaker Max Q5000 (Thermo Fisher

Scientific Inc., Waltham, MA, USA) at 150 rpm and 37 °C for 2 h to complete the intestinal phase.

Subsequently, the micellar fractions were homogenized and centrifuged at 3900 × g for 20 minutes at 4˚C (Granado-Lorencio et al., 2007) using an Allegra X-12R centrifuge (Beckman Coulter, USA). The supernatant was filtered through 0.2 µm nylon membrane (Agilent Technologies, USA). The filtrates were stored at -20 °C under an atmosphere of nitrogen until analysis. The bioaccessibility was calculated as the ratio between the concentration of carotenoid (mg/L) in the micellar phase and its concentration in the MFJB as shown in the following equation:

$$\%Bioaccessibility_{carotenoids} = \frac{[Carotenoids]_{micellar\ fraction}}{[Carotenoids]_{sample}} \times 100 \qquad (1)$$

2.4. Extraction of carotenoids

2.4.1. Undigested samples

The extraction method was done as explained in detailed elsewhere using hexane: acetone (1:1) (Stinco et al., 2014) and saponification was carried out according to the method described in Stinco et al. (2012) using methanolic KOH (30% w/v) for 1 h under dim light and at room temperature.

2.4.2. Micellar fractions

The micellar fractions (ca. 16-17 mL) were gently mixed with 3 ml of mixture of hexane:acetone (1:1 v/v) and centrifuged for 10 min at 3900 x g. The extractions were performed until colour exhaustion. Upon centrifugation, the upper coloured layers containing the carotenoids were recovered and evaporated to dryness at temperature below 30°C. To obtain unesterified carotenoids, the extracts were dissolved in 300 µl of dichloromethane and treated with 300 µl of methanolic KOH (30% w/v) for 1 h under dim light at room temperature. Subsequently, they were washed with 5% NaCl and water to remove any trace of base. The coloured extract obtained was concentrated to dryness in a rotary evaporator at temperature below 30 ºC and dissolved in 50 μL of ethyl acetate prior to their injection in the HPLC system. The injection volumes were 2.5 and 5 μL for the MFJBs and the micellarized fractions respectively. The analyses were performed in triplicate.

2.5. HPLC Analysis of carotenoids

The HPLC analyses were carried out according to the method described by Stinco et al. (2012). The identification of carotenoids was made by comparison of their chromatographic and UV/vis spectroscopic characteristics with those of standards either isolated from appropriate sources or semisynthesized in accordance to standard procedures as explained elsewhere (Meléndez-Martínez et al., 2007).

The quantification was carried out by external calibration from the areas of the chromatographic peaks obtained by DAD detection at the following wavelengths: 285 nm for phytoene and α-tocopherol, 350 nm for phytofluene and retinol and 450 nm for the rest of the CARS (lutein, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene). The total content was assessed as the sum of the content of individual pigments.

The vitamin A activity of the MFJB samples was expressed in terms of retinol activity equivalents (RAE) (Food and Nutrition Board, 2000).The following formula was used for obtaining the RAE value and the results were referred to 100 mL of MFJB:

$$RAE = \frac{\mu g\ \beta\ carotene}{12} + \frac{\mu g\ \ \beta\ cryptoxanthin + \mu g\ \ \alpha\ carotene}{24} \qquad (2)$$

2.6.Contribution to the Recommended Daily Intake (RDI)

The contribution of the different MFJBs analysed to the recommended daily intakes were calculated as %, from the values obtained for total vitamin A and α-tocopherol and the Recommended Dietary Allowances established by the Institute of Medicine (IOM, 2001).

Vitamin A was calculated as the sum of retinol and provitamin A carotenoids (RAE) as indicated above (2). For calculations, the RDA values considered were: for vitamin A 900 µg/d for men and 700 µg/d for women and 600 and 400 µg/d for children from 9-13 and 4-8 years old respectively. For α-tocopherol 15 µg/d for men and woman and 11 and 7 µg/d for children from 9-13 and 4-8 years old respectively.

2.7.Statistical Analysis.

Results were given as mean and standard deviation of independent determinations. Significant differences between the results were calculated by analyses of the variance (ANOVA). Statistically significant differences ($p<0.05$) were determined using the Tukey multiple comparison procedure. All the statistical analyses were performed with Statistica v.8.0 software (StatSoft, 2007).

