

# Nutritional Value of Italian Pistachios from Bronte (*Pistacia vera*, L.), Their Nutrients, Bioactive Compounds and Antioxidant Activity

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## Abstract

This study gives an overview on the nutritional value, bioactive compounds and antioxidant activity (ABTS and FRAP) of Bronte's pistachio (*Pistacia vera*, L., cv. Bianca) from Sicily (Italy). Bronte's pistachios are rich in fat, protein, dietary fiber, trace elements (Fe, Zn, Cu, Mn) and minerals (Ca, P, K, Mg, Na). Lipids mainly consist of MUFA (33.8 g/100g), primarily oleic acid (32.4 g/100g); PUFA is mostly represented by linoleic acid (7.49 g/100g). Bronte's pistachios are also a valuable source of bioactive compounds such as total polyphenols (501.5 mg/100g), lutein (1.26 mg/100g),  $\beta$ -carotene (0.18 mg/100g),  $\gamma$ -tocopherol (19.2 mg/100g) and phytosterols (134.4 mg/100g). Among phytosterols, the main is  $\beta$ -sitosterol (86% of total content), followed by  $\Delta^5$ -avenasterol (6.3%). Phytic acid content is 1763 mg/100g; Ins(1,2,4,5,6)P<sub>5</sub> and Ins(1,2,3,4,5)P<sub>5</sub> have been detected too (31 mg/100g and 10 mg/100g, respectively). The antioxidant activity was determined both in hydrophilic and lipophilic fraction of pistachios, showing >80% of the total antioxidant activity in the hydrophilic, predominately due to phenolic compounds.

## Keywords

Bronte's Pistachios, Dietary Fiber, Phytic Acid, Inositol Pentaphosphates, Carotenoids, Tocopherols, Phytosterols, Total Polyphenols, Antioxidant Activity

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## 1. Introduction

In the Mediterranean area an intense food trade has taken place for centuries. Pistachios (*Pistacia vera*, L.)

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represent an example of the food exchanged in this area, so for a long time pistachios have become an integral part of the Mediterranean food pattern. Nowadays the consumers' interest in pistachios is focusing on the high content of dietary fibre, minerals and trace elements, monounsaturated fatty acids and valuable bioactive compounds. During the last decades nuts have attracted the attention for their potential health benefits; strong relationships between nut/nut oil-containing diet and protection against cardiovascular diseases [1], developing of type-2 diabetes [2], prostate and colorectum cancers [3] have been reported. The potential health benefits related to nut consumption are mainly associated to the favourable lipid composition and the phytochemical profile. Pistachios are rich in healthy lipids and in bioactive molecules, such as dietary fiber, polyphenols, tocopherols, carotenoids and phytosterols [4] [5]; furthermore pistachios contain stilbenes too [6]. Besides the fore-mentioned healthy properties associated to nut composition, it has been shown that nut consumption leads to increased satiety and low glycemic index, without weight gain [7]. Recently it has been also reported that consumption of pistachios modifies human gut microbiota composition increasing the number of beneficial butyrate-producing bacteria [8].

Most of the Italian pistachio area is concentrated in few territories in the eastern Sicily on the slopes of the Etna volcano, Bronte representing the main production area. The Bronte's pistachio is mainly used for the bagged meat and confectionery industry and to produce pistachio cream, sprinkles and ice-cream. In recent years Bronte's pistachio has also received the attribution of Protected Designation of Origin (PDO) by the European Union (EU 21-2010), a quality label referred to zone of production (Bronte, Adrano and Biancavilla). The aim of the present study was to evaluate the content of proteins, lipids, carbohydrates, minerals (Ca, P, K, Mg, Na), trace elements (Fe, Zn, Cu, Mn) and dietary fiber of Bronte's pistachios (*Pistacia vera*, L., cv. Bianca). Moreover, the content of bioactive compounds such as total polyphenols, carotenoids, tocopherols, phytosterols, phytic acid and the antioxidant activity was determined to evaluate the nutritional value of Bronte's pistachio.

## 2. Materials and Methods

### 2.1. Materials

Italian Pistachios (*Pistacia vera*, L., cv. Bianca) from Bronte (Sicily, Italy) were obtained from the "Consortium for Bronte Pistachio Protection (PDO)", according to the corresponding product specification, e.g. cultivation altitude, crop management (hand-harvesting, sun-drying process). A total of 9 kg of pistachios grown in six farms of the Bronte's area were delivered to laboratory raw and unshelled, packed in vacuum sealed storage bags. Equal amounts of pistachio nuts were randomly grouped into six batches and stored in refrigerator at +4°C until analysis. Each batch was analyzed in triplicate.

