Potential for engineering horticultural crops with high antioxidant capacity

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Abstract

Cellular oxidation index has emerged as an important determinant in defining the fate of a living cell and its susceptibility to disease. Oxidative stress targets include oxidation of DNA, protein and lipids; causing cellular oncogenesis, chronic diseases and premature senescence. The possibility that dietary intervention via nutrition-enriched food may significantly decrease incidence of diet-related diseases has catalysed scientific efforts to understand this relationship, which is fundamental to developing future strategies for stemming disease. Multiple and synergistic interactions among nutrients influence antiproliferative activity of a fruit compared with an isolated antioxidant. Nutritional molecules including vitamins (B, C, E and β-carotene), folates, lycopene, flavonoids, isothiocyanates, glucosinolates, polyphenols, glutathione and minerals contribute to the antioxidative capacity of vegetables, fruits, nuts and various herbs. The true potential of a supplemental antioxidant or crop nutrients in human health benefits is in their accessibility, bioavailability and biological potency. Studies on the absorption, metabolism and in vivo potency of phytonutrients have lagged behind and are only now beginning to provide some results. Also, it is important to bear in mind that the levels of phytonutrients present in horticultural crops are low and significantly influenced by genotype/cultivar, growth condition and developmental stage. In this regard, genetic engineering has become a refined tool to increase the antioxidant and nutrient capacity of economically important crops including fruits and vegetables to the levels favourable not only for a highly nutritional diet but also to enable in-depth studies on the relationships between diet, genetics and metabolism. Together with modern biotechnology, deciphering transcriptome–proteome–metabolome of the new transgenics should provide new knowledge to ease the concerns of the society and open the market for genetically engineered horticulture crops, as is seen by higher sales of Hawaii-grown transgenic papaya in the USA. Also, this knowledge will help us in developing precise strategies for redesigning metabolic pathways so that desired levels of a particular phytonutrient (antioxidant) in crops are achieved.

Keywords: Ageing, Antioxidants, Diet-related diseases, Fruits, Gene constructs, Gene expression, GM fruit, Regulatable promoters


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Introduction

Cellular oxidation index has emerged as an important determinant in defining the fate of a living cell and its susceptibility to disease. Thus, uncontrolled generation of reactive oxygen species (ROS), which have the potential to increase the cellular oxidation index, can lead to deleterious biological phenomenon first explored in the ageing process [1, 2]. ROS are highly reactive free radicals that include singlet oxygen, superoxide, hydroxyl radicals and hydrogen peroxide [3–5]. At high concentrations, they oxidize DNA, protein and lipids, which can lead to cellular oncogenesis, senescence and death (apoptosis) [3, 6, 7]. Examples include neurodegenerative diseases associated with oxidative stress-mediated mitochondrial dysfunction [8, 9], diabetes mellitus disease associated with glucose oxidation [10], protein oxidation-associated Alzheimer’s disease, chronic lung disease, chronic renal failure and sepsis [11]. Compelling data have been obtained on the involvement of mitochondria-mediated oxidative stress in the development of diabetic neuropathy [12].

ROS are mainly produced in mitochondria and peroxisomes, and as by-products of various biosynthetic pathways during normal cellular metabolism. To combat oxidative stress during active metabolism, human body has in place intricate defence systems in the form of enzymes such as superoxide dismutase (SOD), glutathione peroxidase, catalase and peroxiredoxins (Prx), which act as intracellular scavengers to maintain the cellular homeostasis and thereby protect cells [3]. However, this defence system can become inefficient with ageing making cells prone to diseases. The realization that certain nutrients when consumed in specific quantities contribute to optimal health has given credence to the concept that dietary quality is intertwined with disease [13]. The prospect of targeting specific dietary treatment to those predicted to gain the most therapeutic benefit clearly has important clinical and economic consequences, particularly in diseases of high prevalence such as coronary artery disease, hypertension, diabetes, osteoporosis and possibly cancer [14]. Numerous epidemiological and clinical research studies have implied the promise of diets rich in vitamins, minerals and other phytonutrients in lowering the risk of cardiovascular diseases and mortality [15–17]. A review of approximately 200 dietary studies came to the conclusion that fruit and vegetable consumption led to a statistically significant protective effect against various types of cancers [18]. Further, people with a lower intake of fruits and vegetables were found to be at twice the risk of experiencing cancer than those that consumed a higher proportion [18]. Fruit and vegetables are dietary sources of phytonutrients with antioxidant properties that potentially help in reducing the risk of chronic diseases. Therefore, it is understood that while the human health disorders are basically genetic, dietary intervention via nutrition-enriched food can significantly be a preventive strategy to decrease incidence of diet-related diseases.

The beneficial effects of a nutritive diet seem registered via interactions of several antioxidant components present in food, although the nature of these interactions is still a matter of conjecture [19, 20]. Interestingly, such interactions may be determined by the nature of antioxidant response elements (AREs) present in the human genome and are polymorphic [21]. AREs are cis-acting enhancer sequences found in genes encoding antioxidant and detoxifying enzymes [21]. Oxidative stress causes binding of the transcription factor NRF2 (nuclear factor erythroid-derived 2-like 2) to AREs, which mediates activation of the responsive gene(s) and thereby switching on defence mechanisms against oxidative damage. This study also identified a set of ARE single nucleotide polymorphisms (SNPs) that could potentially modulate NRF2-mediated responses, and perhaps also the gene–antioxidant interactions. These developments may explain why a wide variation has been observed in preclinical and clinical studies on responsiveness to food components as modifiers of human health, including cancer risk and tumour. These inconsistencies are likely a reflection of the multifactorial nature of signals that determine the specificity and temporal relationships between dietary constituents as modifiers of molecular events. The identification of molecular sites and elucidation of the corresponding variation in phenotype affected by signals around these sites are critical for identifying those who will benefit or be placed at disease risk. There is therefore a need for a better understanding of the relationship between dietary intervention and disease progression to develop fundamental understanding of disease-related process(es) impacted by one or more dietary constituent [22]. Thus, functional genomics research encompassing nutrigenomics and nutrigenetics will contribute in deciphering not only the functional roles of genes but also to understand how a particular diet or a component thereof influences a particular disease. The focus of nutrigenomics is on cross talk between nutrients and metabolic pathways [23], whereas nutrigenetics is devoted to studies on how the genetic makeup of an individual responds to a specific diet [24]. Advances in our understanding of the interactions between lifestyle and genotype in contributing to health and disease are expected to take us a step closer to achieving the highly desirable goal of personalized nutrition (see [13]).

Thus, we are in an exciting era with advanced technologies available to unravel the potential of interdependence between dietary constituents and prevention of disease. The wide spectrum of the disciplines and research areas involved in the subject matter put constraints on writing an exhaustive review or to do justice to the literature. However, we have attempted to bring a cohesion between the subjects covered to provide a common theme on the nutritional and antioxidant capacity of horticultural crops, nutritive antioxidants, the potential of engineering enhanced nutritional quality in
edible produce, and the contribution of nutritional and quality diet in enhancing quality of life by reducing disease.

**Oxidative Stress, Ageing and Disease**

Ageing and some diseases produce harmful amounts of ROS that cause oxidation of macromolecules impairing their function. ROS are natural by-products of cellular metabolism and in physiological amounts also act as signalling molecules to protect living cells including providing defence against pathogens. In order to contain the autocatalytic nature of ROS to propagate chemically, living cells have developed well-defined antioxidant mechanisms that mitigate the uncontrolled consequences of ROS multiplication. In instances when the antioxidant machinery fails to neutralize the excessive accumulation of ROS and ROS production continues unabated, and when the endogenous level of antioxidant molecules is below the threshold, cells/tissues suffer injury and become prone to pathogenesis [11, 25–28].

