Impact of strawberries on human health: insight into marginally discussed bioactive compounds for the Mediterranean diet

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Abstract

Objective: To review and update the current knowledge on the potential impact of strawberry on human health, with particular attention on compounds and indirect mechanisms of action not exhaustively considered.

Design: Personal perspectives and recent data.

Setting: International.

Results: Our research group was among the few groups that have recently investigated the folate content in fresh, stored and processed strawberries, and the data look very promising. As well, some in vivo evidence of the impact of strawberry intake on the folate status in humans have already been reported, but a new increasing interest on this field is strongly hoped. Furthermore, the hypouricaemic effects previously ascribed to cherry consumption need to be evaluated in respect to strawberry intake. At the moment, inconsistent results come from the few investigations designed at this proposal. In our studies, a great interindividual variability was observed on plasma urate levels in response to strawberry intake, suggesting a putative effect.

Conclusions: The mechanisms responsible for the potential health-promoting effects of strawberry may not be necessarily searched in the activity of phytochemicals. Particularly, a greater interest should be addressed to show whether a prolonged strawberry consumption may effectively improve the folate status and reduce the incidence of folate-related pathological conditions. Furthermore, the hypouricaemic effects of cherries need to be evaluated also in respect to strawberry intake, and the mechanisms of actions and anti-gout potentialities need to be studied in detail.

Future investigations involving human trials should be aimed at following these underestimated scientific tracks.

A growing body of epidemiological studies suggests a consistent association between consumption of diets rich in fruits and vegetables and a lower incidence of chronic diseases, including cancer, cardiovascular and neurodegenerative diseases.

The huge variety of fruits and vegetables available for consumption and their complex composition make difficult the explanation of the potential health benefits, as well as the identification of compounds and mechanisms of action directly implicated in the beneficial effects observed.

In the last few decades, single subgroups of fruits or vegetables have been taken into consideration to facilitate the observation of specific health-promoting or -preventing actions. Furthermore, a deep sifting of the plant compounds potentially expressing biological activities has been conducted, including micronutrients, fiber, minerals and also phytochemicals not designated as traditional nutrients.

Strawberry: an optimal source of phytochemicals

Strawberry (Fragaria×ananassa, Duch.) is among the most popularly consumed berries, both in fresh and processed forms such as jams, yoghurts, desserts or juices. The relevant nutritional value of strawberry fruits has been exhaustively proved10 and correlated to the high content of micronutrients such as minerals, vitamin C and folate which are essential for health, and, more recently, to the high levels and variety of phytochemical constituents.

The potential health benefits of phytochemicals found in strawberries have received ample attention in the recent literature, focusing especially on compounds with
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have been also successfully evaluated for their antiangiogenic and chemopreventive properties, the latter due to the observed ability to inhibit mutagenesis caused by several carcinogens.

Another mechanism of action of strawberry phenolics hypothesised by independent research groups seems to refer to the inhibitory effects of these compounds on enzymes implicated in cancer development, such as phase-II detoxification enzymes and cyclooxygenase enzymes. These findings are of relevant interest when considering that cyclooxygenase enzymes, responsible for the conversion of arachidonic acid to eicosanoids implicated in the development of inflammation, are now supposed to be part of a common pathway in the development of many chronic diseases.

However, most of these enthusiastic findings come from in vitro studies, and little is known on the health effects of strawberry phytochemicals once ingested together with the fruit matrix, absorbed and metabolised. Upto now, few published human studies investigated the metabolism and excretion of strawberry phenolics after ingestion of the whole strawberry fruits. Furthermore, even a future understanding of the mechanisms and entity of tissue distribution and accumulation of phenolics after berry consumption will not ascertain the direct role of these compounds in the health benefits correlated to the whole fruits.

This review has the main objective to emphasise that the mechanisms responsible for the potential health-promoting effects of strawberry may not be necessarily searched in the activity of the deeply studied phytochemicals, and could be more complex and indirect than supposed.

Folate and human health: the strawberry folate as a possible underestimated parameter of nutritional value

Among strawberry micronutrients, the level of vitamin C is generally considered as a parameter of particular interest for the nutritional evaluation of strawberry varieties, and is often included in the pool of chemical measurements conducted for screening purposes and for the evaluation of the breeding strategies.

