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# Bergamot: A source of natural antioxidants for functionalized fruit juices

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ABSTRACT

Bergamot is a common Italian citrus fruit, cultivated almost exclusively to produce essential oils; the juice is considered a waste product, which represents a serious environmental and economic problem for the industries. The aim of this study was to re-evaluate bergamot juice through its chemical characterization and its use to enrich and fortify fruit juices. To investigate this, apples and apricots were used for the laboratory-scale production of fruit juice, following both the traditional industrial recipe and those with the addition of bergamot juice at 10% or 20%, together with or in order to replace the synthetic additives normally used in the industrial process (ascorbic acid and citric acid). The ascorbic acid content and the antioxidant activity were measured during the different steps of juice production and after storage at 37 °C for 15 days to evaluate juice shelf-life. Apricot and apple juices fortified with bergamot juice showed a significant increase in their antioxidant properties and a decreased reduction in ascorbic acid content after the typical production steps. All of the results obtained support the hypothesis that the addition of bergamot juice to juices preserves their ascorbic acid content from thermal degradation and contributes to enhance the antioxidant activity, ensuring a product much richer in antioxidants and ascorbic acid. A preliminary consumer test encouraged the production of bergamot fortified fruit juices. Finally, this is the first time that isorhoifolin and rutin have been detected in bergamot juice.


## 1. Introduction

Bergamot (*Citrus Bergamia* Risso) is a natural hybrid fruit derived from bitter orange and lemon; it is produced almost exclusively in the Reggio Calabria region (South Italy), where the cultivated area is about 1500 ha with an annual production of 25,000 tonnes (Mandalari et al., 2006).

Bergamot is used mostly for production of essential oil, obtained from the peel by wash-scraping the fruit. The bergamot essence, claimed DOP (official designation of Denomination of Protected Origin from the European Union) since 1999, is widely used in the pharmaceutical industries for its antiseptic and antibacterial proprieties, in the cosmetic industries (e.g., in perfumes, body lotions, soaps, aromatherapy) for its intense fragrance and freshness, and in the food industries as aroma for the preparation of sweets, liquors and tea. The juice, on the other hand, and in contrast to that from other citrus fruits, is considered a waste of the essential oil production; in fact, it has not found a real use in the food industries until now because of its bitter taste. The disposal of bergamot juice represents a serious problem for the essential oil industries, because of both the high economic costs and the environmental pollution. As a consequence, the re-evaluation of the usefulness of bergamot juice could represent an important opportunity for the essential oil processing industries, which are interested in a possible alternative commercial use of this product.

In general, citrus juices are a rich source of antioxidant compounds, particularly ascorbic acid and phenols (Dhuique-Mayer, Caris-Veyrat, Ollitrault, Curk, & Amiot, 2005; Gorinstein et al., 2001; Rapisarda, Tomaino, Lo Cascio, Bonina, De Pasquale & Saija, 1999); both of these metabolites have been demonstrated to have important health implications. Ascorbic acid is well known for its strong antioxidant activity (Diplock, 1994), while phenolic compounds have been widely investigated and characterised for their anticancer (Garcia-Closas, Gonzales, Aguda, & Riboli, 1999; Hertog, Hollman, & Van de Putte, 1993; Knekt et al., 1997), anti-inflammatory (Gabor, 1986; Galati, Manforte, Kirjavainen, Forestieri, Trovato & Tripodo, 1994) and cardioprotective (Figoli et al., 2006) properties.

Citrus most recurrent flavonoids include flavanones, flavones and polymethoxyflavones. The structure and the occurrence of flavonoids in citrus, and the biological properties related to these metabolites, have been well described in previous papers (Benavente-García, Castillo, Marin, Ortuno, & Del Río, 1997; Manthey, Guthrie, & Grohmann, 2001).

Although bergamot has been known for several centuries, very few studies are present in the literature on this fruit and, furthermore,

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the majority are focused mainly on the essential oil (Dugo, Cotroneo, Verzera, & Bonaccorsi, 2002; Figoli et al., 2006; Mandalari et al., 2006; Sawamura, Onishi, Ikemoto, Tu, & Phi1, 2006), while the works about bergamot juice are generally lacking (Calabrò, Galtieri, Cutroneo, Tommasini, Ficarra & Ficarra, 2004; Gattuso, Caristi, Gargiulli, Bellocco, Toscano & Lezzi, 2006). Nevertheless, in recent years, the beneficial properties of bergamot juice have been raising interest, and have been the subject of several recent studies (Barbera, Pendino, et al., 1998; Ursino et al., 2006).