### 2.2. Methods

#### 2.2.1. Proximate Analysis

Moisture, protein, lipid and ash were determined according to AOAC methods [9].

#### 2.2.2. Minerals and Trace Elements

Samples were analyzed for mineral (Ca, Mg, Na, K, P) and trace element (Fe, Zn, Cu, Mn) contents by ICP-OES (Optima 3200XL—Perkin-Elmer) after liquid ashing (4 mL HNO<sub>3</sub> + 1 mL H<sub>2</sub>O<sub>2</sub>) of the samples in a microwave digestion system (Milestone, 1200 Mega). Standard Reference Materials: Cabbage (IAEA-359, International Atomic Energy Agency Reference Materials Group) and Haricots vert (BCR 383, Community Bureau of Reference, Brussels) were analyzed as a check on the accuracy of the analysis.

#### 2.2.3. Total Dietary Fiber

Total dietary fiber and fractions, soluble and insoluble, were determined by method of Prosky *et al.* [10].

#### 2.2.4. Phytic Acid

Phytic acid and inositolphosphates content were determined by HPLC following the method of Schlemmer *et al.* [11].

#### 2.2.5. Fatty Acids

Fatty acids were methylated according to the method by Slover and Lanza [12]. and quantified by GC-FID

(AGILENT, 7890A series). Chromatographic separation was accomplished on a DB-23 capillary column (J&W 122 - 2361). Beef/pig fat Blend (BCR CRM 163, Institute of Reference Materials and Methods, Geel, Belgium) and F.A.M.E. mix C4-C24 (Supelco, Bellefonte, PA, USA) were analysed as check of the accuracy of the analysis.

### 2.2.6. Total Polyphenols

Total polyphenols content was determined following the Folin-Ciocalteu method [13]; data were expressed as gallic acid equivalent (GAE).

### 2.2.7. Analysis of Bioactive Compounds in Pistachio Lipid Fraction

The lipophilic bioactive compounds were determined in the oil fraction of pistachios. Whole, unshelled pistachios were grounded in a water cooler mixer to a fine powder in order to obtain a homogeneous representative sample. A cold solvent extraction method of pistachio oil was performed to preserve bioactive molecules from degradation [14]. The yield of the obtained oil was 40% - 50%. Data are presented as mg/100g of fresh nut.

### 2.2.8. Carotenoids

Carotenoid (violaxanthin, lutein and its isomers,  $\beta$ -carotene) content in pistachio oil was quantified by HPLC/PAD according to the method by Pilar Cano [15].

### 2.2.9. Tocopherols and Tocotrienols

The pistachio oil was analyzed by LC/MS-MS (AGILENT 1200 series quaternary pump coupled with an AGILENT 6410 triple quadrupole mass spectrometer) for tocopherols (T) and tocotrienols (T3):  $\alpha$ -tocopherol,  $\alpha$ -tocotrienol,  $\beta$ -tocopherol,  $\beta$ -tocotrienol,  $\gamma$ -tocopherol,  $\gamma$ -tocotrienol,  $\delta$ -tocopherol and  $\delta$ -tocotrienol content was determined without saponification [16].

### 2.2.10. Phytosterols

Phytosterols were extracted from pistachio oil after saponification, as reported in literature methods [17] [18]. Phytosterols were separated on HP-5MS capillary column and then quantified by GC-FID (AGILENT, 7890A series) according to the method by Du and Ahn [19].

### 2.2.11. Antioxidant Activity

Antioxidant activity of Bronte's pistachio was assessed both on hydrophilic and lipophilic fractions. The hydrophilic fraction was extracted from fine grounded pistachios by  $\text{CH}_3\text{OH}/\text{H}_2\text{O}$  (1/1), the residue was utilized for the extraction of the lipophilic fraction, carried out by acetone [20]. Antioxidant activity was assessed by ABTS and FRAP assays:

ABTS assay: the antioxidant activity was evaluated by ABTS method as described by Re *et al.* [21]. The antioxidant activity was expressed as mmol Trolox Equivalent (TE)/kg.

FRAP (Ferric reducing antioxidant power) assay: the method was applied only to the hydrophilic extract. The antioxidant activity was expressed as mmol  $\text{Fe}^{2+}$  equivalents/kg [22].