3. Results and Discussion

3.1.Provitamin A, bioactive carotenoids and tocopherol content.

Table 2 shows the mean levels of the carotenoids quantified in the beverages as well as their vitamin A content expressed as retinol activity equivalent and the tocopherol content.

Meaningful comparisons among the MFJB with respect to the carotenoid profiles are difficult due to the wide range of formulations varying both in the type and proportion of fruits and in the juice processing (concentrated, pasteurized or untreated). The fruit content varied widely from 6% to 50% of the formulation. Besides, the percentage of milk and the addition of additives such as colorants, preservatives and different gelling agents, add more variability (**Table 1**). As a general remark, most of them contained added sugar (sucrosa, fructose or glucose) and added vitamins C, E and vitamin A. Vitamin A can be added as retinol, retinyl acetate, retinyl palmitate or β-carotene and vitamin E as D-α-tocopherol, DL-α-tocopherol, D-α-tocopheryl acetate, DL-α-tocopheryl acetate, D-α-tocopheryl acid succinate, all of them are allowed under the European legislation law (EPC, 2006). α-Tocopherol can also be added as an antioxidant additive (E307).

Total carotenoid content assessed as the sum of the individual pigments varied widely among samples. The values ranged from 0.228±0.022 to 7.158±0.434 mg/L. This wide range may be due to some of the factors mentioned above (type and % of fruit added or β–carotene addition as colorant). Some common fruits which appear in the composition of the MFJBs such as apple, grape, pear, strawberry, kiwifruit, pineapple, and banana are low in carotenoids (Beltrán et al., 2012; Dias et al., 2018). The predominant carotenoid in all the samples was β-carotene. Of the 22 MFJB analysed, 15 declared β-carotene as food colorant (E160a) in the label, practice which is allowed under the European legislation and US legislation (Lehto et al., 2017). The lowest and highest levels of β-carotene (0.025±0.004 and 5.677±0.184 mg/L) were found in samples S7 and S21 both with no declared β-carotene in the label so coming from the fruits included in its formulation. In both samples carrot appeared as an ingredient (**Table 1**) and in S21 also other sources of β-carotene like mango, papaya and guava were present. The other provitamin carotenoids were not detected in some samples. In the samples that contained α-carotene (twelve) the level ranged from 0.004 to 0.646 mg/L and they were all formulated with at least one of these fruits: orange, mango or carrot. β-cryptoxanthin was detected in thirteen samples at levels that ranged from 0.029 to 0.466 mg /L, all prepared with concentrated juices from fruits which contain β-cryptoxanthin as the major carotenoid, such as the pulp of yellow lemon or orange juices from different varieties (Stinco et al., 2016), peach or papaya (Dias et al., 2018). However, the low levels found suggest that the concentration process may affect these carotenoids or that the fruit composition declared in the label may not exactly reflect the real mix of fruits, which probably depends on the market availability of concentrated juices.

The vitamin A contents, expressed as retinol activity equivalents (RAE) was calculated according to the previous results and ranged from 0.42 to 47.66 with a mean value of 18.40±15.60 (µg/100 mL) as shown in **Table 2**. For total Vitamin A, calculated as RAE± retinol values were slightly higher 22.15 ±16.53. Previous studies reported RAE values in Spanish commercial samples ranging from 0.44 ± 0.08 to 34.0 ± 5.98 (Zulueta et al., 2007) which could be considered in the same range.

Lycopene was found at low concentrations in beverages containing Guava (Psidium guajava L.) (S16) and passion fruit (Passiflora edulis) (S10), which is in accordance with data composition reported on these fruits (Mercadante et al., 1999, 1998).

Lutein and zexanthin oscillated in a similar range, from 0.027 to 0.230 mg/L, and from 0.032 to 0.241 mg/L, respectively. The highest level of lutein was detected in a sample containing orange and banana (S13) while the highest level of zeaxanthin was detected in a sample containing peach and orange (S1). Banana contains a very low amount of lutein but no zeaxanthin while peach and orange contain a moderate amount of both lutein and zeaxanthin, but predominating the last one (Dias et al., 2018).