However, also folate plays a crucial role in emphasising the nutritional value of strawberry, when considering that the fruit is among the richest natural food sources of folate.

The term folate refers to a wide group of derivatives of the water-soluble B vitamin (tetrahydrofolic acid) much more frequent in food and in the human body than the more stable synthetic form, folic acid, used as supplement and in food fortification.

It is known that folate is essential for the human body by acting as coenzymes in mediating the transfer of one-carbon units in many reactions critical to the synthesis and

Strawberry phytochemicals and evidence for biological efficacy

Many studies carried on in vitro suggest that strawberry phenolics show a wide range of biological activities ranging from anticancer to anti-inflammatory, neurodegenerative and antioxidant effects.

The anticancer effects of individual or whole strawberry phytochemicals seem to involve multiple synergistic mechanisms of action, including antioxidant protection of DNA from oxidative damage but also other effects beyond antioxidation. For example, strawberry phytochemicals have been implicated in the inhibition of transcription factors, the modulation of gene expression and cell-signalling pathways which are strictly responsible for suppression of cancer cell proliferation, transformation and tumour progression. Strawberry extracts

have high antioxidant properties such as many phenolic compounds, together with the relevant contribution of vitamin C. In particular, some of the actual trends of the scientific research are strongly focused on obtaining in vitro evidence for the biological efficacy of individual phytochemicals present in strawberries. These molecules, in fact, occur naturally both as glycosylated and esterified forms, and the degree and position of glycosylation and conjugation may strongly influence the size, structure, nature and solubility of the final compounds, therefore affecting their absorption, bioavailability, accumulation, metabolism and excretion in the human body.

Several subclasses of phenolics have been reported and well represented in strawberries. Flavonoids are the main phenolic representative class, and the most abundant compounds belonging to the subgroups of anthocyanins, flavonols, querce tin and kaempferol, and flavanos. In addition, strawberries are extremely rich in phenolic acids such as hydroxycinnamic acid derivatives, and are among the popularly consumed foods with most relevant content of ellagic acid, which occurs both in the free form and mainly esterified to glucose in water-soluble polymeric polyphenolic compounds, the hydrollysable ellagitannins.

A huge number of studies have been recently directed to the identification and quantification of phenolic compounds in strawberries, showing a relevant variation in the phytochemical content and the nutritional quality of the fruits depending on several pre- and post-harvest factors. In any case, comparisons among data still result to be problematic, due to methodological differences and the current lack of agreement in the choice of the adequate method to analyse these compounds.
Rapidly dividing cells are most vulnerable to the consequences of folate deficiency, the reason for which megaloblastic anaemia is one of the earlier signs observed in adults, and a dietary insufficiency of folate causes dramatical pregnancy complications during the fetal growth and development (neural tube defects, certain heart defects, limb malformations, etc.). Furthermore, observational studies have found a correlation between a poor folate status and the incidence of several cancers\textsuperscript{(10)}. Folate coenzymes are, in fact, essential in DNA and RNA methylation, and inadequate folate intake may affect the mechanisms of DNA repair and lead to inappropriate expression of critical genes involved in the etiology of cancer.

Folate is also required for the metabolism of several important aminoacids. In particular, the synthesis of methionine from homocysteine is dependent on a folate coenzyme and a vitamin B\textsubscript{12}-dependent enzyme, and a deficient intake of folate can result in increased levels of homocysteine. It has long been recognised that increased plasma levels of homocysteine are associated with an increased risk of cardiovascular and other chronic diseases, and several observational studies indicated that a prolonged decrease of homocysteine results in a risk reduction. In parallel, the results of many studies have associated folate-rich diets with decreased homocysteine levels in plasma, and a direct correlation between adequate folate intake and lower risk of coronary event was already observed\textsuperscript{(11)}. However, the relationship between the two conditions is still unclear and will need more investigations.