### 2.2.12. Data Analysis

All experimental data are presented as the Mean  $\pm$  Standard Deviation of the six analysed pistachio's batches. The linearity of responses of the antioxidant activity analysis was determined using Pearson's correlation coefficient (r).

## 3. Results

The overall nutrient composition, total dietary fiber and its fractions, minerals and trace-elements, fatty acids content of Bronte's pistachio are reported in **Table 1**. Generally, nuts have a rather low moisture content, in the case of Bronte's pistachio it was 5.3%, the protein content was 24.5 g/100g, fat content 49 g/100g, whilst the carbohydrates content was fairly low (**Table 1**). The energy value was 549.5 kcal, 80% coming from fat, 18% from protein and 2% from carbohydrates. A previous study [23] reported for Bronte's pistachio a lower protein content and a higher fat content compared to our findings.

The average protein content detected in Bronte's pistachio in this study was fairly high compared with other

**Table 1.** Macronutrients, dietary fiber, minerals, trace elements and fatty acids content in Bronte's pistachio (f.w.).

Bronte Pistachio		
<b>Proximate</b>		
Moisture	g/100g	5.34 ± 0.4
Ash	g/100g	3.06 ± 0.08
Protein	g/100g	24.5 ± 0.4
Lipid	g/100g	49.0 ± 1.2
Carbohydrates	g/100g	2.8*
Dietary fiber	g/100g	15.3 ± 0.32
Soluble	g/100g	2.2 ± 0.14
Insoluble	g/100g	12.3 ± 0.21
<b>Minerals</b>		
Ca	mg/100g	114 ± 12
P	mg/100g	416 ± 25
K	mg/100g	962 ± 33
Mg	mg/100g	121 ± 7.5
Na	mg/100g	tr
<b>Trace Elements</b>		
Fe	mg/100g	4.5 ± 0.28
Zn	mg/100g	2.1 ± 0.12
Cu	mg/100g	1.2 ± 0.05
Mn	mg/100g	0.3 ± 0.02
<b>Fatty acids</b>		
<b>SFA</b>		
C14:0	g/100g	0.044 ± 0.004
C16:0	g/100g	4.36 ± 0.09
C18:0	g/100g	0.92 ± 0.10
C20:0	g/100g	0.08 ± 0.004
C22:0	g/100g	0.04 ± 0.004
<i>Total</i>		5.44
<b>MUFA</b>		
C16:1	g/100g	0.41 ± 0.002
C18:1 n-9	g/100g	32.45 ± 0.76
C18:1 n-11	g/100g	0.78 ± 0.03
C20:1	g/100g	0.16 ± 0.02
<i>Total</i>		33.80
<b>PUFA</b>		
C18:2	g/100g	7.49 ± 0.75
C18:3	g/100g	0.18 ± 0.05
<i>Total</i>		7.67

\*Each value is the Mean +/- SD of the six analysed batches.