With respect to the colourless carotenoids (phytoene and phytofluene), the highest levels were found in beverages containing in their formulation concentrated juices from carrot, papaya, passion fruit and peach (samples S17, S8 and S16) which contain these compounds in remarkable amounts (Meléndez-Martínez et al., 2018, 2015)

Besides the bioactive carotenoids, others carotenoids were identified and quantified in some samples (data not shown). These compounds correspond to 5,6-epoxycarotenoids (violaxanthin, antheraxanthin and geometrical isomers), 5,8-epoxycarotenoids (luteoxanthin and mutatoxanthin) and zeinoxanthin (monohydroxycarotenoid). All of them are present in the carotenoid profile of orange juice and are related to its colour (Stinco et al., 2012).

α-Tocopherol was detected in 19 of the 22 samples analysed and varied widely among them from 0.708 ±0.479 to 12.867 ± 0.786 with a mean content of 4.551 ± 4.768 mg/L. In eleven, of the twenty-two MFJBs analysed, α-tocopherol had been added as an additive declared on the label (**Table 1).**

In order to assess the contribution to the dietary intake of vitamin A and E from the consumption of one unit (200 mL) of a given MFJB, the recommended dietary allowances (RDA) stablished by the Institute of Medicine (IOM) (Otten et al., 2006) were considered for adults (men and women) and children. Total vitamin A was calculated, and the values obtained are shown in **Table 2.** When expressed as retinol equivalents (µg RE), the vitamin A activity of provitamin A carotenoids is twice the vitamin A activity than when estimations are made in µg RAE, whereas for preformed vitamin A, RAE and RE are equivalent (Tanumihardjo et al., 2016). Vitamin A intake provided by MFJBs on average was 22.15± 16.53 µg /100 mL, showing a wide range from 0.42 to 64.79 µg /100 mL. **Table 3** shows the % of the RDA provided by the consumption of 1 unit (200 mL) of these beverages for vitamin A and vitamin E in different age groups. The age group "1 to 3 years" has not been considered since this type of product is not intended for that population. One daily consumption provides on average from 5% to 11 % of the RDA for vitamin A depending on the age group, with higher contributions in children (4-8 years old). Compare to skim milk or semi-skimmed milk which contain on average 0.5-6 to 12-16 µg RE /100 mL (Herrero et al., 2002), one unit of a MFJB provides on average three times more (range 0.07-7.94) vitamin A than a glass of skim milk, and up to 1.5 (range 0.03-3.97) more vitamin A than a glass of semiskimmed milk. If we compare it with a glass of handmade orange juice (RAE 17.33 ± 2.89) or with a pasteurized orange juice

(RAE 14.1 ± 1.71) (Stinco et al., 2012) it provides a similar amount or, in some cases (S21), almost 3 times more vitamin A.

In relation to the % of RDA for vitamin E provided by the consumption of 1 unit of MFJB, (**Table 3**) it ranges from 5.5% in adults to 11.8% of the RDA in the lowest age group. Compare with a glass of enriched skim milk or semiskimmed milk which provides 21-28% of RDA in adults (Herrero et al., 2002), MFJBs have a significantly lower contribution to the dietary intake of this vitamin. This is reasonable considering the low amount of milk (on average 10%) in the formulation and that fruits have a low content in vitamin E, mainly as α-tocopherol (< 0.1 mg/100 g) (Chun et al., 2006). Recent surveys and systematic reviews point out that the intakes of vitamin E are below the RDA in both developing and industrialized countries for the major part of the population, in particular in younger adults (Péter et al., 2015). It would be interesting to consider the reinforcement of this type of products in order to achieve the adequate intake of this vitamin.

3.2. Bioaccessibility of carotenoids and tocopherol

Figure 1 shows the results of individuals and total bioactives carotenoids bioaccessibilities (as percentage) in the 22 MFJBs studied after the *in vitro* digestion. The level of carotenoids in the micellar fraction was 0.738±0.808 mg/L (**supplementary materials**) significantly ($p<0.05$) lower than in the original samples (2.965±2.126 mg/L). As stated above, besides the carotenoids included in **Table 2**, different epoxycarotenoids (violaxanthin, antheraxanthin, luteoxanthin) have also been identified and quantified (data not shown) in the micellarized fraction but they have not been considered in this discussion, since they are not found in human plasma and their functions remains unknown. However, it can be highlighted that epoxycarotenoids are also incorporated into micelles. A global mean bioaccessibility value for carotenoids (including non-bioactive ones) in MFJB was 26.8 ± 24.0% while for provitamin A carotenoids was slightly higher (32.1 ± 24.7 %) with some differences, which will be considered.