Antioxidant cellular machinery consists of enzymes such as SOD, glutathione peroxidase, catalase and Prx, which scavenge ROS. SOD catalyses the conversion of superoxide to freely diffusible hydrogen peroxide, whereas catalase and glutathione peroxidase convert hydrogen peroxide to water [3]. Prx scavenge peroxides by reducing hydroperoxides and peroxynitrils [29]. As a proof of the concept, Cu²⁺/Zn²⁺ SOD1 was over-expressed in transgenic mice with the result that oxidative injury to cytosolic and mitochondrial proteins was reduced [27]. These authors suggested that SOD1 over-expression reduces direct oxidation of X-chromosomelymphed inhibitor-of-apoptosis protein (XIAP) regulators in mouse brain. They later showed that SOD1 overexpression also reduced the oxidative reaction to phosphorylated proline-rich Akt substrate (pPRAS) that regulates in vitro-induced apoptosis [28].

The free-radical theory of ageing [30] enunciated that ageing caused accumulation of metabolic errors and free-radical damage is a key catalyst in the degeneration of aged tissues. However, until recently, experimental evidence in support of the theory that actual damage caused by free radicals limited the life span of mammals was scanty. To test this hypothesis, catalase gene was over-expressed in the mitochondria of an animal model mouse to study the effect on the life span of the mouse. It was demonstrated that specific targeting of the antioxidant catalase, in this instance to mitochondria, boosted antioxidant index and increased the life span of the animal [31]. Similarly to these studies, oxidative stress in brain damage (and other diseases) and effectiveness of antioxidants have been the subject of numerous papers [32–34]. Strong correlative data based on biomarkers of oxidative damage have pinpointed that diseases that include some types of cancer, hepatic cirrhosis, Alzheimer’s disease, hypercholesterolaemia, rheumatoid arthritis and cystic fibrosis are promoted by oxidation of cellular macromolecules [35–39]. Further, it is thought that specific dietary regimes or supplementation with antioxidants are useful in lowering disease risk and treating such diseases (reviewed in [39, 40]). However, research is needed to determine the right dosage or recommended daily allowance (RDA) for each nutrient (antioxidant), since excessive intake of antioxidants may negatively affect cellular redox homeostasis and interfere with the signalling function(s) of ROS.

Although positive effects of using antioxidant supplements in preventing the onset of age-related and other diseases have been demonstrated in a number of investigations, some dissenting reports have claimed that supplements do not provide protection against oxidative damage in vivo [41–45]. It was found that β-carotene, vitamins A, C and E, or selenium supplementation were not preventive of gastrointestinal cancers, and instead increased mortality was recorded [46]. It was argued, therefore, that antioxidant supplements be considered as medicinal products and be disbursed selectively [46]. However, yet another study concluded that β-carotene supplementation does decrease the risk of developing cancer [47]. It is important to note, in this context, that under certain conditions, a particular antioxidant may not be effective in neutralizing oxidative damage, and that the very nature of antioxidants can make them pro-oxidants under some conditions, particularly in the case of polyphenols [42, 43].

There may well be alternative mechanisms, other than their antioxidative property, by which antioxidants reduce or promote the health risk caused by oxidative stress. One emerging concept is that some antioxidants such as flavonoids may also act as signalling molecules and impact signalling cascades involving reversible phosphorylation. Thus, flavonoids or their metabolites may modulate cellular metabolism differently dependent upon their intracellular concentration – at a low level they might affect ARE-mediated gene expression, while at a higher level cause sustained activation of mitogen-activated protein (MAP) kinases and thereby induce apoptosis [48]. This may also be relevant in context of the findings that a synergistic relationship among different antioxidants (phytonutrients), present in dietary vegetables and fruits, has been proposed to be the reason for their beneficial effects on human health (see below [49]).

**Plant Antioxidants, Antioxidation Index and Factors Involved**

Phytonutrients in fruit and vegetables seem to have synergistic effects on the antioxidant activities and when consumed as part of a diet greatly reduce the risk of chronic diseases, in particular cancer and heart disease [41, 42, 50, 51, 52]. The potential of antioxidants in prolonging life and preventing disease incidence in humans has
intensified research on analysing and enhancing nutritional antioxidant metabolites in food crops. Vegetables, fruits, nuts and various herbs are dietary sources of one or more of the following antioxidants: vitamins (C, B, E and β-carotene), folates, minerals and other phytonutrients (lycopene and polyphenols). Nutritional molecules such as carotenoids, lycopene, flavonoids, vitamin C, isothiocyanates, glucosinolates, pyruvate and glutathione may prevent chronic diseases, including epithelial cancers, cardiovascular diseases, digestive disorders and immune deficiency [53–55] through their characteristic of being non-enzymatic ROS scavengers [3, 56]. A brief description of some of these antioxidants is as follows.

**Carotenoids**

It comprises a class of plant pigments responsible for yellow to red colour of fruits and vegetables, representing more than 600 structurally different molecules. Epidemiological studies have shown an inverse relationship between disease status and the serum level of carotenoids in human body – making carotenoids potential antioxidants with anticarcinogenic activity [57–60]. Other antioxidant carotenoids, lutein and xanthin (xanthophyll) referred to as macular pigments exhibit blue-light filtering activity [61–63]. Lycopene is an unsaturated long-chain carotenoid, a potent antioxidant whose dietary sources include tomato, watermelon, pink grapefruit, apricots and pink guavas [55, 64]. It has greater capacity among the carotenoids to quench singlet oxygen [65, 66]. Lycopene feeding has been found to be associated with reduced risks for developing breast cancer [67, 68], prostate cancer [69] – by modulating androgen activation [70] and coronary heart disease [71, 72].

**Polyphenols**

The second most studied group of phytochemicals after the carotenoids, phenolics include flavones, flavonols, flavanols, isoflavones, flavanones, chalcones, cinnamic acid, anthocyanins and stilbens present in fruits and vegetables. These nutrients show chemoprotective activities [73] and profound effects on neurodegenerative diseases such as Alzheimer’s, Parkinson’s and Huntington’s [74, 75]. Cellular antioxidant activity (CAA) of fruits is significantly correlated to high phenolic content [76]. A normal diet draws two-third of the polyphenolics from flavonoids and the remaining one-third from other phenolic constituents [64]. Although it has been assumed that health-promoting effect of polyphenols is because of their strong antioxidant activity, it is probable that they or their metabolites exert the effects by acting as signalling molecules and modifying kinase cascades that then cause downstream signalling [48]. Indeed, increased plasma antioxidant activity after intake of flavonoid rich diet is caused by uric acid and not the flavonoids themselves [77].

**Stilbenes**

Stilbenes are stress metabolites and known phytoalexins [78]. Resveratrol, a naturally occurring stilbene, has antioxidant activity and is present in high amounts in blueberry and grapes [79, 80]. Resveratrol has a cardioprotective role, exerting a survival signal at 2.5–5 mg/kg (lower doses) by inducing Akt1 and Bcl2 anti-apoptotic protein expression while an increase in resveratrol dose, i.e. >25 mg/kg, led to upregulation of redox proteins and pro-apoptotic protein [80].

**Minerals**

Minerals, e.g., selenium, manganese, copper and zinc, also contribute towards antioxidant potential, primarily as cofactors for antioxidant enzymes [81]. Selenium as a cofactor for glutathione peroxidase has the property of balancing the redox homeostasis during lipid peroxidation by recovering the glutathione peroxidase activity and reducing the peroxidized protein product [82].