The first step in bergamot juice evaluation is the characterization of its antioxidant compounds, in order to understand the potential use of this by-product as a natural additive that has healthful properties. The second objective of this work is to test bergamot juice in a real food system; for this reason, apple and apricot fruit juices supplemented with bergamot juice, together with or in order to replace the ascorbic acid and citric acid additives normally used in the process, were prepared at laboratory scale. These "enriched juices" were subjected to thermal treatment at 100 °C for 30 min and subsequently stored at 37 °C in daylight for 15 days to accelerate the oxidative deterioration of the juices. All samples were analysed to evaluate the antioxidant properties (antioxidant activity and ascorbic acid amount) of juices, both those traditionally prepared and with the addition of bergamot juice. Finally, a preliminary sensory analysis was conducted to evaluate the possible use of bergamot as functional ingredient for fortified fruit juices.

## 2. Materials and methods

### 2.1. Materials

Bergamot juice was obtained from MALARA S.r.l., Campo Calabro (Reggio Calabria, Italy), and from Essences S.r.l., S. Marzano sul Sarno (Salerno, Italy), while apple and apricot pomaces were obtained from a local fruit juice industry. All of the materials were collected, transported in containers that were certified microbiologically safe, and stored at 4 °C in dark conditions for 24 h before their use and analysis.

### 2.2. Fruit juice preparation

Apple and apricot juices were prepared in laboratory-scale batches.

Briefly, as shown in Table 1, the following recipes were prepared: (1) the traditional industrial recipe, (2) 20% bergamot juice replacing the synthetic additives (recipe A), (3) 20% bergamot juice in addition to synthetic additives (recipe B), (4) 10% bergamot juice replacing synthetic additives (recipe C), and (5) 10% bergamot juice in addition to synthetic additives (recipe D).

After preparation, fruit juices were heated at 70 °C for 30 s, bottled and thermally treated at 100 °C for 30 min; part of the fruit juice was stored at 37 °C in daylight for 15 days to reproduce the deterioration of the juice under oxidative conditions, simulating a possible shelf-life.

All samples were analysed for their antioxidant activity and for their ascorbic acid content, monitoring different steps of the juice production: freshly prepared, juices heated at 70 °C for 30 s (before bottling), and thermally treated (100 °C for 30 min) and stored (15 days at 37 °C) juices.

### 2.3. Bergamot juice analysis

#### 2.3.1. Characterization of the bergamot phenolic pattern

Polyphenols were analysed following the procedure reported in literature by Van Der Siluis, Dekker, Skrede, and Jongen (2002) with slight modifications. Briefly, 2 ml of bergamot juice were extracted with 10 ml of 70% methanol solution, agitated, and sonicated for 30 min. The extract was centrifuged for 10 min at 1650*g* at 4 °C; the supernatant was collected while the pellet was extracted a second time using the same procedure. The supernatants were combined, ultracentrifuged at 24,000*g* for 1.5 min, and injected into an HPLC (Shimadzu LC 10, Shimadzu, Kyoto, Japan) with a diode array detector and a Prodigy 5 μ ODS3 100 Å column, 250 × 4.60 mm (Phenomenex, Torrance, CA, USA). The mobile phase was a mixture of water/formic acid (95:5 v/v) (A) and methanol (B). Polyphenol separation was achieved using the following linear gradient: starting condition, 85% A, 15% B; 5 min, 70% A, 30% B; 20 min, 50% A, 50% B; 30 min, 25% A, 75% B; 35 min, 5% A, 95% B; 40 min, 85% A, 15% B, at a constant flow of 1 ml/min. Chromatograms were recorded at 256 nm.