The well-established importance of an adequate daily intake of folate, together with the recent awareness that a genetic variation in folate requirements exist among individuals\textsuperscript{(12)} have emphasised that the recommended dietary allowance (RDA) of folate might have been, for a long time, inadequate in many countries, and sub-optimum for physiological function. In USA, the folate RDA has been recently updated (400 \(\mu\)g/d, for adults) and grain fortification with folic acid is now a successful tool to increase the daily intakes of folate and reach the estimated average requirements. On the contrary, the European folate intake recommendations (200–300 \(\mu\)g/d) are still a probable underestimation of the actual needs, and consumption of natural food source rich in folate would be the better way to prevent folate-related diseases.

**Folate in strawberry: a promising finding**

Today, there are few studies regarding the folate content of strawberries\textsuperscript{(4,13)}, the stability of folate during storage or the evaluation of folate retention after processing\textsuperscript{(14)}. Strålsjö et al. in 2003\textsuperscript{(14)} were the first to address a great interest in the total folate quantification in fresh, stored and processed strawberries. The group emphasised the importance of factors such as the genetic background, the environmental conditions and the year of harvest when evaluating the folate contents in different strawberries, and compared values obtained from fruits of known origin, harvested in different years of production. An optimised and modified commercial radioprotein-binding assay specifically validated for berries was used to measure the folate content in thirteen different Swedish strawberry cultivars harvested in southern Sweden and Poland. The folate levels varied from 30 to 69 \(\mu\)g/100 g of fresh weight (FW), with a mean concentration in all strawberry cultivars of 47 ± 9 \(\mu\)g/100 g of FW (Table 1); water content in the fruits varied between 87 % and 93 %. The values obtained appeared to be comparable to the data reported from previously published studies, where contents ranged from 36\textsuperscript{(15)} to 65\textsuperscript{(16)} \(\mu\)g/100 g of FW, and to values indicated in European food data tables, where folate in strawberries range from 20 to 99 \(\mu\)g/100 g FW. In 2006, our group started the evaluation of folate content in different strawberry varieties and selections cultivated at the experimental field of Marche Polytechnic University\textsuperscript{(4)}. Our study was similarly designed to quantify folate content in fresh strawberries grown at the same field under the same controlled conditions, and to investigate folate retention after storage. We measured as well the variation in water content, which is an important factor to take into consideration when comparing values expressed per FW unit.

The folate levels that we observed are in keeping with values reported previously (Table 1), though we used the microbiological assay as analytical method\textsuperscript{(17)}; also, water content ranged similarly from 87 % to 94 %. Interestingly, higher variation in the folate content was observed among the genotypes analysed, with the selections showing the highest folate contents with respect to all the cultivars studied. Nearly a 7-5-fold difference was measured between the lowest (Adria, 12.8 \(\mu\)g/100 g FW) and the highest value (AN94.414.52, 96 \(\mu\)g/100 g FW), if expressed on a FW basis, with a mean concentration in all strawberry cultivars of 35 ± 27 \(\mu\)g/100 g of FW. The trend maintained when results were compared on a dry weight (DW) basis.

According to the results obtained up to now, the intake of dietary folate through strawberry consumption reveals interesting. For instance, 250–350 g of strawberries (~125 \(\mu\)g folate on average) can supply 40–60 % of the daily European folate intake recommendations (200–300 \(\mu\)g/d)\textsuperscript{(9)} and 30 % of the US recommended daily allowance (400 \(\mu\)g/d). These data look more interesting when highlighting two further considerations. First, the calculations are only based on average values; the strong genetic variation in the folate content found in strawberries should stimulate the agronomic research to specifically define breeding programs, for obtaining and monitoring new folate-enriched selections. Secondly, strawberries reveal optimal folate sources, since often they are consumed fresh or only slightly processed, losses
due to heat or oxidative degradation or leaching are a minor risk. Furthermore, preliminary studies confirm a high retention of folate in strawberry products such as jams or desserts\(^{14}\), showing promising findings for the food industry.

In confirmation of the results observed by Strälsjö \textit{et al.}, also our data indicate a high stability of folate in intact strawberries during a short-refrigerated storage (fruits for 3 d at 4°C and a following day at room temperature)\(^{18}\).