Methods employed to evaluate antioxidant capacity of fruits include (Table 1): oxygen radical absorbance capacity (ORAC) [83, 84], the 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) equivalent antioxidant capacity (TEAC) [85, 86], ferric reducing ability of plasma (FRAP) [87] and total antioxidant oxy-radical scavenging assay (TOSC) [88, 89]. The ORAC and TEAC assays measure the ability of an antioxidant to react with or neutralize free radicals. FRAP assay measures the reduction of ferric ion (Fe³⁺) to ferrous ion (Fe²⁺) in the presence of antioxidants. In the TOSC assay, peroxo radicals generated by thermal hydrolysis of 2,2-azobis (amidinopropane) (ABAP) results in the oxidation of α-keto-γ-methylthiobutyric acid (KMBA) to ethylene, which is then quantified by gas chromatography; antioxidants compete with KMBA for oxyradicals and cause partial inhibition of ethylene formation. Lipid peroxidation is measured by the levels of thio-barbituric acid reactive substances (TBARS), present either in plasma or in low-density lipoprotein [90].

Total antioxidant capacity of fruits and berries analysed by various methods are compared in Table 1. Berries, strawberry, plum, kiwifruit and peach prominently feature as having high antioxidant capacity [89, 91–97]. However, it is important to note that antioxidant capacity of fruits is strongly influenced by genotype/cultivar, growth condition and environment [92, 96, 98, 99]. Wide variations also exist in the content of lycopene, ascorbic acid (vitamin C) and phenolics in different tomato genotypes, at times values being higher in the peel tissues than the pulp [100]. The phenolic (flavonoid and capsaicinoid) content of hot pepper is affected by the colour and maturation stage, the antioxidant activity at red stage being greater than the green mature stage [101]. Likewise, antioxidant capacity associated with stilbenes (resveratrol, pterostilbene and piceatannol) in various selections and cultivars of berries from USA and Canada indicated highest resveratrol content in grapes and berries [102].
analysis highlights the importance of taking into consideration the fact that antioxidant capacity of fruits significantly varies between genotypes, species and developmental stage, which can affect their nutritive value.

Serving size of fruits and vegetables has a direct relevance to the quantity of dietary antioxidants consumed and the total antioxidant capacity this would provide. For example, cranberries with the smallest serving size (55 g) provide the largest amount (373 mg/serving) of phenolics while watermelon provides only 183 mg of phenolics per a serving size of 286 g [103]. Other studies showed that even though phenolic content of apples is in the medium range, their per capita consumption is higher and thus they are the largest contributor of total phenolics consumed by the Americans (see [97]). Epidemiological studies have supported the age-old adage of 'an apple a day keeps the doctor away' and shown that daily consumption of apple is healthy and reduces the risks of lung and colon cancer [104]. Apples are a rich source of a variety of polyphenolic compounds, e.g. flavonols, cinnamic acid, flavanols, dihydrochalcones and anthocyanins [103, 105, 106]; epicatechin and procyanidines polyphenolics are major contributors to the antioxidant capacity of the apple fruit.

### Table 1 Total antioxidant capacity of some fruits as measured by different methods

<table>
<thead>
<tr>
<th>Fruit</th>
<th>FRAP value (mmol/100 g FW)</th>
<th>TEAC value (µmol TE/g FW)</th>
<th>ORAC value (µmol TE/g FW)</th>
<th>TOSC value (µmol vitamin C equivalent/g)</th>
<th>Antioxidant content (mmol/serving)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blackberry</td>
<td>3.99&lt;sup&gt;1&lt;/sup&gt;, 5.07&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td>5.75</td>
</tr>
<tr>
<td>Thornless Blackberry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green</td>
<td>23.4–28.8&lt;sup&gt;4,7&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>177.0&lt;sup&gt;5&lt;/sup&gt;</td>
<td>3.13</td>
</tr>
<tr>
<td>Pink</td>
<td>13.7–15.4&lt;sup&gt;4,7&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>64.4&lt;sup&gt;5&lt;/sup&gt;</td>
<td>3.58</td>
</tr>
<tr>
<td>Cranberry</td>
<td>3.29&lt;sup&gt;1&lt;/sup&gt;, 5.03&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raspberry</td>
<td>2.33&lt;sup&gt;1&lt;/sup&gt;, 3.06&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strawberry</td>
<td>2.16&lt;sup&gt;1&lt;/sup&gt;, 2.17&lt;sup&gt;2&lt;/sup&gt;</td>
<td>13.17&lt;sup&gt;3&lt;/sup&gt;</td>
<td>12.2–17.4&lt;sup&gt;4,7&lt;/sup&gt;</td>
<td></td>
<td>134.1 (Earliglow)&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>Wild strawberry</td>
<td>6.88&lt;sup&gt;2&lt;/sup&gt;</td>
<td>33.17&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blueberry</td>
<td>2.15&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td>2.68</td>
</tr>
<tr>
<td>Kiwi fruit</td>
<td>1.33&lt;sup&gt;1&lt;/sup&gt;, 0.91&lt;sup&gt;2&lt;/sup&gt;</td>
<td>2.72&lt;sup&gt;3&lt;/sup&gt;</td>
<td>6.02&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
<td>0.99</td>
</tr>
<tr>
<td>Apple fruit</td>
<td>0.31&lt;sup&gt;1&lt;/sup&gt;, 0.29&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1.60&lt;sup&gt;3&lt;/sup&gt;</td>
<td>2.18&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
<td>98.6&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Apricot</td>
<td>0.52&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1.39&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peach</td>
<td>1.061–1.248&lt;sup&gt;1,7&lt;/sup&gt;</td>
<td>1.22&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>49.5&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plum</td>
<td>1.06&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td>9.49&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pineapple</td>
<td>1.04&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mango</td>
<td>0.35&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomato</td>
<td>0.31&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Banana</td>
<td>0.2&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td>1.89&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pear</td>
<td>0.18&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td>2.21&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melon</td>
<td>0.15&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td>1.34&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Watermelon</td>
<td>0.04&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td>0.97&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: As a comparison, FRAP value for ground cloves is 125.55 mmol/100 g FW [94].

1According to Halvorsen et al. [94].
2According to Halvorsen et al. [93].
3According to Scalzo et al. [96].
4According to Wang et al. [91].
5According to Sun et al. [113].
6According to Meyers et al. [95].
7Range of values obtained from analysis of different genotypes.

### Antiproliferative Activity Involves More Than One Antioxidant Class

A number of examples attest to a linear relationship between the content of a class of plant nutrients and total antioxidant capacity. Thus, fruits containing higher total phenolic content also show a higher total antioxidant activity [89, 101]. It is also apparent that although antioxidant quality is a measure of the fruit type and its nutrient composition, a synergistic relationship exists among antioxidants present in a mixture [103]. However, as mentioned above, antioxidant capacity of fruits and vegetables is also a function of species, cultivar, developmental stage and growth environment [92, 96, 98, 105, 107–109]. Further, modification of nutritional molecules, for instance, via substitution, glycosylation, or conjugation with other molecules, may introduce another caveat in determining the potential of an antioxidant, which is
particularly applicable to phenolic compounds [110]. A comparison of antioxidant activity of fruit and vegetable extracts indicated that the nature of antioxidants in fruit made them superior to vitamin antioxidants (ascorbate and β-carotene) present in vegetables or to pure phenolics [103].

Proliferation of cells is a characteristic feature of malignant cells. Thus, antiproliferative activity of antioxidants and fruits is commonly used to seek potential anti-cancer agents. Resveratrol was found to have growth inhibitory and antiproliferative activity when tested on in vitro on human promyelocytic leukaemia (HL-60) cells, inducing apoptosis [111]. Other in vitro experiments with different cell lines indicated that pomegranate juice had higher bioactivity than individual polyphenols extracted from the fruit [112]. Likewise, a number of fruit extracts were tested on in vitro proliferation of HepG2 human liver-cancer cells, and showed cranberry to have high inhibitory activity followed by lemon, apple, strawberry, red grape, banana, grapefruit and peach [113]. However, there was no tight correlation between the antiproliferative effects of fruit extracts and their antioxidant capacity. These investigators suggested that a specific phenolic compound or a class of phenolics in fruit is responsible for their antiproliferative activity.