nuts like walnut or hazelnut [24]; on the other hand total fat content was among the lowest compared with other nuts [24]–[26] and with other pistachio varieties [23] [27]. It has been demonstrated that fat content and its composition are strongly influenced by place of origin and species in nuts [23]. Bronte's pistachio is also a good source of dietary fiber and is among the highest fiber nuts [24], the cv. Bianca analyzed in this study contained 15.3 mg/100g, approximately 80% of this was represented by the insoluble fraction (Table 1). A regular consumption of foods rich in dietary fiber, like whole grains and nuts, is considered to have health benefits; an inverse relationship between high dietary fiber intake and reduced coronary heart disease risk has been reported [28]. Nuts are also regarded as a good source of minerals and trace elements, Bronte's pistachio in fact was an excellent source of minerals, among these K (962 mg/100g) and P (416 mg/100g) in major amount (Table 1). Even trace elements were detected in quite high amounts, Fe, Zn and Cu were well represented (4.5, 2.1 and 1.2 mg/100g, respectively), by contrast Mn content (0.3 mg/100g) was lower than that usually found in pistachio (Table 1). The peculiar lavic soil as well as the ground water composition in the area of Volcano Etna where Bronte's pistachios are cultivated, may have contributed to the nut trace element profile. Although Bronte's pistachio is a rich source of dietary fat (Table 1), the most was of unsaturated type (41.47 g/100g, mono-plus polyunsaturated). Among saturated fatty acids (SFA) the main was palmitic acid (4.36 g/100g), followed by stearic (0.92 g/100g) and very little amounts of arachidic, behenic and miristic acids (Table 1). Among monounsaturated fatty acids (MUFA) oleic acid (cis-9 oleic acid) was in the highest amount (32.45 g/100g), followed by cis-11 isomer vaccenic acid (0.78 g/100g), palmitoleic acid (0.4 g/100g) and a little amount of gadoleic acid (0.16 g/100g). Polyunsaturated fatty acids (PUFA) were mostly represented by linoleic acid (7.49 g/100g) followed by little amount of linolenic acid (0.18 g/100g) the plant omega-3 fatty acid (Table 1). Monounsaturated fatty acids (MUFA) represented thus the main fatty acid fraction in Bronte's pistachio (69% of total lipid) followed by PUFA (15% of total lipid) and SFA (11% of total lipid) (Table 1). The ratio unsaturated/saturated was 7.6, indicating a favourable fatty acid composition of the unsaturated fraction, responsible for nut health promoting effects [29]. A similar fatty acids profile of Bronte's pistachio was reported [5] [23]. Phytic acid is the major storage form of phosphorus in plant seeds, pistachios are relatively rich in phytate, ranging from 0.29 to 2.83 g/100g (d.w.) [30]. The contents of phytic acid and inositol pentaphosphates of Bronte's pistachio are reported in Table 2. The mean phytate content of Bronte's pistachio was  $1763 \pm 243$  mg/100g and those of Ins(1,2,4,5,6)P5 and Ins(1,2,3,4,5)P5 were  $31 \pm 16$  mg/100g and  $10 \pm 6$  mg/100g, respectively (Table 2). High phosphorylated inositol phosphates such as phytic acid and inositol pentaphosphates can strongly bind minerals and trace elements under the physiological conditions of the gut, forming low soluble complexes. Thus, the prevalence of inositol pentaphosphates might also contribute along with phytic acid to a lower bioavailability of trace elements and minerals [30]. However the ability to bind minerals can be regarded both as detrimental and beneficial for health. Phytate is a highly important bioactive food compounds, within the last twenty years the nutritional physiological properties of phytic acid have been controversially discussed. On the one hand phytate may strongly interfere into the intestinal absorption of minerals and trace elements, for what it was denominated an antinutrient. On the other hand beneficial properties such as its anticancer, antioxidative and anticalcification activities were reported for phytate [30].

The main bioactive phytochemicals content in Bronte's pistachio is reported in Table 3. Generally phytochemical content in plant food is influenced by genetic and environmental factors, such as soil composition, sun exposure, geographical location, besides post-harvest treatments. Our findings showed a total phenolic content in raw Bronte's pistachio of 501.5 mg/100g expressed as mg of gallic acid equivalent (GAE) (Table 3). This value was higher compared to those reported by Gentile *et al.* [31] who found an average content of 175 mg GAE/100g in Bronte's pistachio. Tsantili *et al.* [32] in a study on eight pistachio varieties from different locations, found Bronte's pistachio as the third with the highest total phenolic content. Total polyphenol content varies widely among nuts, Kornsteiner *et al.* [25] found pistachios among the richest sources (ranging from 492 to 1442 mg GAE/100g). Even higher total polyphenol content, up to 1657 mg GAE/100g, was found by Wu *et al.* [33]. Pistachio is, among nuts, the only containing good amounts of both carotenoids and chlorophylls (Table 3). Lutein was the most represented carotenoid in Bronte's pistachio (1.26 mg/100g), followed by  $\beta$ -carotene (0.18 mg/100g) and violaxanthin (0.04 mg/100g). Also little amounts of lutein isomers, neolutein a and neolutein b were found (respectively 0.02 and 0.01 mg/100g) (Table 3). Giuffrida *et al.* [34] found 7 mg/kg of  $\beta$ -carotene in Bronte's pistachio whole nuts, little amounts of neoxanthin and luteoxanthin were detected too. Bellomo and Fallico [4] reported an average content of 26 mg/100g dry matter of lutein. Kornsteiner *et al.* [25] among 10 selected nuts found carotenoids only in pistachio, detecting only trace amount of  $\beta$ -carotene and less than 5

**Table 2.** Content of phytic acid and inositol pentaphosphates of Bronte's pistachio (f.w.).

	mg/100g
IP6	1763 ± 243
I(1,2,4,5,6)P5	31 ± 16
I(1,2,3,4,5)P5	10 ± 6

Each value is the Mean +/- SD of the six analysed batches.