As previously reported for other nutrients, we observed an apparent increase in folate content during storage; the increase cannot be explained by water loss in the fruits during storage, since the water content was measured both in fresh and stored strawberries, and maintained almost practically unchanged. Furthermore, if a water loss in strawberries occurred during storage, the apparent increase should have similarly regarded the other high-to-moderate stable compounds found in the fruits such as ascorbic acid and some phenolic compounds; however, a common increasing trend was not observed.

Certainly, difficulties in comparing the folate content in strawberries from various studies persist, due to the lack of universally accepted analytical methods. For instance, a recent comparative study observed that the folate content in strawberry, on the basis of high-performance liquid chromatography analysis, may be more than three

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**Table 1** Total folate content in different strawberry varieties and selections. Results from different studies

<table>
<thead>
<tr>
<th>Study reference</th>
<th>Cultivar (country)</th>
<th>Days of storage (+4°C)</th>
<th>Harvest year</th>
<th>DM (g/100g)</th>
<th>Folate content (FW μg/100g)</th>
<th>% RDA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holasová \textit{et al.}\textsuperscript{13}\textsuperscript{†}</td>
<td>Elsanta I (Cze Rep)</td>
<td>–</td>
<td>2004</td>
<td>9.7</td>
<td>54 ± 7</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Elsanta II (Cze Rep)</td>
<td>–</td>
<td>2004</td>
<td>8.8</td>
<td>43 ± 4</td>
<td>21.5</td>
</tr>
<tr>
<td></td>
<td>Honeyoe</td>
<td>–</td>
<td>2004</td>
<td>10.0</td>
<td>54 ± 2</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Dukat</td>
<td>–</td>
<td>2004</td>
<td>8.6</td>
<td>36 ± 2</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Dita</td>
<td>–</td>
<td>2004</td>
<td>9.1</td>
<td>33 ± 2</td>
<td>16.5</td>
</tr>
<tr>
<td></td>
<td>Elista</td>
<td>–</td>
<td>2004</td>
<td>9.3</td>
<td>29 ± 1</td>
<td>14.5</td>
</tr>
<tr>
<td></td>
<td>S. Sengana I (Cze Rep)</td>
<td>–</td>
<td>2004</td>
<td>8.2</td>
<td>25 ± 0.4</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>S. Sengana II (Cze Rep)</td>
<td>–</td>
<td>2004</td>
<td>10.6</td>
<td>29 ± 3</td>
<td>14.5</td>
</tr>
<tr>
<td></td>
<td>Carmen (Cze Rep)</td>
<td>–</td>
<td>2004</td>
<td>9.3</td>
<td>37 ± 5</td>
<td>18.5</td>
</tr>
<tr>
<td>Tulipani \textit{et al.}\textsuperscript{15}\textsuperscript{§}</td>
<td>Alba (It)</td>
<td>–</td>
<td>2006</td>
<td>7.2</td>
<td>24 ± 2</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Irma (It)</td>
<td>–</td>
<td>2006</td>
<td>6.9</td>
<td>33 ± 1</td>
<td>16.5</td>
</tr>
<tr>
<td></td>
<td>Patty (It)</td>
<td>–</td>
<td>2006</td>
<td>5.3</td>
<td>17 ± 1</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td>Adria (It)</td>
<td>–</td>
<td>2006</td>
<td>6.0</td>
<td>13 ± 1</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>Sveva (It)</td>
<td>–</td>
<td>2006</td>
<td>6.4</td>
<td>20 ± 2</td>
<td>10</td>
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<td></td>
<td>AN99.78.51 (It)</td>
<td>–</td>
<td>2006</td>
<td>7.2</td>
<td>19 ± 3</td>
<td>9.5</td>
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<td></td>
<td>AN03.338.51 (It)</td>
<td>–</td>
<td>2006</td>
<td>8.9</td>
<td>35 ± 1</td>
<td>17.5</td>
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<tr>
<td></td>
<td>AN94.414.52 (It)</td>
<td>–</td>
<td>2006</td>
<td>12.5</td>
<td>96 ± 10</td>
<td>48</td>
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<tr>
<td></td>
<td>AN00.239.55 (It)</td>
<td>–</td>
<td>2006</td>
<td>8.4</td>
<td>69 ± 3</td>
<td>34.5</td>
</tr>
</tbody>
</table>

Swe, Sweden; Pol, Poland; Cze Rep, Czech Republic; It, Italy; DM, dry matter; FW, fresh weight; RDA, Recommended Dietary Allowance.