Total carotenoids bioaccesibilities (%) in the micellarized samples of MFJBs are shown in **Figure 1A**. The wide range of bioaccessibilities observed could be due, as explained previously, to the variability in the formulations. On one hand to be absorbable in the human body, carotenoids need to be released from the food matrix and dispersed into the lipid phase in the stomach, where a fine emulsion structure is created. In this case the type of fruit and the juice processing may have an influence in the bioaccessibility of the carotenoids (Lemmens et al., 2014). In three of the four samples with the highest bioaccessibilities (>50 %), orange juice (not from concentrate) appeared as ingredient on the label besides other fruits. Thermal treatment is known to influence both the content and bioaccessibility of carotenoids as shown in previous studies (Cilla et al., 2012; Stinco et al., 2012).

In a next step, the carotenoids (which are solubilized in the lipid emulsion droplets) need to be incorporated into mixed micelles, together with free fatty acids, monoglycerides and bile salts. Most fruits and vegetables are naturally low in lipids so the addition of lipids may play a key role in this process (Lemmens et al., 2014). In the MFJBs analysed the proportion of milk added is variable (around 10%) and it is always skim milk, so the effect on the bioaccessibility is difficult to work out. Recent studies by da Costa and Mercadante (2017), have shown an increase in the bioaccessibility of carotenoids in relation to the amount of milk added in a milk fruit-beverage from cajá. The authors have attributed the enhancement in the bioaccessibility of carotenoids to the protein fraction of the milk, rather than to the milk-fat. They have hypothesized that the phosphopeptides derived from the digestion may have a stabilization effect on the micelle. Similarly, Granado-Lorencio et al. (2009) have also shown that the addition of milk to fruit juices increases the bioaccesibility of xanthophylls and α-tocopherol "*in vitro*" although "*in vivo*" this increase did not reach statistical significance.

On the other hand, in water-milk based products, like MFJBs, it is necessary to add emulsifiers and gelling agents for texture and stability reasons. In the label of the commercial MFJBs, two types of gelling agent are reported: pectin and different types of gums. According to our results samples containing pectin showed a significantly ($p<0.05$) higher bioaccessibility (40.7 ± 24.3%) than those containing gum (arabic, xanthan or guar) (7.4 ± 6.9%). The water-soluble dietary fibers are widely used as gelling agents, thickeners, stabilizers, and emulsifiers in a variety of foods. They are known to alter the lipid absorption (mainly cholesterol) by mechanism related with the adsorption of bile acids, the repression of digestive enzymes, and the prevention of the absorption of lipids through the wall of the small intestine (Jesch and Carr, 2017). Several studies have demonstrated a reduction to different extend in the bioaccessibility of carotenoids due to the presence of pectin (Verrijssen et al., 2016, 2015) or gum (Park et al., 2018) but the mechanism has not been fully explained.

Other factor that may influence the overall bioaccessibility of carotenoids is sugar addition. In the commercial MFJBs analysed, sucrosa, glucose, fructose or a syrup containing both are declared in the label. Also sucralose is present in some beverages. Sugar addition (<7%) has been reported to enhance bioaccessibility of carotenoids more in water based beverages than in those containing milk fat (da Costa and Mercadante, 2017).

Considering that orange juice with different thermal treatments was present in different proportions in all the MFJBs analysed, we have compared the mean bioccessibility values of MFJBs with those obtained by the same *in vitro* method for orange juice reported by Mapelli-Brahm et al. (2018). While the mean bioaccessibility for total carotenoids reported for fresh orange juice is 7.57 ± 0.28%, for pasteurized orange juice is almost 3.5 higher (25.9 ± 4.4%) (Mapelli-Brahm et al., 2018). This value is slightly lower than the mean bioaccessibility obtained for MFJBs (32.1 ± 24.7 %) but with a greater variability in these samples due to all the factor discussed above, ones enhancing the bioaccessibility (sugar addition, milk proteins and juice processing) while others reducing it (dietary fiber and gelling agents), besides the different types and proportion of fruits added.

