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Bioactive compounds in blood oranges (*Citrus sinensis* (L.) Osbeck): Level and intake

Biagio Fallico [a,*], Gabriele Ballistreri [b], Elena Arena [a], Selina Brighina [a], Paolo Rapisarda [b]

[a] *Dipartimento di Agricoltura, Alimentazione e Ambiente – Università degli Studi di Catania, Via S Sofia 98, 95123 Catania, Italy*
[b] *Consiglio per la Ricerca in Agricoltura e l'analisi dell'economia agraria – Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee (CREA-ACM), Corso Savoia 190, 95024 Acireale, Italy*

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ABSTRACT

Both the composition and the intake of antioxidants (anthocyanins, ascorbic acid and hydroxycinnamic acids) were reported for all blood oranges including the single cultivars (*Moro*, *Tarocco* and *Sanguinello*) and industrially produced juices. The mean values of the studied bioactive compounds in the edible part oranges were: 9.6 mg/100 g of orange edible part for the anthocyanins; 8.1, 0.7, 1.3, 3.8, 2.5 mg/100 g for total hydroxycinnamic acids, caffeic, sinapic, ferulic and coumaric acids, respectively and 59.1 mg/100 g for ascorbic acid. The consumption of blood oranges contributes to a daily intake of: 9.4 mg/d (up to 55 mg/d) of anthocyanins and 58.5 mg/d (up to 340 mg/d) of vitamin C, respectively. Data suggest that the 50% of consumers, males and females, receive more than the 70% and 90% of EAR value of vitamin C, respectively. The 25% of males and the 40% of females has an intake higher than the EAR.

1. Introduction

The health benefits of a diet rich in vegetables and fruits have been recognised, and following such a regimen is widely encouraged. The World Health Organisation recommends that individuals consume 400 g or five portions of vegetables and/or fruit per day (WHO, 2004). Consuming oranges, particularly blood oranges, has gained increased interest due to their high phenolic compound and anthocyanin content; these molecules are important in the non-energetic portion of the human diet. Epidemiological studies suggest that the consumption of anthocyanins lowers the risk of cardiovascular disease, diabetes, arthritis and cancer due both to their anti-oxidant and anti-inflammatory activities (Prior & Wu, 2006), as well as can play a role in cancer prevention (Wang & Stoner, 2008). Vitamin C is one of the most powerful water-soluble antioxidant. It can quench a variety of reactive oxygen species and reactive nitrogen species in aqueous environments. Evidence for *in vivo* antioxidant functions of ascorbate include the scavenging of reactive oxidants in activated leukocytes, lung, and gastric mucosa, and diminished lipid peroxidation (National Academy of Sciences, 2000). Phenolic acids play a role against oxidative stress, inflammation, cancer and diabetes (De, Baltas, & Bedos-Belval, 2011; Kylli et al., 2008; Nagasaka et al., 2007).

Blood oranges are constituted by three main cultivars (*Moro*, *Tarocco* and *Sanguinello*) and represent the most important citrus product of southern Italy, utilising approximately 170,000.00 ha and producing approximately 3.4 million tons per year (Tribulato, Maccarrone, & La Rosa, 2007).

The literature describing the chemical composition of blood oranges and their juice has grown in recent decades; studies have been carried out concerning the profile and level of anthocyanins (Maccarone, Maccarone, Perrini, & Rapisarda, 1983; Maccarone, Maccarone, & Rapisarda, 1985a, 1985b), vitamin C (Rapisarda, Bellomo, & Intelisano, 2001), hydroxycinnamic acids (Fallico, Lanza, Maccarone, Nicolosi Asmundo, & Rapisarda, 1996; Rapisarda, Carollo, Fallico, Tomaselli, & Maccarone, 1998), the antioxidant activity and the role of the most important constituents (Arena, Fallico, & Maccarone, 2001a; Rapisarda et al., 1999) and the thermal stability (Arena, Fallico, & Maccarone, 2000; Arena, Fallico, & Maccarone, 2001b). Moreover, the actions of blood oranges and their extracts *In Vivo* have also been reported. In fact, blood orange juice consumption determined a significant increase of antioxidants both in plasma (Guarnieri, Riso, & Porrini, 2007; Riso et al., 2005) and in urinary excretion (Giordano et al., 2012). Recently, the risks of pesticide consumption from Sicilian blood oranges (Fallico, D'Urso, & Chiappara, 2009), the contributions of anthocyanins and artificial

* Corresponding author.
E-mail address: bfallico@unict.it (B. Fallico).

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colorants to the visible colour (Fallico, Arena, Chiappara, & Ballistreri, 2010) and the assessment of Allura Red (Fallico, Chiappara, Arena, & Ballistreri, 2011) exposure from the consumption of red juice-based products and red soft drinks in Italy, have been evaluated.

Estimating nutrient intake is an important aspect of monitoring a population's nutritional status (Sette et al., 2013); these estimations identify groups who are nutritionally at risk due to their insufficient or excessive intake of specific nutrients and facilitate the targeting, planning and evaluation of nutrition intervention programmes, as well as the establishment of dietary recommendations, food regulations and nutritional policies (Gibney & Sandström, 2001). Moreover, when attempting to provide sufficient information for consumers to make informed choices and reap the full benefits from healthy foods, it is necessary to know the variations in the antioxidant, vitamin and other bioactive food component contents caused by natural or industrial processes (Dekker & Verkerk, 2003). To obtain the above values, reliable databases both for contaminants and for nutrients are critical. However, due to the variations in nutrients, as well as the difficulties in developing and maintaining food composition tables and training personnel (Hollman, Witthöft, Busstra, Elburg, & Hulshof, 2009), these databases are currently rather challenging (INFOODS, 2014). Methods for compiling food composition tables and databases are clearly described in international papers and guidelines (EFSA., 2008; FAO, 2003; Holden, Seema, & Patterson, 2002; Turrini, 2000).