*According to the European RDA for adults (200 μg/die).

†Data obtained by radioprotein-binding assay.

§Data obtained by high-performance liquid chromatography analysis after enzymatic treatments.

\textsuperscript{1}Data obtained by microbiological assay.

\textsuperscript{1}According to the European RDA for adults (200 μg/die).
times higher than the content reported by microbiological analysis\textsuperscript{16}. The lack of information on folate content concerns several kind of food plants, and the main limitations to this useful assessment seem to be related to the persistent analytical difficulties encountered, together with unresolved methodological discrepancies. The identification of naturally occurring folates is, in fact, complicated by the large number of possible derivatives present in food (150 different forms of folate exist) and the oxidative instability of the molecules; a variable analytical accuracy is also observed among different methods of measurement generally used.

Therefore, the increasing awareness of the relevance of folate content in strawberry as an interesting parameter of nutritional quality makes strongly desirable, in the near future, the optimisation of accurate and reliable analytical methods of choice, in order to confirm and strengthen the existing data.

Evidence of strawberry consumption and folate status

In the recent past, the first pilot studies that aimed to investigate the possible effects of a prolonged daily consumption of strawberries (a medium serving of 6–8 fruits per day) on plasma folate, homocysteine and C-reactive protein (CRP) levels were carried on. CRP is a general marker of inflammation, but recent research associate increased plasma levels of this protein with higher risk of future stroke, myocardial infarction and other cardiovascular pathologies.

These studies demonstrated that a regular, moderate consumption of strawberries may result in a statistically significant increase in plasma folate levels\textsuperscript{19,20}. On the contrary, the results suggested that the small amount of strawberries provided might not be sufficient to observe significant decreases in serum homocysteine and CRP; and in future investigations human subjects with confirmed elevated basal levels should be chosen for testing.

Furthermore, future human studies similarly designed should include the evaluation of eventual changes in the red blood cell folate concentrations, since this parameter has been shown to better correlate with liver folate stores. A parallel monitoring of the serum levels of vitamin B\textsubscript{12} could be interesting, to check for the presence of suboptimal serum concentrations of vitamin B\textsubscript{12}, which could independently cause a persistent buildup of serum homocysteine.

Strawberry consumption and urate levels: a controversial matter

As previously reviewed, the beneficial effects of fruits have been attributed, in part, to the antioxidant properties of phenolic compounds and vitamin C. The antioxidant capacity of whole fruit extracts (TAC, total antioxidant capacity) has become an important parameter for the evaluation of the nutritional quality of several fruits, and reflects the global concentration of antioxidant compounds in the food matrix.

The consumption of flavonoid-rich foods is almost always associated with substantial increases in plasma or serum TAC. However, the finding is difficult to be reconciled with the small increases of concentrations of flavonoids and other antioxidant phenolic compounds. Interestingly, recent studies have observed an increase in serum concentrations of urate, which is a major contributor to the plasma antioxidant power, in response to the consumption of several kinds of fruits. The increase was surprising since the foods under investigation did not contain uric acid. However, considering the role of the metabolism of fructose in urate endogenous production, recent studies hypothesised that fructose in fruits such as apples may be responsible for the increase in plasma urate and thus, in the antioxidant capacity observed in humans after apple consumption\textsuperscript{21}.

On the other hand, clinical case reports of patients with gout showed that consumption of a daily serving of cherries and cherry products for up to 3 months reduced plasma urate to normal levels and alleviated arthritic pain and gout. It is not known what compounds or which synergistic combination in cherries might be responsible for these actions. Anyway, recent experimental studies conducted to determine the extent of these effects on healthy subjects have confirmed the decrease in plasma urate after cherry consumption, supporting the reputed anti-gout efficacy of cherries\textsuperscript{22}.

Is there any evidence for the effects of strawberry intake on the serum levels of urate? Does strawberry behave like apples or like cherry?