3.3.Bioactive Carotenoids Bioaccessibility

The bioaccessibility of β-carotene ranged from 1.2 to 73.9 % (25.8 ± 21.4%). The highest value corresponded to samples containing β-carotene added as colorant (S8 and S12).

With respect to β-cryptoxanthin (**Figure 1B**), the two beverages with the highest bioaccessibilities (86.8 ± 6.2% and 82.3 ± 1.9%) contained more than 40% of concentrated juices from orange, apple, pineapple and lemon (S11 and S14) and pectin as gelling agent. The lowest was found for S1 (9.8 ± 0.9%), a beverage with a low content of peach and orange concentrated juice (6%) and arabic gum as gelling agent.

Bioaccessibility is a relative value, thus the amount of juice in the formulation has no influence on its value. As an example, the sample S21 showed one of the lowest carotenoid content (0.484 mg/L), but the highest bioaccessibility (58.6 ± 11.4%).

The mean bioaccessibility for β-cryptoxanthin was 44.2 ± 27.7%. This result is in agreement with earlier reports for β-cryptoxanthin in a skimmed milk-fruit beverage (54%) (Cilla et al., 2012) and in orange juices 43.7 ± 12.8% (Stinco et al., 2012). Concerning α-carotene, it was present only in 9 samples and the mean bioaccessibility was 35.5 ± 25.7%. These results are consistent with previous studies which suggest that β-cryptoxanthin is better absorbed than other provitamin A carotenoids despite the presence of fat (Burri et al., 2016).

Regarding the bioaccessibility of macular carotenoids (lutein and zeaxanthin), the mean percentage transferred into the micellar fraction were 42.3 ± 24.0 % and 45.3 ± 22.8% respectively (**Figure 1C**). It has been shown that the bioaccessibility of xanthophylls (lutein, zeaxanthin and β-cryptoxanthin) from fruits is better than from green vegetables (O'Connell et al., 2007). Similar results were reported for the macular carotenoid bioaccessibility in orange juices (zeaxanthin 40.4% and lutein 39.5%) (Stinco et al., 2012) and in skimmed milk-fruit based beverages (43% for lutein and 33% for zeaxanthin) (Cilla et al., 2012).

The bioaccessibility of phytoene and phytofluene (colourless carotenoids), were 38.6 ± 27.4% and 37.1 ± 26.7%, respectively (as shown in **Figure 1D**). To the best of the authors' knowledge, bioaccessibility of phytoene and phytofluene has not been previously reported in milk-fruit juice beverages. Jeffery et al. (2012) reported that the bioaccessibility of phytoene varied from 47 to 96% in nine raw carotenoid-storing fruits and vegetables.

Finally, the bioaccessibility of provitamin A carotenoids showed a high variability in the different beverages analysed. The mean bioaccessibility for RAE was 27.2 ± 21.8%, while for α-tocopherol was 33.5 ± 35.7%.

In general, the bioaccessibility of bioactive carotenoids in increasing order was the following: β-carotene < α-carotene. <phytofluene =phytoene < lutein = β-cryptoxanthin < zeaxanthin .These results are in agreement with those of Granado-Lorencio et al. (2007) who reported that the xanthophylls were more efficiently transferred into supernatants than tocopherols and β-carotene.

As discussed above, the comparison of the results obtained is not straightforward whatsoever due to the marked differences both in composition (% fruit, % milk, type of fruit juice, fat and fiber content, etc) and additives (colorants and vitamins).

The results of this work shows that the bioaccessibility vary widely among beverages for each carotenoid, depending on the fruit from which it comes from, besides of the addition of milk and additives. In a recent study, Jeffery et al. (2012) ranked several fruits and vegetables considering the carotenoid relative bioaccessibility from highest to lowest as follows: papaya, watermelon, mango, tomato, carrot, butternut squash, sweet potato, melon and grapefruit.