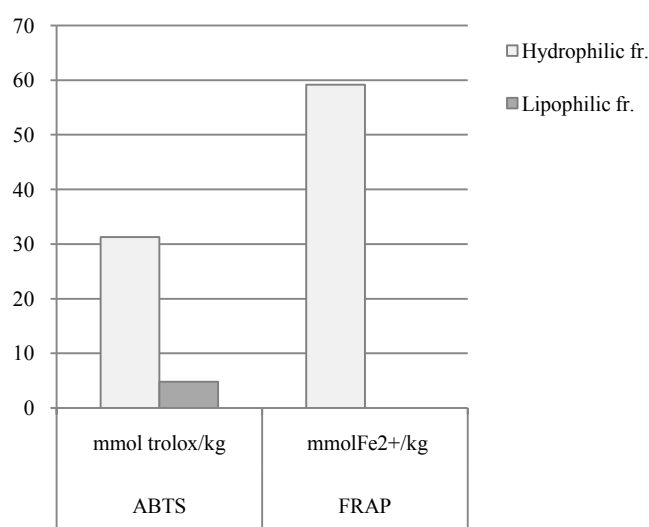
**Table 3.** Content of total polyphenols, carotenoids, tocopherols and plant sterols of Bronte's pistachio (f.w.).

Bronte Pistachio		
<i>Proximate</i>		
Total polyphenols	mg/100g GA	501.5 ± 64.3
Carotenoids		
Lutein	mg/100g	1.26 ± 0.17
$\beta$ -carotene	mg/100g	0.18 ± 0.05
Violaxanthin	mg/100g	0.04 ± 0.01
Neolutein a	mg/100g	0.02 ± 0.01
Neolutein b	mg/100g	0.01 ± 0.00
Tocols		
$\alpha$ -tocopherol	mg/100g	0.77 ± 0.12
$\alpha$ -tocotrienol	mg/100g	0.13 ± 0.02
$\gamma$ -tocopherol	mg/100g	19.19 ± 5.31
$\gamma$ -tocotrienol	mg/100g	2.60 ± 0.48
$\delta$ -tocopherol	mg/100g	0.43 ± 0.09
$\delta$ -tocotrienol	mg/100g	0.13 ± 0.03
$\beta$ -tocopherol	mg/100g	0.04 ± 0.01
$\beta$ -tocotrienol	mg/100g	0.03 ± 0.01
Sterols		
Campesterol	mg/100g	7.44 ± 0.16
Stigmasterol	mg/100g	2.08 ± 0.33
$\beta$ -sitosterol	mg/100g	116.4 ± 9.14
$\Delta^5$ -avenasterol	mg/100g	8.52 ± 0.76
Total sterols	mg/100g	134.44 ± 7.47

EData were the Mean +/- SD of the six analysed batches. GA, gallic acid. Carbohydrates = calculated by difference. To convert percentages of total fatty acids in % of edible portion the conversion 0.956 was applied.

mg/100g of lutein in the oil extract. The USDA database [24] reported lutein + zeaxanthin content of 1.2 mg/100g in pistachios.