Other studies also did not find a correlation between total phenolic or flavonoid contents of a fruit and its antiproliferative activity. For example, total antioxidant capacity (from total phenolics and flavonoids) in fruit extracts of eight different strawberry cultivars was not correlated with their antiproliferative activity [95]. Similar conclusions were arrived at from studies with raspberries [114]. Like the variability in the antioxidant capacity of fruits as a function of species, cultivar, developmental stage and growth environment, antiproliferative activity of strawberry fruits was also found to be greater in fruit immediately after harvest than after storage [115]. Thus, among other things that may also be involved, multiple and synergistic interactions among chemicals promote antiproliferative activity of a plant compared with an isolated antioxidant [51, 64, 112].

In vivo and in vitro studies have shown that antiproliferative activity of ascorbic acid likely results from inhibition of expression of genes involved in cell division [116]. An antioxidant index consisting of α-tocopherol, ascorbic acid and β-carotene was correlated to age-related macular degeneration, suggesting that high plasma levels of α-tocopherol can be potentially protective against this disease [117]. Changes in the antioxidant capacity in blood plasma of the test subject before and after feeding a potential antioxidant nutrient or fruit can provide direct information on the effective bioavailability of an antioxidant chemical or antioxidant-rich fruit. Thus, plasma antioxidant capacity has been considered a kinetic biomarker of phenolic bioavailability [110]. Interestingly, polyphenols [118, 119] and dietary flavonols [120] have been found to bind human lower density lipoproteins (LDL), cellular oxidation of which has been considered a key step in atherogenicity [121]. Enrichment of LDL levels and their protection against oxidation were positively correlated with the intake of grape juice [122, 123], tea or red wine [124], or an antioxidant nutrient like vitamin E (tocopherol) [125]. Similarly, processed tomato products are good bioavailable sources of lycopene, whose intake causes protection of plasma lipoproteins, lymphocyte DNA and serum proteins against oxidative damage. This protection is linked to increases in plasma lycopene levels, subsequently leading to reduction of prostate-specific antigen, upregulation of connexin expression and decrease in prostate tumour proliferation – all anticarcinogenic effects [55]. Fruit consumption has great potential in protecting LDL from oxidation and thus has tremendous health value [103]. It is important to note that this obvious potential is likely to be realized by increasing the levels of antioxidants in horticultural crops, either via marker-assisted breeding strategies or by genetic manipulation (see below). Positive affirmation also comes from a study that demonstrated the antioxidant potential of red apples with skin on: 100 g of these were found to be equivalent to 1.5 g vitamin C [126].

**Genetic Engineering to the Rescue of Enhancing Antioxidants and Nutrients in Produce**

Factors that are critical in determining whether or not a dietary intervention will help conquer a diet-related disease include: (a) the knowledge on the quantity required of each antioxidant nutrient, commonly known as the RDA; (b) the bioavailability of nutrients; (c) novel genotypes of foods, e.g. fruits and vegetables, enriched in a particular nutrient or a group of nutrients with high antioxidant potential (summarized in [40, 127]). Other limitations in our knowledge needed to help biologists, nutritionists and physicians in alleviating diet-related diseases include: (a) nutritional education not in tune with new technology to enable nutritionists to develop applications for nutrigenomics and nutrigenetics; (b) not many nutritionists interact and work interdisciplinarily with geneticists, molecular biologists and bioinformaticians in the development of research strategies and (c) nutritional recommendations are not based on metabolic outcomes [128]. The refined tools of genomics, proteomics and metabolomics are now in vogue to enable nutritionists to develop in-depth studies on the relationships between diet, genetics and metabolism.

Although vegetables and fruits are common sources of phytonutrients, the levels may not be sufficient in a fruit of one species to fulfil the RDA in a full or complete diet. Moreover, the ‘green revolution’, which encompassed developing improved germplasm and optimizing growing conditions, led to increased crop production and contained losses from pests [129–131]. However, types of antioxidants present and their content in the developed germplasm, in most cases, were not determined.

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Therefore, in traditional breeding research, nutritional quality attributes got left far behind those of yield and biotic resistance. Increased public awareness of nutritional benefits of antioxidants to human health has produced a demand and opened up a market for specialty, highly nutritious crops. Thus, definitive basic information on quality attributes in horticultural crops has just begun to emerge. A better understanding of the basic metabolism and key processes involved is needed to enable scientists to develop strategies for specifically improving the nutritional quality of crops including fruits. How each phytonutrient level is regulated is a science we know little about! Also, unambiguous quantification of phytonutrients requires collaboration with expert chemists/biochemists, investment of additional resources and appropriate equipment.

The antioxidant activity and nutritional quality of fruits in human diet vary with the species, cultivar, amount as well as various tissues of the fruit (e.g. peel, flesh and other tissues) and form of consumption (fresh, juice or processed) [98, 132, 133]. The recognized phytonutrients are neither concentrated in a single crop nor are their levels in diverse crops at par with the prescribed RDA were established. Attempts have been made to biofortify food with increased antioxidant levels by using traditional breeding strategies [134–136]. These methods involve crosses and back crosses for several generations which is a highly time consuming process and require high genetic variations of the trait and heritability [137]. A recent experiment highlights other limitations of conventional breeding approach, to improve flavonoid content in tomato. In cultivated tomato, the expression of a key gene for the production of flavonoids, viz., the chalcone isomerase (CHI) gene expression is blocked in the peel and suppressed in the pulp affecting the production and accumulation of flavonoid–quercetin. Wild *Lycopersicon* accessions do express the CHI gene and one of the lines was crossed with a cultivated tomato species to develop a non-transgenic, metabolic strategy to produce flavonoid-rich hybrids [138]. Indeed, hybrid tomato developed by such a cross showed 11.8-fold increase in flavonoid–quercetin in F1 hybrids (0.33 mg flavonoid/g fruit). However, further progeny analysis was not possible because the hybrids were invariably seedless. Another study [139] used light reflective mulches to enhance the ellagic acid (polyphenol) and ascorbic acid contents in strawberry. The ellagic acid was found to be 60–341 mg/g frozen weight (FW). However, it is difficult to assess the actual increase because the units these authors used are not comparable with a range of 0.16–2.07 mg/g dry weight generally present in strawberry (Table 2).