Phenolic compounds were identified and confirmed by an LC/MS/MS analysis. For this purpose, chromatographic separation was performed using an HPLC apparatus equipped with two Series 200 micropumps (Perkin Elmer, Wellesley, MA, USA), an UV/VIS series 200 (Perkin Elmer, Wellesley, MA, USA) detector set at 256 nm, and a Prodigy 5 μ ODS3 100 Å column (250 × 4.6 mm, particle size 5 μm) (Phenomenex, Torrance, CA, USA). The eluents were: A, $water_{(0.2\%\ formic\ acid)}$; B, methanol. The gradient program was the same as described above. The LC flow was split, and 0.2 mL/min was sent to the mass spectrometer; the injection volume was 20 μL.

MS/MS analyses of bergamot juice extracts were performed with an API 3000 triple quadrupole mass spectrometer (Applied Biosystems, Toronto, Canada) equipped with a Turbo Ion Spray source working in the negative ion mode. The analyses were performed using the following settings: drying gas (air) was heated to 400 °C, and the capillary voltage (IS) was set to −4000 V. To obtain identifying information on the metabolites, information-dependent acquisition (IDA) was used. Acquisition (IDA) was carried out using the range *m/z* 50–1100 with a cycle time of 0.5 s and a step size of *m/z* 0.2; the collision energy was set to −60 V. Chromatograms were recorded at 256 nm. Identified compounds were analyzed by MRM (Multiple Reaction Monitoring).

### 2.4. Fruit juice analysis

#### 2.4.1. Ascorbic acid

Fruit juice samples (both traditionally prepared and those with added bergamot juice) were centrifuged at 1650*g* for 5 min at

**Table 1**
Fruit juice composition

| | Traditional recipe | | Recipe A | | Recipe B | | Recipe C | | Recipe D | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Apple fruit juice | Apricot fruit juice | Apple fruit juice | Apricot fruit juice | Apple fruit juice | Apricot fruit juice | Apple fruit juice | Apricot fruit juice | Apple fruit juice | Apricot fruit juice |
| Pomace | 43.61 | 28.36 | 43.61 | 28.36 | 43.61 | 28.36 | 43.61 | 28.36 | 43.61 | 28.36 |
| Sugar solution (40%) | 22.03 | 26.08 | 22.03 | 26.08 | 22.03 | 26.08 | 22.03 | 26.08 | 22.03 | 26.08 |
| Additives | 0.50 | 0.50 | 0 | 0 | 0.50 | 0.50 | 0 | 0 | 0.50 | 0.50 |
| Water | 33.86 | 45.06 | 25.56 | 14.36 | 25.06 | 13.86 | 35.56 | 24.36 | 35.06 | 23.86 |
| Bergamot juice | 0 | 0 | 20 | 20 | 20 | 20 | 10 | 10 | 10 | 10 |

4 °C. The supernatant was used for determination of the ascorbic acid content by titration with DIF (2,6-dichlorophenolindophenol sodium salt hydrate) according to the AOAC Official Method (1995), chap. 45. Results were expressed as mg ascorbic acid/ml juice.

*2.4.2. Antioxidant activity*

An aliquot of the traditional and "enriched" fruit juice samples was centrifuged at 1650*g* for 5 min at 4 °C, the supernatant was collected and analysed to assess antioxidant capacity of juices using three different methods based on the evaluation of the free radical scavenging capacity.

The first assay performed was the *N*,*N*-dimethyl-*p*-phenylenediamine (DMPD) method described in the literature by Fogliano, Verde, Randazzo, and Ritieni (1999). In presence of $Fe^{3+}$, a coloured DMPD radical cation is generated; antioxidant compounds able to transfer a hydrogen atom to $DMPD^{\cdot+}$ cause a decolouration of the solution measured by the decrease in absorbance at 505 nm.

The second test is based on the ability of antioxidant molecules to quench the $ABTS^{\cdot+}$ (2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation), a blue–green chromophore with characteristic absorption at 734 nm; the addition of antioxidants to the preformed radical cation reduces it to ABTS, determining a decolorization (Pellegrini, Re, Yang, & Rice-Evans, 1999).

Finally, antioxidant capacity was analysed by DPPH (α,α-diphenyl-β-pycrylhydrazyl) assay (Brand-Williams, Cuvelier, & Berset, 1995). The $DPPH^{\cdot}$ solution has a deep violet colour, radical scavenging activity of antioxidant compounds can be measured spectrophotometricallynat 517 nm by the loss of the absorbance as the pale yellow nonradical form is produced.