The botanist Linnaeus reported that after eating almost nothing but strawberries, his gout was dramatically relieved. Also, the French herbalist Messegue recommended a strawberry regime lasting several days for individuals suffering from gout or kidney stones.

However, beyond the anecdotal reports the questions are still unanswered. The effect of strawberries and other berries at this proposal is still unclear, and the matter controversial. There is a general underestimation of the importance of these data, confirmed by the limited number of investigations focused on these topics.

Few studies have been conducted to verify the serum level concentration before and after strawberry consumption, but they generally led to inconsistent results\textsuperscript{22,23}, due to the differences encountered among the experimental designs and the analytical methods used. Our group carried out sequential human studies inviting volunteers to acute and prolonged consumptions of relevant amount of strawberries (Table 2)\textsuperscript{24}. We observed statistically significant increases in the plasma...
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Table 2  Plasma total antioxidant capacity measured by FRAP and TEAC assays, and RP-HPLC serum ascorbate and urate levels during the strawberry consumption studies carried out by our group. Results are expressed as mean values and SEM (n 11 in study 1; n 12 in study 2).

<table>
<thead>
<tr>
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<th>Study 1: acute consumption of strawberries</th>
<th>Study 2: prolonged consumption of strawberries</th>
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<tr>
<td><strong>Experimental design</strong></td>
<td>After a fasted night, eleven healthy subjects consumed 1 kg of strawberries in 10 min. Heparinised plasma was obtained immediately before (baseline) up to 3 h after the consumption of the fruits</td>
<td>Twelve healthy volunteers consumed 500 g of strawberries per day, for 16 d, preferably at mid-morning and mid-afternoon between meals. During the study, the subjects were invited to maintain their normal dietary habits, to daily update a personal diet diary and to reduce the intake of polyphenols-rich food the dinner before each blood sampling. Blood was obtained after an overnight fast in two baseline timepoints, after 4, 8, 12, 16 d of strawberries supplementation and 1 month after the end of the study (wash-out)</td>
</tr>
<tr>
<td><strong>Timepoints</strong></td>
<td>Baseline</td>
<td>1 h</td>
</tr>
<tr>
<td>FRAP (μmol TE/l)</td>
<td>34.8 ± 3</td>
<td>40.0 ± 2*</td>
</tr>
<tr>
<td>TEAC (mmol TE/l)</td>
<td>3.2 ± 0.1</td>
<td>3.2 ± 0.1</td>
</tr>
<tr>
<td>Ascorbate (μmol/l)</td>
<td>28.8 ± 1</td>
<td>49.5 ± 1*</td>
</tr>
<tr>
<td>Urate (μmol/l)</td>
<td>124.4 ± 13</td>
<td>124.5 ± 13</td>
</tr>
</tbody>
</table>

FRAP, ferric reducing antioxidant power; TEAC, trolox equivalent antioxidant capacity; RP-HPLC, reverse phase high-performance liquid chromatography; TE, trolox equivalents.

*Significantly different from baselines, P<0.05.

Acknowledgements

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In the acute study, we measured the serum urate uptake 3 h after the consumption of strawberries. Therefore, data from the current studies cannot definitively exclude a putative hypouricaemic effect from strawberry intake. A putative hypouricaemic effect from strawberry intake might be reflected in the relationships of serum folate, plasma homocysteine, and serum urate levels. The present work had the main objective to emphasize the potential long-term hypouricaemic effect of strawberries on subjects with high basal levels of urate in serum.

However, the high content found in strawberry, but no significant increase in the serum folate levels of homocysteine, and whether increasing dietary folate intake actually results in decreased risk of birth defects and adult cardiovascular, neurodegenerative and proliferative diseases.

Secondly, the hypouricaemic effects previously ascribed to the consumption of berry fruits need to be confirmed for anti-folate potentialities and studied in detail, in order to benefit of these relevant findings.

In conclusion, we observed that the mechanisms responsible for the potential health-promoting effects of strawberry may be not necessarily in the activity of the deeply studied phytochemicals or micronutrients such as vitamin C, and could be more complex and indirect than supposed. Therefore, data from the current studies cannot definitively exclude a putative hypouricaemic effect from strawberry intake.
References