Tocopherols and tocotrienols ( $\alpha$ -T,  $\beta$ -T,  $\gamma$ -T,  $\delta$ -T;  $\alpha$ -T3,  $\beta$ -T3,  $\gamma$ -T3,  $\delta$ -T3) content in Bronte's pistachio is reported in Table 3. The predominant vitamin E isoform was  $\gamma$ -tocopherol (19.19 mg/100g) followed by  $\gamma$ -tocotrienol (2.6 mg/100g). Alpha tocopherol was present in good amounts (0.77 mg/100g), while the content of its correspondent  $\alpha$ -tocotrienol isomer was much lower (0.13 mg/100g). Delta tocopherol and  $\delta$ -tocotrienol were 0.43 mg/100g and 0.13 mg/100g, respectively. The  $\beta$ -isoforms were detected in traces (less than 0.1 mg/100g). Concentrations found in this study for  $\alpha$ - and  $\gamma$ -tocopherols were higher than those found by both Gentile *et al.* [31] and Ballistreri *et al.* [14] in *Pistacia vera*; the greatest difference regarded the  $\gamma$ -tocopherol concentration, in the present study it was up to three times higher. Alpha-tocopherol content varied greatly among nuts, the highest sources of  $\alpha$ -tocopherol are almond and hazelnut, by contrast both pistachio and walnut are richest in  $\gamma$ -tocopherol [24] (USDA, 2010). Kornsteiner *et al.* [25] found both  $\beta$ - and  $\gamma$ -tocopherol as predominant tocopherols in 7 nut types, pistachios showing the highest values. Phytosterol content in Bronte's pistachio is reported in Table 3. Up to 134.44 mg/100g of total sterols were detected. The dominant phytosterol in Bronte's pistachio was  $\beta$ -sitosterol (about 86% of total content), followed by  $\Delta^5$ -avenasterol (6.3%) and campesterol (5.5%), little amount of stigmasterol (1.5% of total content) was also detected (Table 3). Other unidentified sterols were observed (data not shown), accounting for less than 2% of total phytosterol content. The higher  $\Delta^5$ -avenasterol content found in Bronte's variety compared to other pistachio varieties was also confirmed by Arena *et al.* [5]. Phillips *et al.* [35] found phytosterol content in nuts ranging from 95 to 270 mg/100g and quantified also other sterols, unidentified, contributing to less than 10% to the total content. The USDA database [24] reports slightly higher amount of total phytosterol content in pistachio (214 mg/100g) compared to our findings. The individual sterol content found in this study was similar to that reported by Derewiaka *et al.* [26]. The contribution of hydrophilic and lipophilic fractions to the total antioxidant activity of Bronte's pistachio is described in Figure 1. Hydrophilic fraction contributed with over 80% to the total antioxidant activity (31.29 mmol trolox/kg, ABTS assay), the remaining 20% was due to the lipophilic fraction (4.84 mmol trolox/kg, ABTS assay) (Figure 1). This was probably due to the abundant phenolic content of pistachios, in fact a good correlation ( $r^2 = 0.80$ ) was found between the total phenolic content and the antioxidant activity of the hydrophilic fraction, using the Pearson correlation factor. The antioxidant activity of the hydrophilic fraction was also measured by FRAP method (Figure 1). Results were found comparable to those from ABTS, taking into account that trolox antioxidant activity is twice  $\text{Fe}^{2+}$  antioxidant activity. Generally good correlation is found between the two methods, as they utilize the same single electron transfer mechanism. Present data on the total antioxidant activity of the cv. Bianca of *Pistacia vera* L. were higher than those reported by Gentile *et al.* [31] (28.06 mmol trolox/kg), but lower than those reported by Tsantili *et al.* [32] (130 mmol trolox/kg) and by Tomaino *et al.* [36] (243



**Figure 1.** Antioxidant activity of Bronte's pistachio hydrophilic and lipophilic fractions measured by ABTS and FRAP assays.



mmol trolox/kg). Pellegrini [37] reported for pistachios a contribution to the total antioxidant activity, due to the free forms of the molecules present in the hydrosoluble extract, close to that reported in this study. Wu *et al.* [33] also reported antioxidant activity values in both lipophilic and hydrophilic fractions of pistachios very similar to those found in this study. In a study aimed at assessing the antioxidant content of over 1000 foods, pistachio was ranked among the top 50 foods with the highest antioxidant activity [38].

#### 4. Conclusion

Pistachios are a worth component of a healthy dietary pattern providing high protein, unsaturated fat, dietary fiber, micronutrients and a variety of bioactive compounds like sterols, tocopherols and lutein. Fat content, although high in Bronte's pistachios (about 15 g per 30 g serving), is most of unsaturated type (41.47 g/100g, mono-plus poly-unsaturated). Among PUFA linoleic acid, an  $\omega$ -6 essential fatty acid is well represented; the last Italian consumption survey [39] indicates a median intake of linoleic acid of 8.1 g/die, thus one pistachio's serving (30 g) provides 2.25 g of it, accounting for 28% of the daily intake. This favourable fatty acid profile is accompanied by a rich bioactive molecule's content; this further supports the protective health effects of a regular nut consumption. Dietary fiber is a key component of a healthy dietary pattern; one serving (30 g) of Bronte's pistachios supplies 4.6 g of dietary fiber, which is about 18% of the current Italian recommendation for daily fiber intake [40]. Bronte's pistachios are also a valuable vehicle in the diet of several bioactive molecules with beneficial effects on health, e.g. one serving (30 g) supplies 150 mg of total polyphenols, 40.3 mg of sterols and 5.8 mg of  $\gamma$ -tocopherol. The high concentration, especially of hydrophilic bioactive molecules, strongly contributes to the antioxidant activity of pistachios. Our findings suggest that a regular pistachio's consumption improves well-being and seems to significantly contribute to the health preserving Mediterranean diet.

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