Antioxidant activity values were calculated from L-Ascorbic acid standard curve and results were expressed as mmol ascorbic acid equivalents/ml juice.

*2.5. Consumer test*

A preliminary consumer test was conducted in the laboratory with 60 untrained panelists (68% women, 32% men, all them between 18 and 55 years of age). The principal selection criterion for consumer recruiting was that subjects had to be regular consumers of juice at least twice a week.

The new apricot and apple juices supplemented with 10% and 20% bergamot juice were served in glasses that were 50% transparent and were marked with a randomised numerical code. Consumers were asked to express a judgment using the following ratings: hedonic parameters, colour, visual viscosity, flavour intensity and taste, and intent to purchase. The response was recorded using a 7 points hedonic scale (1, extremely dislike; 2, moderately dislike; 3, slightly dislike; 4, neither like nor dislike; 5, slightly like; 6, moderately like; 7, extremely like) for the four hedonic parameters analysed, and a 5 points scale (1, no purchasing intention; 2, probably no purchasing intention; 3, indifferent to purchasing the product; 4, likely purchasing intention; 5, surely purchasing intention) for the intent to purchase. Moreover, as an option, the consumers were invited to explain in writing the reasons for their choices.

*2.6. Statistical Analysis*

Results are given as the mean ± standard deviation of six independent determinations. One-way analysis of variance (ANOVA) was used to compare the means. Differences were considered to be significant at $P < 0.05$. All statistical analyses were performed with SPSS 13.0 (LEAD TOOLS (c) 1991–2000, LEAD Technologies, Inc.).

## 3. Results and discussion

*3.1. Phenolic compounds in bergamot juice*

The phenolic pattern of bergamot juice was the first objective attained in this work. The main peaks identified by HPLC by co-injections with pure standard were narirutin (r.t. 18.8 min), naringin (r.t. 19.8 min), isorhoifolin (r.t. 21.6 min) and rhoifolin (r.t. 22.6 min); rutin (r.t. 20.4 min), on the other hand, was present in lower amounts. LC/MS/MS analysis confirmed the presence of these metabolites and allowed the identification of additional compounds: neoeriocitrin, neoponcirin, hesperidin and neodiosmin. The spectrometric parameters are briefly summarized in Table 2.

*Citrus* species are characterized by different types and amounts of phenolic compounds (Marini & Balestrieri, 1995). The literature data report that naringin, neohesperidin and, to a lesser extent, neoheriocitrin, are the main flavonoids found in bitter orange (Leuzzi, Caristi, Panzera, & Licandro, 2000); hesperidin, narirutin and, in small amount, didymin in sweet orange (Leuzzi et al., 2000; Mouly, Gaydou, Faure, & Estienne, 1997); heriocitrin, diosmin and hesperidin in lemon (Caristi, Bellocco, Panzera, Toscano, Vadalà & Leuzzi, 2003); and naringin, narirutin and, in small amounts, hesperidin and neohesperidin, in grapefruit (Rouseff, Martin, & Youtsey, 1987). Comparing the results of these studies with the main compounds detected in this work, it is evident that the presence of naringin and narirutin is common in *Citrus* fruit, linking bergamot to orange and grapefruit in particular; on the other hand, rhoifolin and isorhoifolin, two flavonoids detected in great amounts in bergamot juice, seem quite particular to this species, as they are present only in very small amounts in orange, tangerine and grapefruit, while they are absent in lemon (Kawaii, Yasuhiko, Eriko, Kazunori, & Masamichi, 1999). Gattuso et al. (2006) report that neoeriocitrin and naringin are the main flavonoids in bergamot juice; this study, which considered the relative percentage of each identified peak, confirms neoeriocitrin as one of the main flavonoids in bergamot (15%), while narirutin and naringin together represent only 14% of the total flavonoids.