### Table 2 Phytonutrient levels in various fruit crops

<table>
<thead>
<tr>
<th>Fruit crop</th>
<th>Cultivar/variety</th>
<th>Nutrient</th>
<th>Concentration or fold range</th>
<th>Antioxidant activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raspberry</td>
<td>5</td>
<td>Anthocyanin Phenolics</td>
<td>454–1972 µg/g FW 2060–2670 µg/g FW</td>
<td>15.9–28.2 µmol TE/g (ORAC)</td>
<td>92</td>
</tr>
<tr>
<td>Blackberry (thornless)</td>
<td>3</td>
<td>Anthocyanin Phenolics</td>
<td>1335–1716 µg/g FW 2040–3260 µg/g FW</td>
<td>20.3–24.6 µmol TE/g (ORAC)</td>
<td>92</td>
</tr>
<tr>
<td>Strawberry</td>
<td>8</td>
<td>Anthocyanin Phenolics</td>
<td>233–453 µg/g FW 950–1520 µg/g FW</td>
<td>12.2–17.4 µmol TE/g (ORAC)</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Anthocyanin Flavonol Phenolics</td>
<td>4-fold 2.7-fold 1.8-fold</td>
<td>nd</td>
<td>230</td>
</tr>
<tr>
<td>Tomato</td>
<td>12</td>
<td>Lycopene Vitamin C Phenolics</td>
<td>48.3–141 µg/g FW (peel) 20.4–69.4 µg/g FW (pulp) 85.5–560 µg/g FW (peel) 84.0–324 µg/g FW (pulp) 104–400 µg/g FW (peel) 92.0–270 µg/g FW (pulp)</td>
<td>0.64–2.3 mM (FRAP)</td>
<td>100</td>
</tr>
<tr>
<td>Strawberry</td>
<td>45</td>
<td>Ellagic acid</td>
<td>60–341 µg/g FW</td>
<td>nd</td>
<td>139</td>
</tr>
<tr>
<td>Blueberries (lowbush) wild</td>
<td>135</td>
<td>Anthocyanin Phenolics</td>
<td>1270–2100 µg of C3-glu equiv/g FW 3460–4120 µg of GA equiv/g FW</td>
<td>51.5–90.1 µmol TE/g FW</td>
<td>231</td>
</tr>
<tr>
<td>Blueberries (highbush) cultivated</td>
<td>80</td>
<td>Anthocyanin Phenolics</td>
<td>927–1480 µg of C3-glu equiv/g FW 1650–2160 µg of GA equiv/g FW</td>
<td>33.2–58.2 µmol TE/g FW</td>
<td>231</td>
</tr>
<tr>
<td>Apple</td>
<td>10</td>
<td>Total phenol Chlorogenic acid</td>
<td>1.6-fold 10-fold</td>
<td>nd</td>
<td>232</td>
</tr>
<tr>
<td></td>
<td>46</td>
<td>Chlorogenic acid Epicatechin Procyanidine B2</td>
<td>21–350.53 mg/l 0–206.54 mg/l 0–247 mg/l</td>
<td>nd</td>
<td>233</td>
</tr>
</tbody>
</table>

nd=not determined.
Genetic engineering is a powerful tool to introduce favourable changes in metabolic pathways of plants to improve the quantity and bioavailability of phytonutrients with particular attention to antioxidants [40, 127, 140]. Together with modern biotechnology, transcriptome–proteome–metabolome deciphering a crop plant should eventually lead to tissue-specific and development-specific redesign of metabolic pathways in order to accumulate desired, or close-to-the-desired, levels of a particular phytonutrient. Particular emphasis needs to be given to regulate expression of introduced genes using regulatable promoters, stabilizing the progeny for several generations, and field tests to confirm that the transformed crop performs equally well or even better as the non-transformed cultivar. Transgenic crops add to the genetic resources that can be used to obtain insightful knowledge about genetic, biochemical and physiological regulation of various metabolic pathways and functional metabolites and provide an applied angle, in making available highly nutritious, ‘specialty crops’ to the public [140].

Various vegetable and fruit crops have been transformed with the objective to enhance the concentration of a useful nutrient metabolite [141–144]. However, in some instances, increase in a specific nutrient was found to have come about at a certain cost, for instance, by affecting yield or another nutrient. As an illustration, overexpression of cystathionine γ-synthase (CγS) gene, which encodes the first enzyme in methionine biosynthetic pathway, in combination with methionine-rich 15-kD β-zein resulted in 2- to 6-fold increase in free methionine as well as methionine content of the zein protein fraction in potato tuber. However, the resultant transgenic plants suffered from severe growth retardation, lower tuber yield and reduced amount of anthocyanins in tubers [145]. A few examples of successfully manipulated horticultural crops and their enhanced antioxidant nutrients are provided in Table 3, and the information is summarized below.

**Tomato**

**Carotenoids**

Tomato is a major dietary source of lycopene (25.73 μg/g FW – USDA database) and contains other antioxidants, vitamin C, flavonoids and tocopherols [140, 146]. Its heavy consumption, reports of its health benefits, ease of genetic transformation and relatively small genome size have made tomato a model crop for genetic engineering for nutrition enhancement, particularly carotenoids and polyphenolics [140, 147]. Simultaneously, intensified efforts have been made on elucidating the biosynthesis pathways and regulatory steps in limiting antioxidant content of the fruit.

Lycopene accumulation in tomato fruit was linked to a differential regulation of phytoene synthase (PSY), phytoene desaturase (PDS), lycopene beta (LCY-b) and lycopene epsilon (LCY-e) cyclases [148–151]. Expression of the genes encoding PSY and PDS proteins, and lycopene production, increase during ripening while that of LCY-b and LCY-e, which convert lycopene to either β- or δ-carotene, declines precipitously [152]. Expression of β-cyclase gene under the control of a fruit-specific promoter resulted in a 7-fold increase in β-carotene, whereas its silencing via antisense expression doubled the increase in lycopene [153; Table 3]. Various research groups [153–155] have successfully used LCY β-cyclase gene under fruit-specific promoters to increase the β-carotene content of tomato fruit (Table 3). Constitutive expression of PDS-ctl also resulted in high level of β-carotene [155]. Expression in tomato of the bacterial crtB gene that encodes PSY when engineered with the fruit polygalacturonase promoter led to 5–10-fold increase in total PSY enzyme activity and a significant increase in phytoene, carotene and lycopene [156]. The use of a bacterial gene was advantageous in that this avoided the possibility of reducing the carotenoid content through co-suppression and gene silencing by a tomato homologue [157] and the use of fruit-ripening promoter, instead of the constitutive CaMV35S, avoided producing a dwarf phenotype [158]. It has become clear that the use of regulatable promoters with heterologous genes overrides endogenous control of carotenoid pathway genes, enabling higher accumulation of carotenoids during fruit ripening [127, 140]. Expression of yeast SAM decarboxylase (ySAMdc) fused to the ripening-inducible promoter E8 in homozygous lines of tomato led to accumulation of higher polyamines, which caused enhancement of fruit juice quality and 2–3-fold higher lycopene accumulation compared with the controls [159]. The use of fruit-specific Pds promoter with lycopene β-cyclase (b-Lcy) and β-carotene hydroxylase (b-Chy) genes resulted in a 12-fold (63 μg/g FW) increase in β-carotene over the control (5 μg/g) [160]. These transformants also accumulated β-cryptoxanthin and zeaxanthin (downstream product in β-carotene pathway).

RNAi-mediated suppression of an endogenous photomorphogenesis regulatory gene, DET1, which down-regulates light mediated development, increased carotenoids and flavonoids without affecting other fruit quality parameters [161]. Combination of fruit-specific promoters with RNAi technology to down-regulate UV-DAMAGED DNA BINDING PROTEIN-1 (DDB1) significantly increased lycopene and total carotenoid levels in tomato concomitant with increased number of plastids ([162]; Table 3). This study also showed that tomato culin CUL4 gene regulates plastid number and carotenoid accumulation.