Recently a specific database for phenols in foods (Phenol-Explorer) has been developed (Rothwell et al., 2013). It gives also details concerning the effect of processing and/or cooking. But, there are very few data concerning blood oranges.

The aims of this paper are twofold. One is to build composition tables concerning bioactive compounds, using a probabilistic approach, that focus on anthocyanins, hydroxycinnamic acids and vitamin C in total blood oranges (all samples), single cultivars (*Moro*, *Tarocco* and *Sanguinello*) or industrial juices (Not From Concentrate (NFC) and Reconstituted From Concentrate (RFC). Secondly, to use these data to assess the dietary intake of these compounds by blood oranges consumers.

2. Materials and methods

2.1. Development and managing of database

Excel® 2003 spreadsheets containing the concentrations of the bioactive compounds in the edible part of blood oranges (juice and pulps) were compiled: anthocyanins (expressed as mg of cyanidine-3-glucoside/100 g), hydroxycinnamic acids (total, caffeic, sinapic, ferulic and coumaric acid (mg/100 g)); ascorbic acid (mg/100 g). All data involved were presented as follows when available: all samples (total), organic or conventional, industrial samples (NFC, Not From Concentrate; RFC, Reconstituted From Concentrate) and single cultivar (*Moro*, *Tarocco* and *Sanguinello*). When information about the clone was available (e.g., *Tarocco Gallo*, *Tarocco Meli* or *Doppio Sanguigno*), the sample was assigned to the cultivar, (e.g., *Tarocco* or *Sanguinello*). Samples have been classified, according to the International Food Code systems (FoodEx2 and LanguaL), as A01CS and B4313, respectively. The industrial juices, NFC and RFC, since they were not adjusted in colour, pulps and sugars/acidity ratio, typical of commercial (ready to drink) blood orange juices, have been included in the above classification.

In this study, only direct analyses and data from peer-reviewed papers were used. Data from public databases and web data were deliberately excluded for two reasons: first, evaluating the data quality would be impossible; and second, it was necessary to avoid counting the same data many times from different sources.

Table 1
Quality assessment of used data.

	Quality Assessment of Data	
	Category	Score **Mean**; ds; Min; Max
Anthocyanins	1. Food Description	**4**; 0.5; 4; 5
	2. Component Identification	**5**
	3. Sampling Plan	**2**; 2; 0; 5
	4. Number of Analytical Samples	**3**; 1; 1; 3
	5. Sample Handling	**5**
	6. Analytical Method	**5**
	7. Analytical Quality Control	**3**
	Total Quality Index (TQI)	**27**
Hydroxycinnamic acids	1. Food Description	**4**; 0.5; 4; 5
	2. Component Identification	**5**
	3. Sampling Plan	**2**; 2; 0; 5
	4. Number of Analytical Samples	**3**; 1; 1; 3
	5. Sample Handling	**5**
	6. Analytical Method	**5**
	7. Analytical Quality Control	**3**
	Total Quality Index (TQI)	**27**
Ascorbic Acid	1. Food Description	**4**; 0.5; 4; 5
	2. Component Identification	**5**
	3. Sampling Plan	**2**; 3; 0; 4
	4. Number of Analytical Samples	**2**; 1; 1; 3
	5. Sample Handling	**5**
	6. Analytical Method	**5**
	7. Analytical Quality Control	**2**
	Total Quality Index (TQI)	**25**

Because both individual and composite samples were available, weighting factors (Sioen et al., 2007) were used: $wi,_{Total} = wi,_{unit} * wi,_{meas}$, where $wi,_{uni}$ is the number of units per sample and $wi,_{meas}$ is the number of measurements per sample. The final number of samples for the compound *i* is given by the following expression:

$$wi, Final = \sum_i wi, Total$$

The mean (M_{group}) and standard deviation (SD_{group}) used for each composite compound were calculated as follows:

$$Mgroup = \frac{\sum Wi, T * Mi}{Wi, F}$$

$$SD^2group = \frac{(\sum Wi, T - 1) * S_i^2 + \sum (M_i - M_{group})}{Wi, F - 1}$$

where Mi and Si are the mean and the standard deviation of compound i, respectively.

The data also include the evaluation of sampling plan, the number of samples, their handling and the analytical methods. The quality of data, reported in each paper, was evaluated according to EuroFIR (2014). This system uses 7 quality categories (1. Food description; 2. Component Identification; 3. Sampling Plan; 4. Number of Analytical Samples; 5. Sample Handling; 6. Analytical Method; 7. Analytical Quality Control). Each category gives a score and the sum of the 7 category scores results in the Total Quality Index (TQI) of the used data. The results are in Table 1.

2.2. Simulations and scenarios

Simulations were run (@Risk 5.5, Palisade Inc.) to obtain estimations (distribution probability) both for the concentrations of each compound and the estimation of dietary intakes. The conditions of each simulation were as follows: the number of iterations = 10.000