In addition, the new compounds identified in this study appear to be very interesting and innovative. Previously, Gionfriddo, Postorino, and Bovalo (1996) and Postorino, Poiana, Pirrello, and Castaldo (2001) reported neoheriocitrin, naringin, narirutin, neohesperidin and traces of hesperidin in bergamot juice, but not isorhoifolin, rhoifolin and rutin, which were identified in this study. Recently, rhoifolin was also identified by Dugo, Lo Presti, Ohman, Fazio, Dugo and Mondello (2005) when samples were extracted following a particular procedure using a microHPLC column, and by Gattuso et al. (2006); moreover, the same authors (Dugo et al., 2005; Gattuso et al., 2006) reported the presence of diosmetin, luteolin, apigenin and chrysoeriol glucosides. Isorhoifolin and rutin, on the other hand, have never been detected in bergamot juice until now.

**Table 2**
Molecular weight and fragmentation negative ions of identified flavonoids by LC/MS/MS

| Flavonoid | m/z | Fragmentation ions |
|---|---|---|
| Neoeriocitrin | 597 | 451, 289 |
| Narirutin | 581 | 383, 273 |
| Rutin | 609 | 301 |
| Naringin | 579 | 452, 271 |
| Isorhoifolin | 579 | 271 |
| Hesperidin | 610 | 463, 301 |
| Rhoifolin | 579 | 271 |
| Neodiosmin | 609 | 303 |
| Neoponcirin | 593 | 285 |

### 3.2. Fruit juices

#### 3.2.1. Ascorbic acid

Data obtained for fresh traditional and "enriched" juices are summarized in Table 3. As is evident, the ascorbic acid content in the fresh "enriched" apple juice always increased significantly; the only exception was represented by the addition of 10% bergamot juice without the traditional additives, which presented values similar to the traditional recipe. Concerning apricot juice, the results looked different. The addition of bergamot juice increased ascorbic acid content when combined with synthetic additives, while it decreased the ascorbic acid content when used alone. This result can be explained by considering that, in traditional apricot juice, the amount of ascorbic acid is principally due to the additives, as the pomace is diluted to 28%. As a consequence, the effect of bergamot juice is less evident in the apricot juice than in the apple juices, where the pomace is diluted only to 43%. The addition of bergamot juice as a functional ingredient allows the enrichment of the ascorbic acid concentration of traditional juice to 10%.

The ascorbic acid content during different steps of the production process is also reported in Table 3. As expected, in the traditional juices, ascorbic acid content decreased slightly after heating, but it was strongly affected by the thermal process.

Interestingly, the "enriched" juices showed a different behaviour, as the ascorbic acid content decreased less markedly after thermal treatment. Moreover, it is important to note that the ascorbic acid content in the "enriched" juice after thermal treatment is much more elevated with respect to the calculated theoretical value, considering the percentage decrease in ascorbic acid for a single traditional fruit juice and the bergamot juice. Specifically, this increase was about 80% and 60% on average in "enriched" apple and apricot juices, respectively. This experimental result suggests that bergamot bioactive components have an active role to reduce the thermodegradation of ascorbic acid content.

In the "enriched" juices, ascorbic acid was more protected from degradation after juice storage at 37 °C for 15 days, too. The reduction observed in juices was according to literature data, maintaining that vitamin C content in fruit juices decreases during storage, depending on storage conditions, such as temperature, oxygen and light access (Al-Zubaidy & Khalil, 2007; Kabasakalis, Siopidou, & Moshatou, 2000; Zerdin, Rooney, & Vermue, 2003). Klimczak, Malecka, Szlachta, and Gliszczynska-Swiglo (2007) report that ascorbic acid content in commercial orange juice was reduced by 81% after 6 months storage at 38 °C.

In conclusion, although in the first step (fresh juice), the addition of bergamot juice in the fruit juices showed a slight increasing effect on the ascorbic acid content (about 10%), after thermal treatment and storage, it contributes markedly to the preservation of ascorbic acid content, ensuring a high content of this compound in the final product, differently from the synthetic additives. This data can be explained by considering that the bergamot juice brings a very important contribution in terms of bioactive compounds protecting or stabilizing juice ascorbic acid; among them, authors suppose that a significant role can be attributed to bergamot polyphenols. Supporting this hypothesis, in literature Miller (1998) also underlines the importance of phenolic compounds in the prevention of ascorbic acid from oxidative degradation.

#### 3.2.2. Antioxidant activity

The antioxidant capacity measured by DMPD, ABTS and DPPH assays is shown in Tables 4–6, respectively.