**Polyphenols**

Tomatoes in general contain small amounts of flavonoids, most of which are located in the peel of the fruit [163]. Attempts to increase total phenolics (flavonoids and
<table>
<thead>
<tr>
<th>GM fruit</th>
<th>Target nutrient</th>
<th>Gene introduced</th>
<th>Promoter used</th>
<th>Transgenic versus wild-type (WT)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato</td>
<td>β-carotene</td>
<td>β-Lcy/Arabidopsis + βChy/Pepper</td>
<td>Pds (Tomato fruit specific)</td>
<td>63 µg/g FW (WT: 5 µg/g FW)</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DET1/Tomato (RNAi)</td>
<td>P119 (Tomato fruit specific)</td>
<td>20.5 µg/g FW (WT: 2.4 µg/g FW)</td>
<td>161</td>
</tr>
<tr>
<td></td>
<td></td>
<td>β-Lcy/Arabidopsis</td>
<td>Pds</td>
<td>57.0 µg/g FW (WT: 8.0 µg/g FW)</td>
<td>153</td>
</tr>
<tr>
<td></td>
<td>Lycopene β-cyclase/Erwinia herbicola</td>
<td>crt1/Erwinia uredovora</td>
<td>atpl (chloroplast targeted)</td>
<td>286 µg/g DW (WT: 69 µg/g DW)</td>
<td>154</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CRTB/E. uredovora</td>
<td>PG (fruit-specific)</td>
<td>520 µg/g DW (WT: 271 µg/g DW)</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CRY2/Tomato</td>
<td>CaMV3S3</td>
<td>170 µg/g DW (WT: 33 µg/g DW)</td>
<td>156</td>
</tr>
<tr>
<td></td>
<td>Lutein</td>
<td>CRY2/Tomato</td>
<td>CaMV3S3</td>
<td>101 µg/g DW (WT: 78 µg/g DW)</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>Lycopene β-cryptoxanthin, zeaxanthin</td>
<td>B-Lcy/Arabidopsis (antisense)</td>
<td>Pds</td>
<td>36 µg/g DW (WT: 23 µg/g DW)</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>Anthocyanin</td>
<td>Del/Snapdragon + Ros1/Snapdragon</td>
<td>E8 (ripening-inducible)</td>
<td>2830 µg/g FW (WT: not detected)</td>
<td>170</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lycopene β-SAMDC, Spe2/Yeast</td>
<td>E8 100–150 µg/g FW (WT: 25–40 µg/g FW)</td>
<td>159</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lycopene B-Lcy/Arabidopsis (antisense)</td>
<td>Pds</td>
<td>97 µg/g FW (WT: 54 µg/g FW)</td>
<td>153</td>
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<tr>
<td></td>
<td></td>
<td>Lycopene CrtB/E. uredovora</td>
<td>PG</td>
<td>2000 µg/g DW (WT: 1238 µg/g DW)</td>
<td>156</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CRY2/Tomato</td>
<td>CaMV3S3</td>
<td>1354 µg/g DW (WT: 775 µg/g DW)</td>
<td>164</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CRL/Tomato (RNAi)</td>
<td>CaMV3S3</td>
<td>300 µg/g FW (WT: 100 µg/g FW)</td>
<td>162</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HP1/Tomato (RNAi)</td>
<td>TFM7</td>
<td>47 µg/g DW (WT: 28 µg/g DW)</td>
<td>156</td>
</tr>
<tr>
<td></td>
<td>Phytotoxins</td>
<td>CortB/E. uredovora</td>
<td>PG</td>
<td>47 µg/g DW (WT: 28 µg/g DW)</td>
<td>156</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Isoflavone/genistin</td>
<td>GmIFS2/Soybean</td>
<td>0.45 nmol/g FW (peel) (absent in WT)</td>
<td>165</td>
</tr>
<tr>
<td></td>
<td>Phytotoxins</td>
<td>STS/Vitis vinifera</td>
<td>Pd3Ss (Tomato-fruit specific)</td>
<td>10.4 µg/g FW (whole fruit) (WT: not detected)</td>
<td>166</td>
</tr>
<tr>
<td></td>
<td>Deoxychalcones</td>
<td>CHS/Petunia + CHR/Alfalfa</td>
<td>Pd3Ss</td>
<td>37.5 µg/g FW (peel) (absent in WT)</td>
<td>156</td>
</tr>
<tr>
<td></td>
<td>Flavonoids/luteolin aglycon</td>
<td>CHI/Petunia + FNS-II/Gerbera</td>
<td>Pd3Ss</td>
<td>265 µg/g FW (peel) (absent in WT)</td>
<td>156</td>
</tr>
<tr>
<td></td>
<td></td>
<td>STS/Vitis vinifera</td>
<td>Pd3Ss</td>
<td>340 µg/g FW (peel) (absent in WT)</td>
<td>156</td>
</tr>
<tr>
<td></td>
<td>Flavonoids/rutin</td>
<td>CHI/Petunia</td>
<td>CaMV3S3</td>
<td>150 µg/g FW (peel) (absent in WT)</td>
<td>156</td>
</tr>
<tr>
<td></td>
<td>Flavonoids/kaempferol</td>
<td>CHI/Petunia</td>
<td>CaMV3S3</td>
<td>900 µg/g FW (peel) (WT: 56.25 µg/g DW)</td>
<td>163</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.6–3.5-fold over WT</td>
<td>TFM7</td>
<td>16520 µg/g DW (WT: 250 µg/g DW)</td>
<td>163</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.6–3.5-fold over WT</td>
<td>TFM7</td>
<td>150 µg/g FW (peel) (absent in WT)</td>
<td>156</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.6–3.5-fold over WT</td>
<td>TFM7</td>
<td>16520 µg/g DW (WT: 250 µg/g DW)</td>
<td>163</td>
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<tr>
<td></td>
<td></td>
<td>2.6–3.5-fold over WT</td>
<td>TFM7</td>
<td>150 µg/g FW (peel) (absent in WT)</td>
<td>156</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.6–3.5-fold over WT</td>
<td>TFM7</td>
<td>16520 µg/g DW (WT: 250 µg/g DW)</td>
<td>163</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.6–3.5-fold over WT</td>
<td>TFM7</td>
<td>150 µg/g FW (peel) (absent in WT)</td>
<td>156</td>
</tr>
<tr>
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<td></td>
<td>2.6–3.5-fold over WT</td>
<td>TFM7</td>
<td>16520 µg/g DW (WT: 250 µg/g DW)</td>
<td>163</td>
</tr>
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<td></td>
<td>2.6–3.5-fold over WT</td>
<td>TFM7</td>
<td>150 µg/g FW (peel) (absent in WT)</td>
<td>156</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.6–3.5-fold over WT</td>
<td>TFM7</td>
<td>16520 µg/g DW (WT: 250 µg/g DW)</td>
<td>163</td>
</tr>
<tr>
<td>GM fruit</td>
<td>Target nutrient</td>
<td>Gene introduced</td>
<td>Promoter used</td>
<td>Transgenic versus wild-type (WT)</td>
<td>Reference</td>
</tr>
<tr>
<td>----------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>--------------</td>
<td>---------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Tomato</td>
<td>trans resveratrol</td>
<td>StSy/V. vinifera</td>
<td>CaMV35S⁷</td>
<td>26 μg/g FW (absent in WT)</td>
<td>167</td>
</tr>
<tr>
<td></td>
<td>Glycosylated resveratrol</td>
<td>StSy/V. vinifera</td>
<td>CaMV35S⁷</td>
<td>0.58 μg/g FW (peel) (WT: 0.26 μg/g DW)</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td>trans-piceid</td>
<td>StSy/V. vinifera</td>
<td>CaMV35S⁷</td>
<td>126.58 μg/g FW (peel) (absent in WT)</td>
<td>168</td>
</tr>
<tr>
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<td>trans-resveratrol</td>
<td>StSy/V. vinifera</td>
<td>CaMV35S⁷</td>
<td>48.48 μg/g FW (peel) (absent in WT)</td>
<td>168</td>
</tr>
<tr>
<td>Potato</td>
<td>Carotenoid</td>
<td>Or</td>
<td>cauliflower</td>
<td>GBSS (tuber-specific)</td>
<td>31 μg/g DW (WT: 5.5 μg/g DW)</td>
</tr>
<tr>
<td></td>
<td>Carotenoids</td>
<td>CrtB/E. uredovora</td>
<td>Patatin (tuber-specific)</td>
<td>35 μg/g FW (WT: 5.6 μg/g FW)</td>
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<tr>
<td></td>
<td>β-carotene:</td>
<td>CrtB, CrtB and CrtY/E. uredovora</td>
<td>Pat1 (tuber-specific)</td>
<td>114 μg/g DW (WT: 5.8 μg/g DW)</td>
<td>175</td>
</tr>
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<td>Carotenoids</td>
<td>LCY-e/potato (antisense)</td>
<td>Patatin B33</td>
<td>47 μg/g DW (WT: 0.013 μg/g DW)</td>
<td>176</td>
</tr>
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<td>β-carotene</td>
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<td></td>
<td>12.27 μg/g DW (WT: 4.67 μg/g DW)</td>
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<td>Zeaxanthin:</td>
<td>DFR/Petunia hybrida</td>
<td>CaMV35S⁷</td>
<td>0.99 μg/g DW (WT: 0.262 μg/g DW)</td>
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<tr>
<td></td>
<td>Flavonoids/ chlorogenic acid</td>
<td>Pelargonidin</td>
<td>CaMV35S⁷</td>
<td>2.5 mg/g DW (WT: 1.5 mg/g DW)</td>
<td>182</td>
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<td></td>
<td>Petunidin</td>
<td></td>
<td></td>
<td>0.4 mg/g DW (WT: 0.1 mg/g DW)</td>
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</tr>
<tr>
<td></td>
<td>Kaempferol</td>
<td></td>
<td></td>
<td>3.0 mg/g DW (WT: 0.5 mg/g DW)</td>
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</tr>
<tr>
<td></td>
<td>Caffeoylquinic acid</td>
<td>StMtf1</td>
<td>CaMV35S⁷</td>
<td>16.5 mg/g FW (WT: 11.5 mg/g FW)</td>
<td>236</td>
</tr>
<tr>
<td></td>
<td>α-tocopherol</td>
<td>SmTmff⁶/S. tuberosum</td>
<td>Tuber-specific</td>
<td>1.8 mg/g DW (WT: 0.451 mg/g DW)</td>
<td>174</td>
</tr>
<tr>
<td></td>
<td>α-tocopherol</td>
<td>CrtB-TP/E. uredovora</td>
<td>Patatin</td>
<td>7.5 μg/g FW (WT: 4.4 mcg/g FW)</td>
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</tr>
<tr>
<td></td>
<td>Caffeoylquinic acid</td>
<td>AtHPPD/Arabidopsis</td>
<td>CaMV35S⁷</td>
<td>1.038 μg/g FW (WT: 0.208 ng/g FW)</td>
<td>184</td>
</tr>
<tr>
<td></td>
<td>α-tocopherol</td>
<td>AtHPT/Arabidopsis</td>
<td>CaMV35S⁷</td>
<td>0.579 μg/g FW (WT: 0.208 μg/g FW)</td>
<td>184</td>
</tr>
<tr>
<td></td>
<td>Amino acids/total protein</td>
<td>AmA1/Amaranthus hypochondriacus</td>
<td>CaMV35S⁷ and GBSS (tuber specific)</td>
<td>16.5 mg/g FW (WT: 11.5 mg/g FW)</td>
<td>236</td>
</tr>
<tr>
<td></td>
<td>Brassica</td>
<td>β-carotene</td>
<td>e-CYC/Brassica napus (RNAi)</td>
<td>P35S⁷ (constitutive)</td>
<td>90.76 μg/g FW (WT: 0.49 μg/g FW)</td>
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<tr>
<td></td>
<td>Lutein</td>
<td></td>
<td></td>
<td>76.22 μg/g FW (WT: 3.30 μg/g FW)</td>
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<tr>
<td></td>
<td>Carotenoid</td>
<td>crtB/bacteria</td>
<td>Napin (seed specific)</td>
<td>1000 μg/g FW (WT: 33 μg/g FW)</td>
<td>198</td>
</tr>
<tr>
<td></td>
<td>β-carotene</td>
<td>DET1 (RNAi suppression)</td>
<td>seed specific</td>
<td>857 μg/g FW (WT: 5 μg/g FW)</td>
<td>199</td>
</tr>
<tr>
<td></td>
<td>Carotenoid</td>
<td>ctrl + ctrl/bacteria</td>
<td>CaMV35S⁷</td>
<td>7 μg/g FW (WT: 0.5 μg/g FW)</td>
<td>238</td>
</tr>
<tr>
<td></td>
<td>Ketocarotenoid</td>
<td>idt + ctrlW + ctrlZ (synthetic)/marine bacteria + ctrlE + ctrlB + ctrl + ctrlY/Pantoea ananatis</td>
<td>CaMV35S⁷, napin or FAE1 (seed specific)</td>
<td>412–657 μg/g FW (WT: 22 μg/g FW)</td>
<td>239</td>
</tr>
<tr>
<td></td>
<td>Oilseed</td>
<td>α-tocopherol</td>
<td>γ-TMT/Arabidopsis</td>
<td>CaMV35S⁷</td>
<td>60–190 μg/g FW (WT: absent)</td>
</tr>
<tr>
<td></td>
<td>γ-tocopherol</td>
<td></td>
<td></td>
<td>62% (WT: 9.8%)</td>
<td>195</td>
</tr>
<tr>
<td></td>
<td>Flax</td>
<td>Carotenoid</td>
<td>crtB/P. ananatis</td>
<td>CaMV35S⁷ and FAE¹</td>
<td>156.3 μg/g DW (WT: 8.4 μg/g DW)</td>
</tr>
<tr>
<td></td>
<td>Carrot</td>
<td>Ketocarotenoid</td>
<td>CrtO/Haematococcus pluvialis</td>
<td>Ubi/CaMV35S/RolD</td>
<td>70% of total carotenoid converted to ketocarotenoids (absent in WT)</td>
</tr>
</tbody>
</table>

