

Antioxidant Activity and Quality of Apple Juices and Puree After *in vitro* Digestion

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Abstract

Dietary recommendations include the consumption of fresh apples and processed apple based products mainly for the antioxidant properties associated to the polyphenols, and vitamin C content. Thermal treatment, generally used to extend shelf life of fruit based foodstuff, can affect the quality. 5-hydroxymethylfurfural (5-HMF), reducing sugars, ascorbic acid, and the total antioxidant activity in bio available fraction after *in vitro* digestion, have been evaluated in 16 apple-based nectars (fruit content: 30-60%), 15 apple-based juices (fruit content 100%) and 5 apple-based puree. Observed data indicate a 5-HMF values ranging from 0.06 mg/L in juices to 28.61 mg/L in nectars. The reducing sugar amount did not vary significantly between the three analysed typology of apple derivatives, while the ascorbic acid content was quite high compared to reported literature data. The antioxidant activity after an *in vitro* digestion showed values ranging from 0.21 to 7.68 mmol of Trolox in juices, and puree, respectively.

Keywords: apple juice, apple puree, quality, anti oxidant activity, trolox, vitamin C, 5-HMF

1. Introduction

Quality of apple juices and puree can be affected by different factors like cultivar, geographical region, climate, cultivar practices, harvest (Picinelli, 1997), storage conditions (Addie, 2005; Perales, 2008), and processing (Van Der Sluis, 2004; Kadakal, 2003; Valdramidis, 2010). The incorporation of apples and their derivatives in the diet mainly for their antioxidant properties, associated to the polyphenols and vitamin C content, is considered useful since these compounds can contribute to reduce the risk of coronary heart disease, carcinogenesis, aging processes, and can inhibit human low density lipoprotein oxidation (Boyer, 2004; Pearson, 1999; Dembinska-Kiec, 2008). Consumer trends show an increasing interest for fruit juices with a high natural antioxidants content, e.g. vitamins and polyphenols.

The parameters considered relevant to describe the overall quality of fruit juices and their processed derivatives are the amount of 5-hydroxymethylfurfural (5-HMF), the reducing sugars and ascorbic acid content, and the total antioxidant activity. On the other hand, thermal treatment, generally used to extend shelf life of fruit products, can also affect these parameters descriptors of the quality. The most important transformations during the processing concern the loss of antioxidant compounds, non-enzymatic browning reactions (Rattanathanalerk, 2005), and the formation of undesirable products like 5-hydroxymethylfurfural (5-HMF).

Various analytical methods have been reported to evaluate the real amount of antioxidant bio-available compounds in juices and puree, and an *in vitro* digestion procedure is needed (McDougall, 2005a; McDougall, 2005b; Perales, 2008; Ryan, 2010).

The first aim of the present study is to investigate selected parameters considered relevant to describe the quality, like 5-HMF, reducing sugars, ascorbic acid, total antioxidant activity in bio available fraction after *in vitro* digestion in 36 commercially available fruit derivatives, namely apple juices and apple puree obtained starting from conventional and organic agriculture cultivated fruits.

2. Methods

2.1 Chemicals

Fehling's reagents A and B, 2,6-dichlorophenolindophenol sodium salt hydrate (DIF), potassium chloride (KCl), potassium thiocyanate (KSCN), monosodium phosphate (NaH_2PO_4), sodium sulphate (Na_2SO_4), sodium chloride (NaCl), sodium acid carbonate (NaHCO_3), urea, α -amylase, hydrochloric acid (HCl), pepsin, pancreatin, bile salts, 2,2'-azinobis(3-ethylbenzothiazolinesulfonate) diammonium salt (ABTS), potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$), were obtained from Sigma-Aldrich (Steinheim, Germany). Acetonitrile, water, acetic acid for chromatography were purchased from Merck (Darmstadt, Germany). De-ionized water ($< 18 \text{ M}\Omega \text{ cm}$ resistivity) was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Chromatographic solvents and water were degassed for 20 min using a Branson 5200 (Branson Ultrasonic Corp., CT, USA) ultrasound device.

2.2 Sample Selection

The analysed juices and puree have been purchased from the local market (Napoli, Italy) and are commercialized by known Companies. A total of 36 products, samples numbered from 1 to 36, have been analysed, and they include three categories: 16 apple-based nectars (fruit content: 30-60%), 15 apple-based juices (fruit content: 100%) and 5 apple-based puree.