Using the DMPD method, an extraordinary high activity was observed in bergamot juice, values were about 2800 and 1300 times greater than data obtained for apple and apricot juices, respectively. Then, the addiction of bergamot contributed to a considerable increase in the antioxidant activity in both of the juice types. In a more thorough evaluation, this increase was valuable as the values obtained in the fresh juices were greater than the ones theoretically calculated (on average, by about 20%).

**Table 3**
Ascorbic acid content (mg/ml) in fruit juices

| | Fresh | | Heated | | Thermally-treated | | Shelf-life | |
|---|---|---|---|---|---|---|---|---|
| | Apple fruit juice | Apricot fruit juice | Apple fruit juice | Apricot fruit juice | Apple fruit juice | Apricot fruit juice | Apple fruit juice | Apricot fruit juice |
| Traditional recipe | 0.192 a,C | 0.234 cD | 0.185 a,bC | 0.172 cC | 0.060 aB | 0.036 aB | 0.003 aA | 0.009 aA |
| Bergamot juice | 0.295 cC | 0.356 eD | 0.274 cC | 0.331 fC | 0.207 dB | 0.250 fB | 0.050 dA | 0.060 eA |
| Recipe A | 0.215 bD | 0.186 bD | 0.199 bC,D | 0.136 bC | 0.162 cB,C | 0.078 cB | 0.069 eA | 0.043 cA |
| Recipe B | 0.280 cD | 0.347 eD | 0.256 cC,D | 0.308 eC | 0.226 dB,C | 0.124 eB | 0.078 fA | 0.051 dA |
| Recipe C | 0.172 aD | 0.162 aD | 0.159 aC,D | 0.108 aC | 0.128 bB,C | 0.057 bB | 0.024 bA | 0.035 bA |
| Recipe D | 0.230 b,cC | 0.319 dD | 0.201 bC | 0.274 dC | 0.143 b,cB | 0.095 dB | 0.033 cA | 0.037 b,cA |

Lower case represents the comparison among the different juices for each treatment (compare values within each column); capitals represents the comparison among the four treatments (compare among rows). Means with the same letters did not show significant differences ($P < 0.05$).

**Table 4**
Antioxidant activity (mmol ascorbic acid/ml) of fruit juices evaluated by DMPD

| | Fresh | | Heated | | Thermally-treated | | Shelf-life | |
|---|---|---|---|---|---|---|---|---|
| | Apple fruit juice | Apricot fruit juice | Apple fruit juice | Apricot fruit juice | Apple fruit juice | Apricot fruit juice | Apple fruit juice | Apricot fruit juice |
| Traditional recipe | 2.79 aA | 5.65 aA | 2.80 aA | 4.75 aA | 3.03 aA | 4.85 aA | 3.01 aA | 4.63 aA |
| Bergamot juice | 81.46 dA | 82.71 dA | 104.76 dA | 92.77 eA | 112.17 dA | 119.31 dA | 108.33 dA | 111.91 dA |
| Recipe A | 23.14 cA | 24.91 cB | 23.92 cA | 26.62 cB | 22.74 cA | 21.24 cA | 23.37 cA | 21.82 cA |
| Recipe B | 23.78 cA | 26.68 cB | 22.81 cA | 29.33 dC | 23.98 cA | 22.76 cA | 23.79 cA | 23.54 cA |
| Recipe C | 13.05 bA | 18.00 bA,B | 13.49 bA | 20.11 bB | 16.00 bA | 17.42 bA | 14.35 bA | 14.89 bA |
| Recipe D | 13.43 bA | 19.50 bA | 14.75 bA | 24.49 cB | 17.80 bA | 18.87 bA | 15.42 bA | 16.44 bA |

Lower case represents the comparison among the different juices for each treatment (compare values within each column); capitals represents the comparison among the four treatments (compare among rows). Means with the same letters did not show significant differences ($P < 0.05$).