Considering bioaccessibility of provitamin A carotenoids and vitamin E in the MFJBs after digestion, the contribution to the RDA is drastically reduced by 40% for vitamin A and about 13% for vitamin E (**Table 3**). Compare to a handmade orange juice or pasteurized orange juice (Stinco et al., 2012) the content of vitamin A in the micellar fraction is about 50% higher for the MFJBs than for the orange juice. It must be taken into account that retinol is highly bioaccessible.

Conclusion

To sum, MFJB carotenoid composition shows a great variability depending on the type and amount of fruit. The great variability in biaccesibilities is also related to the complex matrix, in which factors like gelling agent, sugar addition or milk fat can enhance or interfere in the carotenoid absorption. Considering that β-carotene is present in all the samples MFJBs can be a valuable alternative to increase vitamin A intake in children with low fruit intake. Compared to milk (skim and semiskimmed) MFJBs also provide more vitamin A and more bioaccesible than some fruits. Considering the aspects discussed, and knowing the bioaccessibility of elements in different types of foods, consumers have the choice to balance their diet in a healthy way.

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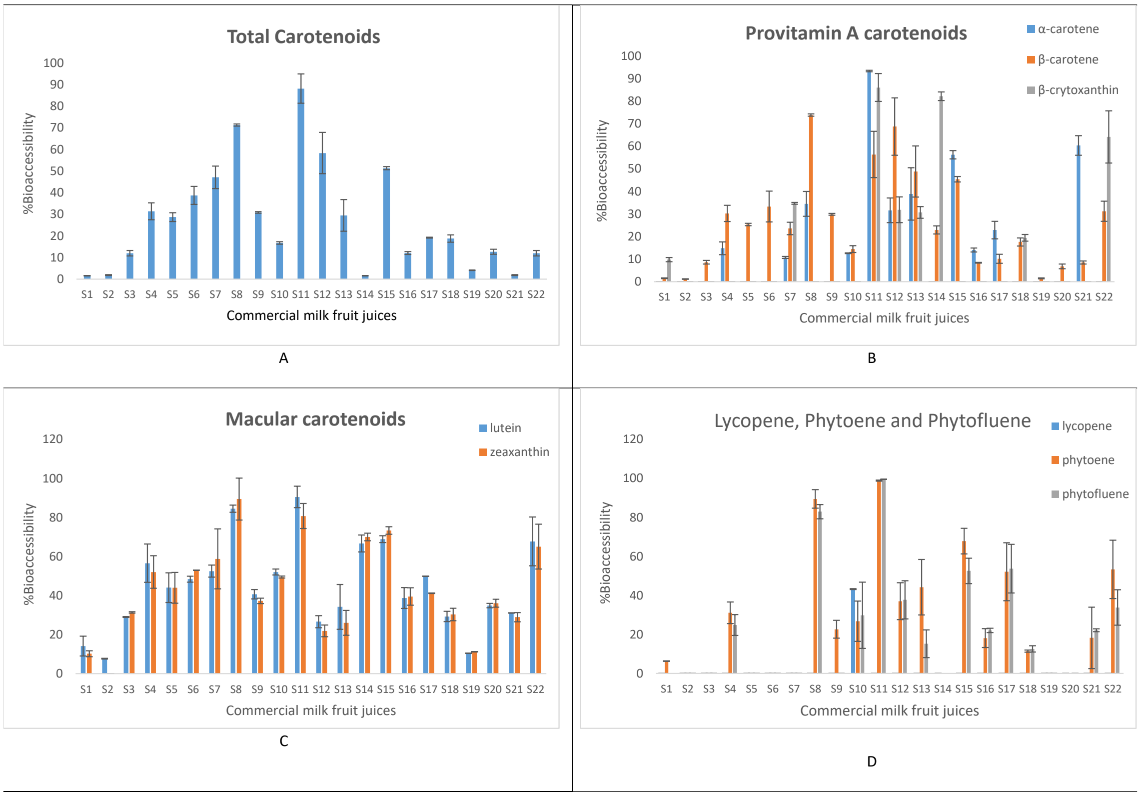
Figure captions

Figure 1. Mean bioaccesibilities (%) of total and bioactive carotenoids in commercial milk fruit juices.

Figure 1.



A: Total carotenoid, B: Provitamin A carotenoids, C: Macular carotenoids, D: Lycopene, Phytoene and Phytofluene