Table 3 (Cont.)

References:
1. http://www.cabi.org/cabreviews
2. 10 Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources
Table 3. Folate and pteridine contents while result in 2-fold and 3–140-fold increases, respectively, part) under the control of a fruit-specific promoter gene (based on the sequence of its mammalian counterpart) under the control of a fruit-specific promoter gene (based on the sequence of its mammalian counterpart). Polyphenol content [167] as well as resveratrol and piceid contents [168] increased in tomato transformed to constitutively express the grape (Vitis vinifera L.) StSy gene, which encodes a key enzyme in the trans-resveratrol biosynthesis, stilbene synthase. The increased antioxidant metabolites led to higher antioxidant capacity of the transgenic fruit. Increase in resveratrol content (2.5-fold) in tomato was also achieved by the constitutive expression of Arabidopsis hmgr1 gene encoding 3-hydroxy3-methylglutaryl CoA reductase [169].

Another study fused fruit ripening E8 promoter to regulatory genes to explore their potential in increasing the antioxidant capacity of tomato fruit [170]. The two genes used are: a basic helix-loop-helix Del1 and Ros1, aMYB related transcription factor, from snapdragon, both of which interact to induce anthocyanin biosynthesis in snapdragon flowers [171, 172]. The resultant transgenics had purple coloration in both flesh and peel, accumulating 2.38 mg/g of FW anthocyanin in both peel and flesh, while anthocyanin was undetectable in the non-transgenic control fruit [170]. Also, this led to a 3-fold increase in the antioxidant capacity of the transgenic fruit.