2.3 5-HMF Determination

5-HMF was analysed by HPLC. The extraction from samples was performed by adding to 1 mL of juice/puree sample and 1 mL of purified water in an Eppendorf tube. The solution was centrifuged at 13000 rpm for 5 minutes and 20 μL of the aqueous phase were analysed by HPLC. A Spherclone (Phenomenex) column (size 250 x 4,60 mm, pore size: 5 μm) at a flow rate of 1 mL/min in isocratic conditions. The mobile phase was a mixture of acetonitrile in water (5 % v/v) and the UV detector was set at 280 nm. The 5-HMF was quantified using the external standard method within the range 0.025-75 mg/L. In these conditions the retention time for 5-HMF was 7.2 minutes as reported in Figure 1 (A). For reference the retention time of the standard is reported in Figure 1 (B). The limit of detection (LOD) of the method was 0.010 mg/L. Limit of quantification (LOQ) was 0.030 mg/L. All the analyses were performed in triplicate.

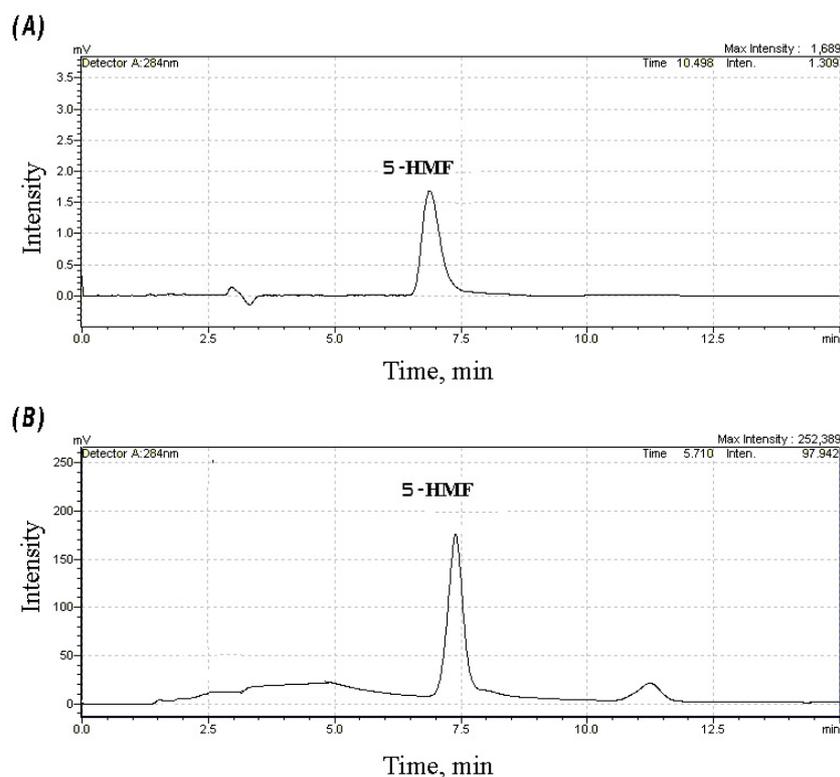


Figure 1. 5-HMF retention time for sample 2 (A) and retention time of the used standard (B)

2.4 Reducing Sugars Analysis

The determination of the reducing sugars was done according to the Fehling's titration method, the Official method of sugar analysis (ICUMSA, 1994), as described in the following.

Twenty grams of the samples were weighted and transferred in a flask. The volume was adjusted to 150 mL by adding purified water. After a few minutes to allow the sugar dissolution, 10 mL of lead acetate and the minimum amount of sodium oxalate were added. The volume of the resulting solution was adjusted to 200 mL, and the solution shaken, filtered and transferred in a burette for the titration.

Five mL of Fehling reagent A, 5 mL of Fehling reagent B and 40 mL of purified water were transferred in a flask. The solution was heated up to boiling point and the solution was added drop by drop till the nearly complete de-coloration of the Fehling reagent. Two drops of methylene blue were added, and the boiling continued for 3 minutes. The solution from the burette was added till the blue coloration of the indicator disappeared and the solution toned to a red colour. Each sample was analysed in triplicate.

2.5 Ascorbic Acid Determination

Ascorbic acid determination was carried out according to the AOAC official method (AOAC, 1990) by titration with a solution prepared by weighting 50 mg of 2,6-dichlorophenol-indophenol (DIF) and dissolving them in 50 mL H₂O added with 42 mg of NaHCO₃. The volume was adjusted to 200 mL. DIF was diluted with H₂O (in a ratio 1:30 for samples with poor vitamin C content and 1:5 for samples with higher vitamin C content, respectively). Five mL of an aqueous solution of 10% acetic acid, CH₃COOH, solution were added to 2 mL of sample and this solution was titrated with DIF up to the onset of a permanent and soft pink colour.

2.6 In vitro Digestion Procedure

Eight different products among the total 36 analysed, were selected for the evaluation of antioxidant activity after an *in vitro* digestion procedure: namely apple juice (sample 8), apple with pear and banana juice (sample 13), apple and banana juice (sample 16), apple and pear with rice drops nectar (sample 28), green apple with aloe nectar (sample 31), apple and peach with flesh puree (sample 32), apple puree (sample 33), apple and soft fruit high-fibres puree (sample 35).

The *in vitro* digestion model used has been adapted from the one recently described by Versantvoort et al. (Versantvoort, 2004) with slightly modifications. Each sample underwent an initial saliva/pepsin/HCl digestion for 2 h at 37 °C, to simulate the mouth and the gastric conditions, followed by a digestion with bile salts/pancreatin for 2 h at 37 °C to simulate duodenal digestion. The samples were mixed with 6 mL of artificial saliva constituted by a mixture of KCl at a concentration of 89.6 g/L, KSCN, 20 g/L, NaH₂PO₄, 88.8 g/L, NaSO₄, 57 g/L, NaCl, 175.3 g/L, NaHCO₃, 84.7 g/L, urea, 25 g/L, and 290 mg of α-amylase dissolved in 80 mL purified water. The pH of the solution was adjusted to 2 with HCl 6 N. Immediately after its preparation, the artificial saliva was added with 0.5 g of pepsin (14,800 U) dissolved in the minimum quantity of HCl 0.1 N, and then incubated at 37 °C in an orbital shaker (250 rpm) (Infors AG CH-4103, Bottmingen, Switzerland) at 55 rpm for 2 h.

After the gastric digestion, the pancreatic digestion was simulated. The pH of the solution was increased to 6.5 with NaHCO₃ 1 N, and 5 mL (1:1; v/v) of pancreatin at a concentration 8 mg/mL, and bile salts at a concentration 50 mg/mL, dissolved in 20 mL of water, were added. The solution was incubated at 37 °C in an orbital shaker (55 rpm) for 2 h and homogenized. Thirty mL of the mixture were then centrifuged at 4000 rpm at 4°C for 1h. The supernatant, constituting the physically bio accessible fraction, was collected and the antioxidant activity was immediately evaluated.

2.7 Antioxidant Activity Evaluation

The procedure used for the reagent 2,2'-azino-bis-3-ethylbenzotiazolin-6-sulfonic acid (ABTS) preparation was described by Pellegrini et al. (Pellegrini, 1999); a concentrate solution of the reagent (stock solution) was prepared dissolving 9.6 mg of ABTS in 2.5 mL of water and adding 44 mL of a solution made by dissolving 37.5 mg of potassium persulphate, K₂S₂O₈, in 1 mL of water. The stock solution was kept in the dark at 4 °C for 8 h before use; the work solution was obtained from the stock solution by dilution using a 1:88 (v/v) ratio. Dilution was adjusted depending on the measured absorbance at wavelength 734 nm (A₇₃₄) in the work solution, until a value between 0.7 and 0.8. Subsequently, 100 µL of sample and 1 mL of work solution were added, and A₇₃₄ was measured exactly after 2 min and 30 sec. Calibration curve for ABTS was obtained using 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), a water-soluble analog of α-tocopherol, as standard. Antioxidant activity was expressed as Trolox equivalent mmol.

2.8 Statistical Analysis

All data were analysed with respect to the variance using SPSS 11.0 software (SPSS Inc., Chicago, IL, USA). The significance of differences between experimental and control groups was determined by the Student's *t* test. Differences were declared significant at $P < 0.05$.

3. Results

Table 1 reports the experimental results relative to the quality descriptors that have been evaluated in this study, namely: 5-HMF, reducing sugars, ascorbic acid, and anti oxidant activity. The reported values refer to each analysed category: juices, nectars, and puree. Some of the analysed products are constituted by apple mixed to other fruits or enriched with fibres or fortified. As it is shown in Table 1, the observed data refer to a wide range of products allowing to evaluate the quality descriptors and also suggesting that exist possible sources of variability in quality parameters depending on the analysed apple based product.

Table 1. Experimental results for the evaluated parameters: 5-HMF (mg/L), reducing sugars (g/100 mL), ascorbic acid (mg/100 mL), antioxidant activity (mmol Trolox), identified as quality descriptors. Commercial brand names for the analysed samples, numbered from 1 to 36, have been omitted

Sample number	Sample typology	5-HMF (mg/L)	Reducing sugars (g/100mL)	Ascorbic acid (mg/100 mL)	Antioxidant activity (mmol Trolox)
Juice 100%					
1	apple bio ^(a)	6.47±1.02	3.37±0.73	16.56±3.15	5.08±0.08
2	apple and carrot bio ^(a)	0.06±0.01	1.52±0.38	18.89±4.07	1.79±0.03
3	apple and red fruits	2.04±1.43	3.05±0.98	15.17±2.98	5.40±0.02
4	apple	1.39±0.31	3.63±0.99	17.02±3.34	4.56±0.02
5	apple and red fruits vitamin enriched	5.49±0.82	2.88±1.23	18.86±2.97	3.60±0.03
6	apple bio ^(a)	0.11±0.02	3.37±1.32	16.57±3.32	1.99±0.02
7	apple bio ^(a)	1.83±0.43	3.37±1.42	13.79±2.97	2.23±0.01
8	apple	18.12±2.92	3.37±1.23	18.83±4.33	2.39±0.02
9	apple from Trentino ^(b)	0.30±0.02	3.37±1.42	14.24±3.41	0.21±0.01
10	apple	0.70±0.31	2.52±0.45	11.92±2.34	0.21±0.02
11	apple with ginger	<LOD	0.43±0.08	14.24±1.18	1.77±0.05
12	apple juice	<LOD	4.05±1.15	14.24±2.34	0.56±0.01
13	apple with pear and banana	1.96±0.23	3.16±1.12	14.23±3.01	1.42±0.03
14	apple	5.17±0.92	3.37±1.40	14.24±2.31	0.68±0.05
15	apple	5.26±0.98	2.23±0.65	12.38±1.89	0.51±0.04
Nectars					
16	apple and banana	0.36±0.05	2.39±0.45	25.40±2.43	1.84±0.02
17	apple with pulp	1.41±0.63	3.16±0.76	25.87±3.21	2.05±0.07
18	apple with pulp	1.25±0.57	1.81±0.43	15.43±2.91	2.21±0.07
19	apple and banana	0.30±0.01	3.37±0.80	19.82±2.47	1.63±0.02
20	green apple	3.00±1.52	1.85±0.32	16.57±1.94	1.25±0.03
21	apple, carrots and lemon	0.24±0.02	3.61±1.73	18.89±2.33	1.85±0.07
22	apple	1.67±0.04	3.49±1.52	13.31±2.89	2.05±0.08
23	apple bio ^(a) with pulp	2.27±0.73	2.88±0.85	14.24±2.32	2.09±0.06
24	apple vitamin enriched light	1.16±0.57	2.65±0.97	30.52±3.88	0.85±0.04
25	apple and green fruits	0.88±0.07	2.88±1.42	14.24±3.04	0.46±0.04
26	apple and white fruits	1.32±0.10	2.88±1.20	14.24±2.88	0.95±0.07
27	apple	2.34±0.21	2.88±1.16	11.92±3.43	0.38±0.06
28	apple and pear with rice drops	7.76±1.23	4.05±1.32	15.17±3.50	0.53±0.04
29	apple and kiwi	2.01±0.84	3.37±1.25	16.56±3.45	0.46±0.02
30	apple vitamin enriched	28.61±3.56	0.25±0.02	14.24±3.24	1.55±0.05
31	green apple with aloe	0.95±0.12	3.37±1.14	17.96±2.33	0.37±0.01
Puree					
32	apple and peach with pulp	<LOD	3.89±1.62	15.17±3.53	3.56±0.06
33	apple	3.37±0.88	4.05±1.76	16.57±4.34	3.68±0.07
34	apple	0.14±0.02	3.40±1.43	14.24±2.80	5.81±0.08
35	apple and soft fruit high-fibers	12.28±1.67	0.54±0.04	21.22±3.69	7.68±0.08
36	apple and soft fruit	4.75±1.71	4.74±1.41	11.92±2.61	4.65±0.06

^(a)fruits from biological agriculture.

^(b)apple from Trentino (Region in North of Italy) producer of high quality apple.

5-HMF is naturally formed as a Maillard reaction (MR) product (Ames, 1998), and from dehydration reaction of hexoses in mild acidic conditions (Kroh, 1994). MR include condensation between reducing sugars and amino acids, also called “caramelization reaction”; in the same conditions also the ascorbic acid and pigments disappear (Cohen, 1998; Damasceno, 2008). 5-HMF has been considered a heat-induced-marker for a wide range of carbohydrate-containing foods, and is considered a marker for monitoring the heating process during food factory processing. This compound is formed from cyclization and dehydration of the 3-deoxyosone, a dicarbonyl intermediate that can be formed by the direct caramelization reaction through the Maillard reaction by 1,2 enolization of the Amadori product.

Results for 5-HMF in analysed samples show that the highest detected level in apple juices was 18.12 mg/L (sample 8) and the lower value was 0.06 mg/L (sample 2) with an average value of 3.76 mg/L. Two of the 15 analysed apple juices were characterized by level of 5-HMF below the LOD (sample 11 and 12). The highest detected value in nectars was 28.61 mg/L (sample 30) while the lower was 0.24 mg/L (sample 21) and the average value for this commercial category was 3.47 mg/L. In apple puree samples, one out of five analysed samples evidenced levels of 5-HMF below the LOD (sample 32). Values were between 0.14 and 0.24 mg/L and the average value was 5.14 mg/L. Three analysed samples exceeded limit indicated for 5-HMF (samples 8, 30 and 35).

5-HMF content in apple juice concentrate properly produced and stored, was reported to be considerably lower than 10 mg/100 g (Babsky, 1986). More recently, Çetin Kadakal et al. (Kakadal, 2003), reported levels of 5-HMF in a range between 2.07 mg/L and 10.14 mg/L after heat treatment and evaporation of apple juices.

More in general, and with reference to 5-HMF content in fruit derived foodstuff, Ulbricht (Ulbricht, 1984), suggested a value up to 150 mg/day/person, while the Scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC) estimated an intake of 1.6 mg/day/person (EFSA 2005; Capuano, 2011). The International Federation of Fruit Juice Processors (IFFJP) recommended a maximum concentration of 5-10 mg/L 5-HMF in fruit juices and 25 mg/L in concentrates (Wagner, 2006).

Relatively to reducing sugars, no significative differences between the three analysed categories can be observed. The highest level in apple juices was 4.05 g/100 mL detected in sample 28, and the lower value was 0.43 g/100 mL in sample 11. The average value for this category was 2.91 g/100 mL. The levels of reducing sugars in apple nectars, were comprised in a range between 0.25 g/100 mL (sample 28) and 4.05 g/100 mL in sample 30. The average value was 2.81 g/100 mL. For the apple puree, the highest level was 4.74 g/100 mL (sample 36) while the lower value was 0.54 g/100 mL (sample 35) and the average was 3.32 g/100 mL.

The observed data are in agreement with previously reported values of reducing sugar amount in fruit juices. Klockow et al. (Klockow, 1994) measured levels between 2.27 and 2.43 g/100 mL, while Karadeniz and Ekşi (Karadeniz, 2002) reported the levels of glucose in apple juices ranged between 0.93 and 3.22 g/100 mL while for fructose values were in a range 6.61-9.60 g/100 mL. Rodriguez et al. (Rodriguez, 2001) reported values of glucose between 2.78 g/100 mL and 3.18 g/100 mL and more recently Chinnici et al. (Chinnici, 2005) reported for glucose values between 2.46 g/100 mL and 6.27 g/100 mL, and, for fructose, values between 2.22 and 7.54 g/100 mL. Eisele and Drake (Eisele, 2005) reported an average value of 2.01 g/100 mL and 5.69 g/100 mL for glucose and fructose, respectively. Our data do not vary significantly between the three analysed typology of apple derivatives suggesting that the factory processing does not influence significantly the total reducing sugar content.

The antioxidant ability of the ascorbic acid, vitamin C, is very well known, however it is not generally included in foodstuff nutritional labels. As a reference value, it could be observed that Elkins et al. (Elkins, 1997), in a compositional characterization of commercially produced pineapple juice concentrate, reported for citric acid a value of 3%. Chinnici et al. (Chinnici, 2005), reported in a more recent study; amounts varying from 0.52 to 5.61 g/L for ascorbic acid content.

In the analysed juices, a product containing a mixture of apple and carrot (sample 2), was the richest in vitamin C, with a level of 18.89 mg/100 mL. The lower quantity, 11.92 mg/100 mL, was observed in sample 10. The average value was 15.41 mg/100 mL. In one case, the sample 31, the value reported on the packaging label for vitamin C content (24 mg/100 mL) was higher than the measured level (17.96 mg/100 mL). This could be attributed to the storage or transportation of the product: a fraction of the ascorbic acid present in the juice degraded probably due to high temperature exposition. In analysed nectars, the highest detected level for vitamin C was found in a product that, according to nutritional label, was fortified with vitamins. Referring only to the apple nectars, the highest observed value (non enriched products) was 13.31 mg/100 mL for sample 22, and the lower value was 11.92 mg/100 mL, sample 36. The average value was 18.01 mg/100 mL. In apple puree, the

maximum value was 21.22 mg/100 mL, detected in sample 35, a product containing apple and soft fruit enriched with natural fibres; the lower value was 11.92 mg/100 mL, observed in sample 36, and the average value was 15.82 mg/100 mL. In all analysed samples, the ascorbic acid content was quite high compared to reported literature data, and did not significantly vary between the three products typologies analysed, the lower values being observed in apple nectars as in can be seen in Table 1.

Van der Sluis et al. (Van der Sluis, 2002) described the effect of producing apples juices on polyphenolic antioxidant content, and activity. Raw juice obtained by pulping and straight pressing or after pulp enzyming had an antioxidant activity that was only 10 and 3%, respectively, compared to the antioxidant activity of the fresh apples. Most of the antioxidants were retained in the pulp rather than being transferred into the juice. In apple juice, 45% of the total measured antioxidant activity could be ascribed to the antioxidants still contained in the juice.

For the analysed samples, the levels of antioxidant activity in juices showed levels in a range between 0.21 (samples 9 and 10), and 5.40 mmol Trolox for a product containing apple and red fruits (sample 3). The average value was 2.16 mmol Trolox. In the case of nectars, values were between 0.37 (sample 31) and 2.21 mmol Trolox in a product containing also fresh apple fruit (sample 18), while the average value was 1.28 mmol Trolox. In apple puree, the levels were highest compared with the other analysed categories; amounts were between 3.56 (sample 32) and 7.68 mmol Trolox, observed in a product containing apple and fresh fruit and also fibers enriched (sample 35), with an average value of 5.08 mmol Trolox.

The effects of the *in vitro* digestion on the antioxidant activity for 8 selected products of different typologies are reported in Figure 2. There were considerable differences in the effects of an *in vitro* digestion procedure on the different juice kind. Five among the artificially digested products showed an increase of the antioxidant activity after the *in vitro* procedure (samples 13, 16, 28, 31, and 33) in a range between 0.05% (sample 16) and 2.04% (sample 31). On the opposite, samples 8, 32, and 35 showed a decrease of antioxidant activity, with values between 0.03 (sample 32) and 0.38% (sample 35).

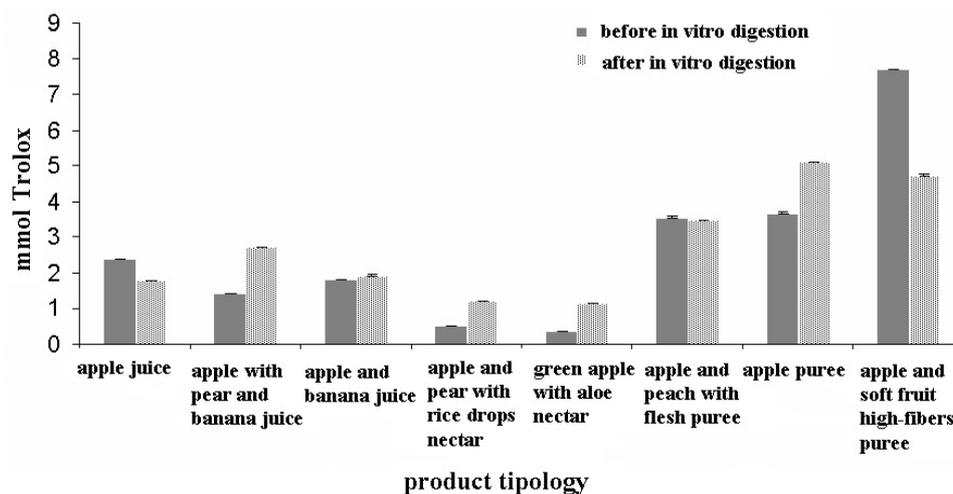


Figure 2. Antioxidant activity (mmol Trolox) after and before *in vitro* digestion for 8 selected products belonging to different typologies

Observed data, represented in Figure 2, partially agree with previously reported studies. This allows to speculate on the possibility that exposition to the *in vitro* digestion conditions, could cause a part of the active compounds to assume a different structure with different chemical properties. In this case causing a possible underestimation of the total antioxidant compounds amount after the *in vitro* digestion could be possible. Reported studies in fact seem to suggest that it is possible to measure an increase or a decrease of the antioxidant activity.

Perales et al. (2008) observed that the bio-accessible fractions (maximum soluble fraction in simulated gastrointestinal media) of beverages obtained after an *in vitro* gastrointestinal digestion, had antioxidant activities significantly lower ($p < 0.05$) than the original beverages. The loss of antioxidant activity was always

lower than 19%, thus indicating the stability of the total antioxidant capacity under the applied conditions.

Recently however Ryan and Prescott (Ryan, 2010) reported that it is possible to observe an increase in the antioxidant capacity of red fruit juices after *in vitro* digestion. This could be due to an increase in anthocyanins content. Bermúdez-Soto et al. (Bermúdez-Soto, 2007) found an increase in a number of polyphenols after the gastric phase of the *in vitro* digestion process, while after the pancreatic digestion phase these antioxidants were degraded by the alkaline value of the pH. McDougall et al. (McDougall, 2005a; McDougall, 2005b) found a decrease in antioxidants after *in vitro* digestion when analysing specific antioxidant compounds, rather than total antioxidant capacity.

4. Conclusions

The data observed for 5-HMF are in general higher than the suggested values as estimated by the International Federation of Fruit Juice Processors (IFFJP): maximum concentration of 5-10 mg/L 5-HMF in fruit juices and 25 mg/L in concentrates as reported by Wagner (Wagner, 2006). Our observed values range from 0.06-18.12 mg/L in juices, from 0.24-28.61 mg/L in nectars, and from 0.14-0.24 mg/L in puree, and could be attributed to a strong thermal treatment during the processing and manufacturing of the fresh apple fruits. Our data for the reducing sugar amount do not vary significantly between the three analysed typology of apple derivatives suggesting that the factory processing does not influence significantly the total reducing sugar content. In fact a range 0.43-4.05 g/100 mL, 0.25-4.05 g/100 mL, and 0.54-4.74 g/100 mL, for apple juices, nectars and puree, respectively, do not indicate any alteration of the sugar content related to thermal treatment during the processing or to the storage conditions.

In all analysed samples, the ascorbic acid content was quite high compared to reported literature data, and did not significantly vary between the three products typologies analysed, the lower values being observed in apple nectars. Interestingly a value of 11.92 mg/100 mL of vitamin C was measured as the minimum content of this compound in juices, nectars and puree. The higher levels were, 18.89, 13.31, 21.22 mg/100 mL for juices, nectars and puree, respectively. The observed data could indicate a limited impact of thermal treatments and heat exposure on the ascorbic acid content.

Measured values for the antioxidant activity after an *in vitro* digestion partially agree with previously reported studies. Reported studies in fact seem to suggest that it is possible to observe an increase or a decrease of the antioxidant activity. Our data seem to indicate that industrial processing could have a major impact on the antioxidant activity of the analysed foodstuff. In fact, observed values are in the range 0-21-5.40, 0.37-2.21, and 3.56-7.68 mmol of Trolox for juices, nectars and puree, respectively. Data seem to indicate that thermal treatment, involved in the apple puree making process, affects more the anti oxidant activity.

Declaration of Interest

Authors have no conflict of interest, in particular no financial, consulting and personal relationships with other people or organizations that could influence (bias) the author's work.

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