**Table 5**
Antioxidant activity (mmol ascorbic acid/ml) of fruit juices evaluated by ABTS

| | Fresh | | Heated | | Thermally-treated | | Shelf-life | |
|---|---|---|---|---|---|---|---|---|
| | Apple fruit juice | Apricot fruit juice | Apple fruit juice | Apricot fruit juice | Apple fruit juice | Apricot fruit juice | Apple fruit juice | Apricot fruit juice |
| Traditional recipe | 2.848 bB | 1.878 bB | 2.932 bB | 1.980 cB | 3.130 bB | 1.832 cB | 2.170 b,cA | 1.230 aA |
| Bergamot juice | 2.428 aB | 2.432 cB | 2.662 aC | 2.671 eC | 2.194 aB | 2.198 dB | 1.894 aA | 1.904 dA |
| Recipe A | 3.309 cB | 1.856 bC | 3.291 cB | 1.786 bB,C | 3.681 cC | 1.631 bA,B | 2.399 cA | 1.488 bA |
| Recipe B | 3.885 dB | 2.698 dC | 3.489 cB | 2.757 eC | 3.768 cB | 2.411 eB | 2.432 cA | 1.622 cA |
| Recipe C | 3.094 b,cB | 1.527 aB | 3.228 bB | 1.414 aB | 3.067 bB | 1.441 aB | 1.974 a,bA | 1.159 aA |
| Recipe D | 3.723 dB | 2.381 cB | 3.471 cB | 2.455 cB | 3.573 cB | 2.341 eB | 2.271 b,cA | 1.461 bA |

Lower case represents the comparison among the different juices for each treatment (compare values within each column); capitals represents the comparison among the four treatments (compare among rows). Means with the same letters did not show significant differences ($P < 0.05$).

**Table 6**
Antioxidant activity (mmol ascorbic acid/ml) of fruit juices evaluated by DPPH

| | Fresh | | Heated | | Thermally-treated | | Shelf-life | |
|---|---|---|---|---|---|---|---|---|
| | Apple fruit juice | Apricot fruit juice | Apple fruit juice | Apricot fruit juice | Apple fruit juice | Apricot fruit juice | Apple fruit juice | Apricot fruit juice |
| Traditional recipe | 1.196 bB | 0.974 cC | 1.319 bB,C | 1.002 cC | 1.371 bC | 0.884 cB | 0.326 aA | 0.196 aA |
| Bergamot juice | 0.862 aC | 0.851 bC | 0.729 aC | 0.715 aC | 0.574 aB | 0.569 bB | 0.305 aA | 0.295 cA |
| Recipe A | 1.741 dB | 0.890 bC | 1.732 dB | 0.851 bC | 1.728 cB | 0.442 aB | 0.383 aA | 0.221 a,bA |
| Recipe B | 1.703 dB | 1.157 dC | 1.716 dB | 1.143 dC | 1.744 cB | 0.959 dB | 0.348 aA | 0.301 cA |
| Recipe C | 1.386 cB | 0.787 aC | 1.519 cC | 0.851 bC | 1.665 cD | 0.384 aB | 0.321 aA | 0.256 a,bA |
| Recipe D | 1.328 b,cB | 1.116 dC | 1.520 cC | 1.132 dC | 1.451 bB,C | 0.879 c,dB | 0.311 aA | 0.288 b,cA |

Lower case represents the comparison among the different juices for each treatment (compare values within each column); capitals represents the comparison among the four treatments (compare among rows). Means with the same letters did not show significant differences ($P < 0.05$).

No significant differences were observed among the juices during the different steps of the industrial process, with the only exception being apricot juices, which showed a lower antioxidant activity when compared with the fresh juices after thermal treatment. The extraction, during processing, of antioxidant bioactive compounds may contribute to the antioxidant activity of thermally processed juices. Finally, no different values were shown after storage at 37 °C for 15 days in apple juices.

When measuring the antioxidant activity by ABTS and DPPH methods, different results were obtained. Bergamot juice exhibited lower antioxidant capacity compared to apple and apricot juices with both the tests, thus determining a different response in the antioxidant capacity of enriched fruit juice compared with previous data.