Folates

Overexpression of a synthetic GTP cyclohydrolase I (gchI) gene (based on the sequence of its mammalian counterpart) under the control of a fruit-specific promoter resulted in 2-fold and 3–140-fold increases, respectively, in folate and pteridine contents while p-aminobenzoate (PABA) content was reduced [173]. The Arabidopsis aminodeoxychorismate synthase (ADCS) gene, which
catalyses the first step of PABA synthesis, was fruit-specifically expressed in tomato, resulting in 19-fold more PABA accumulation than in the controls. A genetic cross between the pteridine and PABA over-expressing transgenic lines led to 25-fold more folate in the progeny, concentrations at par with the RDA for adults [173].

**Potato**

**Carotenoids**
The *Erwinia* PSY crtB gene fused to a tuber-specific promoter was introduced in potato and several-fold increase in the contents of violaxanthin, lutein and β-carotene was obtained [174]. A similar strategy used to introduce bacterial mini-pathway carotenoid genes into potato resulted in achieving substantial amounts of carotenoids including β-carotene and lutein ([175]; Table 3). Antisense silencing of potato β-carotene hydroxylase CHY1 and CHY2 genes, which metabolize β-carotene, led to 4–7-fold increase in lutein but β-carotene level was enhanced by 38-fold [176].

The cauliflower *Or* gene, *Orange* gene mutation responsible for β-carotene accumulation in cauliflower, when introduced in a tuber-specific manner into potato led to a 6-fold higher level of total carotenoids in transgenic versus the controls [177, 178].

Ketocarotenoids are important nutraceuticals for human health [179]. An algal bkt1 gene, encoding a β-ketolase enzyme, was overexpressed in potato tuber with an aim to engineer ketocarotenoid biosynthesis in a plant storage organ. This resulted in ketocarotenoid accumulation up to 14 μg/g DW [180].

**Polyphenols**
To increase the content of health promoting polyphenolic compounds such as kaempferol and chlorogenic acid, potato plants were transformed with a modified MYB transcription factor. The activation of the flavonoid biosynthetic pathway resulted in 100-fold increase in phenolic acids [181]. Constitutive expression in potato of petunia dihydorflavonol reductase (DFR) gene, which encodes a key enzyme in flavonoid biosynthesis, significantly enhanced anthocyanins and phenolics [182].

**Tocopherols**
Tocopherols are also among potent antioxidants with anticarcinogenic properties and have a potential to be used as chemotherapeutics [183]. Overexpression of *crtB* gene in potato tuber resulted in a substantial increase in the α-tocopherol content [174]. *Arabidopsis* HPPD and HPT genes were tested in tobacco and their overexpression also increased α-tocopherol content by 106–266% [184].

**Apple**

**Polyphenols**
To engineer stilbene biosynthesis in the apple fruit, grape stilbene synthase gene, Vst1, was expressed in apples. This led to a several-fold accumulation of piceid (trans resveratrol glucoside) ([185]; Table 3). The increase in piceid correspondingly led to higher amounts of hydroxycinnamic acid and flavonols in the transgenic apple, while neither resveratrol nor any of its derivatives were found in the non-transgenic apple fruit.

**Kiwi**

**Piceid**
Stilbene synthase gene from three different *Vitis* species when expressed constitutively (CaMV35S promoter) in kiwifruit led to resveratrol glucoside (piceid) accumulation in tomato leaves [186]. However, this study did not determine the piceid concentration in the fruits.

**Strawberry**

**Phenylpropanoids**
A proof-of-the-concept experiment with potential to use genes in phenylpropanoid pathway to enhance antioxidant capacity of strawberry was carried out. Strawberry antisense chalcone synthase (CHS) gene expressed under the control of constitutive promoter, CaMV35S, into a cultivated variety of strawberry (*Fragaria xananassa*) resulted in a differential effect – while expression of genes involved in phenylpropanoid pathway was enhanced and led to increases in cinnamoyl glucose, caffeoyl glucose, feruloyl glucose, p-coumaryl alcohol and p-coumaryl-1-acetate, expression of flavonoid pathway genes was reduced, which resulted in reduced levels of anthocyanins, flavonols and proanthocyanidines [187]. Thus, CHS was found to act in strawberry as a regulator of substrate flow between the two affected pathways. The effect of these changes on the fruit quality or any other parameter was not reported.

**Ascorbic acid**
The NADPH-dependent D-galacturonate reductase, encoded by GalUR, converts D-galacturonic acid to ascorbic acid. *GalUR* expression pattern in strawberry fruits correlates with the pattern of ascorbic acid content. The potential of strawberry *GalUR* to increase the levels of ascorbic acid (vitamin C) was tested by transforming this gene into *Arabidopsis* ([188]; Table 3). Overexpression of *GalUR* resulted in 2–3-fold higher levels of ascorbic acid. The consequence of this expression in the physiology of the transformed plants was not reported.
**Banana**

Banana is a common fruit crop, a good source of vitamins B6 and C, and consumed equally in the tropics as in the sub-tropics [189]. In the developing world, it ranks as the fourth important food crop after rice, wheat and maize, and is widely consumed by adults and children alike. There is a wide diversity of banana [189] together with genetic variability in fruit provitamin A carotenoids, lutein and other micronutrients [190]. Genetic engineering has the potential to further enhance nutritional quality and revenue, and make banana a more valuable source of antioxidants for better human health [191–194].

**Lettuce**

**Tocopherols**

Overexpression of *Arabidopsis* γ tocopherol methyl transferase gene (under the control of constitutive promoter) in lettuce caused an increase in the levels of α-tocopherol compared with the wild-type control ([195]; Table 3).

**Folates**

Synthetic codon-optimized GTP cyclohydrolase I (*gchI*) based on native *Gallus gallus* gene when introduced into lettuce enhanced folate levels. The transgenic lines thus generated had folate content that ranged from 2.1- to 8.5-fold higher than the non-transgenic lines. The high-folate lettuce is sufficient to provide 26% of the RDA for folate, provided its bioavailability and bioactivity are optimal and equivalent [196].

**Carrot**

**Ketocarotenoids**

Carrot roots accumulate high levels of α-carotene and β-carotene, but are deficient in ketocarotenoids. Introduction of a β-carotene ketolase gene from alga *Haematococcus pluvialis* into carrot root resulted in ketocarotenoid accumulation to 2400 μg/g root dry weight [197].

**Canola**

**Carotenoids**

Transformation of canola seed with bacterial PSY (*crtB*) gene using seed-specific napin promoter resulted in orange coloured seed with a 50-fold higher (1000–1500 μg/g FW) total carotenoid level than the wild-type (33 μg/g FW) [198]. Transgenic canola seeds that co-expressed ctrl and *crtB* gene were found to accumulate lycopene (29 μg/g FW) and β-carotene (857 μg/g FW) [199].

**Linseed Flax**

**Carotenoids**

Flaxseed is enriched in α-linolenic acid and lignans. Lignans are sources of phytoestrogens and act as antioxidants due to the presence of α-linolenic acid, a ω-3 fatty acid, which is essential for human health [200]. Overexpression of bacterial *crtb* gene in flaxseeds caused an increase of 7.8–18.6-fold in the carotenoid levels of the seeds but not leaves [201].

**Sweet Potato**

To increase the levels of unsaturated fatty acids in sweet potato, a rich source of vitamin A, ascorbic acid and carbohydrates, a tobacco microsomal fatty acid desaturase gene (*NtFAD3*) was overexpressed using a modified CaMV35S promoter. Transgenic sweet potatoes thus obtained had increased amounts of linolenic acid [202].

**Soybean**

**Tocopherols**

Overexpression of the *Arabidopsis* genes involved in the biosynthetic pathway of α-tocopherol, VTE4 (γ-TMT – γ tocopherol methyl transferase) gene in combination with the VTE3 (2-methyl-6-phytylbenzoquinol methyltransferase) gene, increased by 8-fold the α-tocopherol content in soybean seeds [203]. