

Absorption of strawberry phytochemicals and antioxidant status changes in humans

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Abstract. In this study the antioxidant composition of fresh and stored strawberries and the bioavailability of the main strawberry bioactive compounds were determined in humans. In addition we have investigated plasma total antioxidant capacity. On 13 healthy volunteers, blood samples were collected before and after acute ingestion of fresh and stored strawberries, 300 g respectively. Ferric Reducing Antioxidant Power (FRAP) values after consumption of fresh and stored strawberries showed a significant increase ($P < 0.05$) at 5 and 8 hours time interval, while significantly decreased TRAP (Total Radical-Trapping Antioxidant Parameter) values ($P < 0.05$) were found at 8 hours respect to baseline after stored strawberries consumption. After consuming fresh strawberries, plasma levels of α -carotene increased significantly ($P < 0.05$) respect to stored ones. Moreover, consumption of fresh and stored strawberries resulted in a significant increase of vitamin C at 2, 3 and 5 hours ($P < 0.05$). The bioavailable amount of strawberries antioxidant compounds reflects the variations observed in fresh and stored fruits. We could summarize that the global food quality is related to both native quantity of bioactive compounds and storage treatments.

Keywords: Strawberry, domestic storage, bioactive compounds, plasma antioxidant status

1. Introduction

Recently the researchers have focused their attention on the beneficial effects of berry fruits (blueberries, blackberries, strawberries, raspberries) [5, 7, 19, 23, 25, 31], due to their composition in bioactive molecules such as anthocyanins, flavanols and catechins [13, 15, 16, 21, 22, 27, 29, 33, 38, 42, 45]. Studies conducted *in vitro* and *ex vivo* using strawberries, as well as other plant food, indicate that phytochemicals exert a wide range of biochemical actions including antioxidant, immunomodulating, inhibition of platelet aggregation and anticarcinogenic action [2, 30, 32, 41, 43]. Several studies have shown that the antioxidant capacity of strawberries extracts was positively related to their antiproliferative activities [52]. Some constituents of strawberries have shown inhibition of initiation and promotion of the carcinogenic process [20]. Zunino et al. [56] have investigated the ability of purified strawberry fractions to induce apoptotic cell death in leukaemia cells. Zhang et al. [55] have identified strawberry phenolics with antioxidant and human cancer cell antiproliferative activities.

Studies focusing on anthocyanins absorption have shown that the bioavailability of anthocyanins is quite low and their metabolism is still not fully understood [7, 9, 37, 53].

Human studies evaluating the biological properties and bioavailability of strawberry phytochemicals and impact of strawberries consumption on human health are needed.

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Strawberries (*Fragaria × ananassa* Duch.) are a rich source of micronutrients and phytochemicals such as α -carotene, vitamin C and phenolic compounds [14, 49, 50] including ellagic-acid-containing compounds, proanthocyanidins, quercetin, kampferol and anthocyanins, mainly pelargonidin glycosides [8, 54]. The high antioxidant capacities associated with the relative content of bioactive nutrients was influenced by several factors including storage [19], as known, during storage several degradation reactions may occur reducing quality of strawberry [3, 39, 47, 48].

The data reported in this work originate from a wider study in which bioavailability of strawberries antioxidants on humans was investigated [4].

Thus, the aim of present work was to examine the effect of domestic storage on the micronutrient and phytochemical composition of strawberries and to investigate the effects of acute strawberries consumption in humans on total antioxidant capacity and plasma antioxidant levels.

2. Materials and methods

2.1. Chemicals

All solvent were purchased from Carlo Erba (Milan, Italy), BDH (Poole, England) and Merck (Darmstadt, Germany). 2,2'-Azobis (2-amidinopropane) dihydrochloride (ABAP) was purchased from Wako Chemical (Germany), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) was from Fluka (Switzerland). R-phycoerythrin (R-PE), phosphate-buffered saline (PBS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and ascorbic acid were provided by Sigma–Aldrich Srl. Commercial standards were also from Sigma–Aldrich Srl (Milan, Italy). Double distilled water (Millipore, Milan, Italy) was used throughout the study.

2.2. Subjects

Thirteen volunteers (9 males and 4 females) were screened and enrolled in the study according to absence of acute or chronic diseases or metabolic disorders, no smoking habits, no alcohol consumption, no use of drugs, vitamin or mineral supplements for 2 weeks before the investigation. Mean age and BMI of the subjects were 26 ± 7 years and 22.6 ± 2.6 kg/m².

2.3. Strawberries

Strawberries (cv *Favetta*) were grown in southern Lazio (Latina, Italy) by local producers. Fresh or stored strawberries derived from the same genotype and pre-harvest period.

Once received, they were immediately washed and divided into two parts. One half was used as fresh product (fresh strawberries), while the other part was stored at +4°C over a 4-day period and then used as stored strawberries. On the experimental days participants were served with 300 g portions of fresh or stored strawberries. The amount of bioactive compounds ingested by subjects was evaluated using the methods described below.

2.4. Study design

The recruitment of volunteers took place in different centres (Universities, Research Institutes), the study protocol was approved by the University of Rome “La Sapienza” Ethical Committee and all participants gave their written consent.

Three days before the study volunteers followed a low antioxidants diet, excluding some fruits, vegetables and beverages (wash-out). After fasting overnight, venous blood samples were collected (baseline). Following acute ingestion of fresh strawberries (300 g), blood samples were collected at 0.5, 1, 2, 3, 5, 8 hours.

The same procedure was adopted after 4 days administrating 300 g of stored strawberries (at +4°C).

2.5. Samples treatment

Blood samples were collected in EDTA containing tubes. After centrifuging at 3000 rpm for 10 min at 4°C the plasma was stored at –80°C until analysis. Aliquots of plasma were used for the following determinations: total antioxidant capacity, lipidemic profile, uric acid, vitamin A and E, vitamin C, carotenoids.

2.6. Food analyses

2.6.1. Total antioxidant capacity

The Total Antioxidant Capacity was evaluated by the Ferric Reducing Antioxidant Power (FRAP) [6] and Total Radical-Trapping Antioxidant Parameter (TRAP) [17] method. FRAP assay measures the reducing power—the ability of plasma samples to reduce the colorless ferric-tripyridyltriazine complex (TPTZ-Fe³⁺) to its ferrous colored form (TPTZ-Fe²⁺). It is a simple and highly reproducible spectrophotometric assay using Fe(II) as standard. The TRAP was determined according to the method of Ghiselli et al. [17] that was based on the protection provided by antioxidants on the fluorescence decay of *R*-phycoerythrin (lag phase) during a controlled peroxidation reaction.

2.6.2. Carotenoids

Carotenoids were extracted using the method described by Sharpless et al. [46]. The determination of carotenoid concentrations was carried out by high performance liquid chromatography techniques (HPLC) [34].

2.6.3. Phenolics and total ascorbic acid

Phenolics and total ascorbic acid were extracted from strawberries using the methods previously described by Hertog et al [22] and Margolis et al. [35] respectively. The quantitative analyses were performed using an HPLC system equipped with a coulometric detector (ESA model 580, Chemsford, MA, USA). The chromatographic separation was obtained applying the methods described by Serafini et al. [44].

2.6.4. Anthocyanins

Anthocyanins were extracted by a method adapted from Kay et al. [26]. The extracts were analysed by high-performance liquid chromatography with mass spectrometry (HPLC-MS) detection, using the analytical conditions previously described by Vitaglione et al. [51].

2.7. Biological samples assessment

Plasma total antioxidant capacity was measured by both FRAP [6] and TRAP method [17]. Total cholesterol and triglycerides were evaluated using commercial kits. Plasma uric acid was measured by spectrophotometric assay with a commercial kit. The spectrophotometric assay for measuring thiol groups was based on DTNB or Ellman reagent which reacts with SH groups leading to the formation of a colored solution which shows a maximum absorption at 412 nm [24].

The quantitative analysis of plasma carotenoids (lutein and zeaxanthin, cryptoxanthin, lycopene, α -carotene, β -carotene), vitamin A and vitamin E was carried out by HPLC [34]. Total vitamin C was extracted from plasma and quantified according to Margolis et al. [35, 36]. The quantitative analysis was done by HPLC like strawberries analysis.

2.8. Statistical analysis

All data were checked for normal distribution using Shapiro–Wilk's test. *t*-Test for dependent samples was applied for statistical analysis of food and dietary intake. For biological samples statistical analysis was performed using ANOVA test for repeated measures and the Bonferroni's 2-tailed *t*-test matched pairs test assuming the baseline values as reference category. $P < 0.05$ was considered significant.

3. Results and discussion

Strawberry breeding programs are currently used to improve specific agronomic, qualitative and sensorial characteristics and to increase strawberry fruit nutritional quality [11]. To identify a suitable cultivar for the study, different agricultural ecotypes of strawberries were analyzed: strawberry “Aprica” (Lombardia, North Italy), strawberry “Favetta di Terracina” (Lazio, Centre Italy), strawberry “Mara des Bois” (Calabria, South Italy), commercial strawberry (imported from Spain and available on market place).

For each selected product was evaluated the content of the most representative bioactive compounds in strawberries such as polyphenols, carotenoids and vitamin C. TRAP and FRAP were also evaluated as measure of antioxidant capacity.

In Table 1, “Favetta di Terracina” showed a total antioxidant capacity (FRAP 18.80 ± 0.70 mmol/kg and TRAP 10.02 ± 0.68 mmol/kg) and single antioxidants content (7.10 ± 1.70 mg/100 g, 5.0 ± 0.30 mg/100 g, 1.16 ± 0.40 mg/100 g and 55.7 ± 2.5 mg/100 g for coumaric acid, quercetin, kampferol and vitamin C respectively) higher than “Mara des Bois” (FRAP 17.78 ± 0.43 mmol/kg, TRAP 9.87 ± 0.28 mmol/kg, coumaric acid 1.24 ± 0.20 mg/100 g, quercetin 3.10 ± 0.04 mg/100 g, kampferol 1.56 ± 0.59 mg/100 g, vitamin C 49.8 ± 3.5 mg/100 g) and commercial strawberries (FRAP 19.74 ± 0.68 mmol/kg, TRAP 10.34 ± 0.15 mmol/kg, coumaric acid 1.87 ± 0.84 mg/100 g, quercetin 2.06 ± 0.87 mg/100 g, kampferol 1.14 ± 0.01 mg/100 g, vitamin C 44.2 ± 2.8 mg/100 g). Although “Aprica” showed the highest values as wild ecotype (FRAP 62.85 ± 3.23 mmol/kg, TRAP 16.31 ± 1.20 mmol/kg, coumaric acid 0.80 ± 0.31 mg/100 g, quercetin 2.46 ± 0.45 mg/100 g, kampferol 3.38 ± 0.97 mg/100 g, vitamin C 52.0 ± 3.1 mg/100 g), “Favetta di Terracina” was selected to perform the bioavailability study due to the high values of antioxidant compounds and its characteristic of “product at zero Kilometer”.

As reported in Table 2, test meal chemical characterisation (fresh and stored strawberries) includes total antioxidant capacity values and some antioxidant compounds such as vitamin C, α -carotene for carotenoids and polyphenols as well as anthocyanins.

Several studies have shown that the post-harvest factors and storage conditions not only influence the content and composition of phytochemicals in foods, altering the bioavailable amount, but can also modify the chemical structure of the compound [12]. A lot of factors could modify and/or influence the bioavailability of bioactive molecules in humans. The different bioactive components present in strawberries respond differently to conservation treatment, so the storage effect on bioactive molecules present in the strawberries does not seem negligible.

Our results demonstrated the increase of α -carotene, quercetin and kaempferol in stored strawberries and lower concentration in anthocyanins and anthocyanidins in fresh ones (Table 2), while no significant differences were obtained for ascorbic acid content.

These results are in accordance with literature data reporting different effects of storage on bioactive compounds in berries and berry products; some compounds and antioxidant activities in berry products are reported to be unchanged or even to increase during processing or storage [1, 28, 49]. The observed increase in quercetin and kampferol during the storage period could be directly associated to degradation of cell structures and so to their higher extractability [19].

Regarding biological samples, no significant differences were found in cholesterol and triglycerides plasma levels after consumption of both fresh and stored strawberries (data not shown).

Table 3 shows vitamin A, vitamin E, vitamin C and carotenoids plasma levels ($\mu\text{mol/L}$) upon consumption of fresh and stored strawberries. No significant change due to stored strawberries consumption was found in plasma levels

Table 1
Total antioxidant capacity and bioactive compounds in different ecotypes of strawberries (mean \pm s.d.)

Product	FRAP (mmol/kg)	TRAP (mmol/kg)	Coumaric acid (mg/100 g)	Quercetin (mg/100 g)	Kaempferol (mg/100 g)	Vitamin C (mg/100 g)
Mara des Bois	17.78 ± 0.43^b	9.87 ± 0.28^b	1.24 ± 0.20^b	3.10 ± 0.04^b	1.56 ± 0.59^b	49.8 ± 3.5^{ab}
Aprica	62.85 ± 3.23^a	16.31 ± 1.20^a	0.80 ± 0.31^b	2.46 ± 0.45^b	3.38 ± 0.97^a	52.0 ± 3.1^{ab}
Favetta di Terracina	18.80 ± 0.70^b	10.02 ± 0.68^b	7.10 ± 1.70^a	5.0 ± 0.30^a	1.16 ± 0.40^b	55.7 ± 2.5^a
Commercial	19.74 ± 0.68^b	10.34 ± 0.15^b	1.87 ± 0.84^b	2.06 ± 0.87^b	1.14 ± 0.01^b	44.2 ± 2.8^b

Different superscript letters within each column indicate significant differences by Bonferroni's test: a vs b $P < 0.05$.

Table 2
Total antioxidant capacity and bioactive compounds of the experimental meals

	Fresh Strawberries	Stored Strawberries	Statistic
FRAP ($\mu\text{mol Fe}^{+2}/300\text{ g}$)	6.96 \pm 0.41	6.89 \pm 0.48	n.s.
TRAP (mmol/300 g)	2.65 \pm 0.29	2.85 \pm 0.37	n.s.
α -carotene ($\mu\text{g}/300\text{ g}$)	20.58 \pm 5.48	37.53 \pm 7.73	$P < 0.05$
Vitamin C (mg/300 g)	119.7 \pm 23.2	127.3 \pm 30.2	n.s.
Coumaric acid (mg/300 g)	4.44 \pm 0.93	4.38 \pm 1.52	n.s.
Quercetin (mg/300 g)	10.41 \pm 4.57	19.79 \pm 11.46	$P < 0.05$
Kampferol (mg/300 g)	5.57 \pm 1.71	9.44 \pm 3.63	$P < 0.05$
Cyandin 3-glucoside (mg/300 g)	0.20 \pm 0.22	0.20 \pm 0.05	n.s.
Pelargonidin 3-glucoside (mg/300 g)	8.93 \pm 4.20	6.66 \pm 0.97	$P < 0.05$
Pelargonidin malonil-gluc (mg/300 g)	0.28 \pm 0.32	0.22 \pm 0.12	n.s.
Pelargonidin acetyl-gluc (mg/300 g)	0.16 \pm 0.16	0.11 \pm 0.10	n.s.

n.s. = not significant.

Table 3

Vitamin A, vitamin E, vitamin C and carotenoids plasma levels ($\mu\text{mol/L}$) before and after consumption of fresh and stored strawberries. The results are expressed as mean \pm s.e.m. ($n = 13$)

	Baseline	0.5 (h)	1 (h)	2 (h)	3 (h)	5 (h)	8 (h)
Vitamin A							
Fresh	1.92 \pm 0.13	1.81 \pm 0.11	1.74 \pm 0.08	1.81 \pm 0.11	1.79 \pm 0.12	1.73 \pm 0.12	1.75 \pm 0.11
Stored	1.85 \pm 0.11	1.84 \pm 0.12	1.82 \pm 0.13	1.87 \pm 0.12	1.85 \pm 0.11	1.78 \pm 0.12	1.78 \pm 0.12
Vitamin E							
Fresh	21.1 \pm 1.26	21.3 \pm 1.26	20.7 \pm 1.26	20.7 \pm 1.26	21.9 \pm 1.48	20.9 \pm 1.26	20.9 \pm 1.26
Stored	21.7 \pm 1.26	21.3 \pm 1.26	20.5 \pm 1.48	21.7 \pm 1.26	21.7 \pm 1.48	20.9 \pm 1.26	20.7 \pm 1.26
Vitamin C							
Fresh	47.1 \pm 3.4	49.4 \pm 3.4	54.5 \pm 6.2	66.4 \pm 5.1*	61.3 \pm 5.1*	56.7 \pm 3.4*	52.8 \pm 4.5
Stored	42.0 \pm 2.8	52.8 \pm 2.8*	55.8 \pm 5.1*	65.8 \pm 3.4*	65.3 \pm 4.5*	58.4 \pm 4.0*	51.1 \pm 4.0
Lutein plus zeaxanthin							
Fresh	0.21 \pm 0.02	0.19 \pm 0.01	0.19 \pm 0.01	0.20 \pm 0.01	0.21 \pm 0.02	0.20 \pm 0.02	0.19 \pm 0.02
Stored	0.21 \pm 0.02	0.19 \pm 0.01	0.21 \pm 0.01	0.19 \pm 0.01	0.20 \pm 0.02	0.19 \pm 0.01	0.18 \pm 0.01
Criptoxanthin							
Fresh	0.23 \pm 0.06	0.21 \pm 0.05	0.20 \pm 0.04	0.21 \pm 0.05	0.23 \pm 0.05	0.21 \pm 0.05	0.20 \pm 0.04
Stored	0.21 \pm 0.04	0.20 \pm 0.04	0.22 \pm 0.03	0.20 \pm 0.04	0.19 \pm 0.03	0.20 \pm 0.04	0.18 \pm 0.04
Lycopene							
Fresh	0.82 \pm 0.07	0.77 \pm 0.05	0.80 \pm 0.06	0.79 \pm 0.06	0.79 \pm 0.07	0.78 \pm 0.06	0.80 \pm 0.06
Stored	0.80 \pm 0.07	0.80 \pm 0.07	0.89 \pm 0.10	0.83 \pm 0.06	0.80 \pm 0.06	0.83 \pm 0.06	0.86 \pm 0.07
α -carotene							
Fresh	0.08 \pm 0.01	0.08 \pm 0.01	0.09 \pm 0.02	0.09 \pm 0.02	0.11 \pm 0.02 [#]	0.11 \pm 0.02 [#]	0.10 \pm 0.01 [#]
Stored	0.09 \pm 0.01	0.10 \pm 0.02	0.10 \pm 0.02	0.09 \pm 0.02	0.08 \pm 0.02	0.09 \pm 0.01	0.07 \pm 0.01*
β -carotene							
Fresh	0.42 \pm 0.09	0.39 \pm 0.09	0.43 \pm 0.12	0.40 \pm 0.11	0.45 \pm 0.13	0.43 \pm 0.11	0.39 \pm 0.10
Stored	0.39 \pm 0.08	0.39 \pm 0.10	0.43 \pm 0.10	0.41 \pm 0.09	0.37 \pm 0.08	0.40 \pm 0.08	0.31 \pm 0.07

* $P < 0.05$ vs baseline; [#] $P < 0.05$ fresh vs stored.

of vitamin A and vitamin E. After the consumption of fresh or stored strawberries there was a significant increase in vitamin C during entire study time respect to baseline, while plasma peaks of maximum absorption at 2 hours were significantly higher after eating stored strawberries respect to fresh ones.

Table 4

Plasma levels (mean \pm s.e.m.) of thiol groups (SH), uric acid, Ferric Reducing Antioxidant Power (FRAP) and Total Radical-Trapping Antioxidant Parameter (TRAP) before and after ingestion of fresh and stored strawberries

Time (hour)	Thiol groups ($\mu\text{mol/l}$)		Uric Acid (μM)		FRAP ($\mu\text{mol Fe}^{+2}/\text{l}$)		TRAP (mmol/l)	
	Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored
Baseline	429 \pm 20	428 \pm 22	332 \pm 17	349 \pm 23	911 \pm 152	866 \pm 128	1031 \pm 141	1179 \pm 138 [#]
0.5	406 \pm 20	412 \pm 21	332 \pm 29	349 \pm 29	938 \pm 178	926 \pm 133	1060 \pm 203	1178 \pm 148 [#]
1	432 \pm 30	424 \pm 21	308 \pm 29	361 \pm 35	927 \pm 179	933 \pm 149	1125 \pm 131	1101 \pm 195
2	439 \pm 49	425 \pm 23	314 \pm 17	302 \pm 23	948 \pm 182	933 \pm 138	978 \pm 182	1130 \pm 112 [#]
3	430 \pm 40	422 \pm 21	326 \pm 29	326 \pm 17	940 \pm 193	929 \pm 136	1022 \pm 192	1110 \pm 196
5	431 \pm 39	402 \pm 23	380 \pm 23	355 \pm 17	983 \pm 179*	966 \pm 142*	1087 \pm 219	1116 \pm 188
8	409 \pm 20	410 \pm 21	337 \pm 29	343 \pm 29	968 \pm 173*	966 \pm 136*	980 \pm 188	1005 \pm 193*

* $P < 0.05$ vs baseline; [#] $P < 0.05$ fresh vs stored.

Regarding carotenoids plasma levels upon consumption of fresh and stored strawberries, no differences were present.

After consuming fresh strawberries, plasma levels of α -carotene significantly increased ($P < 0.05$) at 3, 5 and 8 hours time intervals respect to stored ones, while consuming stored strawberries α -carotene plasma values significantly decreased after 8 hours compared with baseline. Our published results [4], examining flavonoid and anthocyanins bioavailability after consumption of fresh and stored strawberries have shown that no traces of quercetin and kampferol were revealed in plasma; no anthocyanins plasma levels were present, while coumaric acid, protocatechuic acid and hydroxybenzoic acid were detected. We observed a decrease of Area Under Curve (AUC) in plasma levels of coumaric acid, hydroxybenzoic acid, protocatechuic acid and in the urinary excretion of metabolite after stored strawberries consumption. In addition we proposed a metabolic pathway of pelargonidin-3-glucoside indicating the 4-hydroxybenzoic acid as a major human metabolite.

Table 4 shows the changes of plasma total antioxidant capacity, thiol groups and urate after eating fresh and stored strawberries. We observed high variability among subjects within the experimental period of the study. FRAP values after consumption of fresh and stored strawberries showed a significant increase ($P < 0.05$) at 5 and 8 hours time interval, while a significant decrease in TRAP values was found ($P < 0.05$) at 8 hours respect to baseline after stored strawberries consumption.

No significant differences were observed in both plasma SH groups and uric acid levels.

An increase of FRAP values was obtained according to vitamin C levels over the study time compared with baseline (Table 3). Moreover simple polyphenols produced by the metabolism of bioactive compounds (anthocyanins, flavonoids, proanthocyanidins) could contribute to increase FRAP values [40]. In addition a not significant increase of uric acid levels (contribute to FRAP around 60%) was present at 5 hours from acute consumption of fresh and stored strawberries.

It was previously shown by many researchers that the FRAP method is unable of assaying either thiol- or carotenoid-type antioxidants [10], probably due to this chemical inertness. As reported by Benzie and Strain [6], the main difference between FRAP and TRAP specificities lies in the FRAP lack of sensitivity for SH groups in antioxidants. On the other hand, the significant decrease of plasma TRAP values at 8 hours could depend on decreasing of single antioxidant including thiol groups, ascorbate and urate plasma levels.

Our results highlight the correlation between plasma endogenous antioxidant and circulating levels of dietary phenolics, underlying the antioxidant action of phenolic metabolites. In addition the results showed that the storage seems to influence the content of bioactive molecules and so their bioavailability.

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