The different behaviour shown by bergamot juice can be explained considering that the three tests, apart from differing for the reactive species, are performed in different reaction phases. DMPD measures the antioxidant capacity in hydrophilic environment, while ABTS and DPPH are performed in lipophilic environment; then, DMPD assay is particularly suitable for hydrophilic antioxidants but is less sensitive to hydrophobic bioactive compounds (Fogliano et al., 1999), the opposite for the other two tests. This consideration suggests that the three tests furnished a diverse kind of information as they emphasized differently the antioxidant capacity of hydrophilic and hydrophobic antioxidants compounds; results indicated that in bergamot juice there were especially water soluble constituents provided of enhanced antioxidant capacity, while hydrophobic antioxidants were in the same rank of commercial juice. Similarly, Gil, Tomas-Barberan, Hess-Pierce, Holcroft, and Kader (2000) found that pomegranate juice exhibited much more elevated antioxidant activity when measured with DMPD method compared with values obtained with ABTS and DPPH assays; fractionating the juice components, authors determined that organic acids, and especially citric acid, were the main responsible for the enhanced antioxidant activity with DMPD assay, and that organic acids did not show any free radical scavenging activity with the other two methods. Being bergamot juice a rich font of organic acids, the same conclusion can be hypothesized to justify the elevated antioxidant activity of this juice by DMPD method.

However, even if bergamot juice alone did not show elevated antioxidant capacity when assessed with ABTS and DPPH methods, its addiction in apple and apricot juices together with synthetic additives increased significantly the antioxidant activity of the enriched juices compared with juice prepared following the traditional recipes in both the tests. This was more evident in apple juice, where the addiction of bergamot juice at 20% as an alternative of synthetic additives also determined significant enhanced values of antioxidant activity.

While the antioxidant capacity assessed by DMPD was mainly ascribed to hydrophilic compounds, and especially ascorbic and citric acids, using ABTS and DPPH, in agree with literature works (Miller & Rice-Evans, 1997; Rapisarda et al., 1999), it can be speculated that bergamot polyphenols, and potentially bergamot flavanones, give an important contribute to increase the antioxidant values of the enriched juices.

In conclusion, data obtained clearly confirmed that no single antioxidant method can accurately reflect the antioxidant potency of a food matrix, this because antioxidant compounds may act differently to diverse tests.

However, the addiction of bergamot juice in common juices, like apple and apricot, enhances the antioxidant activity especially when used in combination with synthetic additives, thus because bergamot brings new bioactive compounds allowing the generation of fruit juices that also maintain high nutritional value and health functionality after conventional thermal treatments and storage. Obviously, further studies are required to evaluate the relative contribution of ascorbic acid, phenolic compounds and other possible bergamot metabolites to the improved antioxidant potential of fortified juices. On the other side, this aspect was not the main objective of this study which aimed to investigate the possible alternative use of bergamot juice, an industrial waste with biological proprieties.

### 3.3. Consumer test

Consumer acceptance, expressed as degree of liking, was quite high in both the fortified apple and apricot juices for the following hedonic parameters: colour (ranging from 5.2 to 6.8), visual viscosity (ranging from 5.1 to 6.5) and flavour intensity (ranging from 5.3 to 6.7). As taste is concerned, while the 10% fortified juices generally garnered a positive response (ranging from 4.3 to 6.6 and from 3.6 to 6.1 for apple and apricot fortified juices, respectively), the 20% fortified juices did not (responses ranging from 2.7 to 4.0 and from 2.1 to 3.8 for apple and apricot fortified juices, respectively); this latter negative judgment was explained by the consumers by the presence of a too bitter or astringent taste.

In evaluating the purchasing intention, it can be concluded that both of the fruit juices fortified with the addition of 10% bergamot juice, in particular apple juice, were well accepted by the consumers (average score was 4.7 and 4.2 for apple and apricot juices, respectively); both of these juices could represent a promising opportunity for the industries. On the other hand, the addition of 20% bergamot juice did not garner a positive response from the consumers (average score was 2.3 and 1.2 for apple and apricot juices, respectively), but it must be stressed that the fruit juice composition can be improved by modifying the recipe. Moreover, for the realisation of fortified juices at industrial-scale, bergamot juices could be extracted following a milder procedure, ensuring a less bitter taste (Postorino et al., 2001).

Although the consumer test conducted furnished only preliminary sensorial data, the obtained responses are encouraging for the development of new functionalized juices.

In conclusion, this study is useful in re-evaluating a by-product of bergamot that represents a cost and a possible environmental pollution source, suggesting other industrial mechanisms for producers to explore.

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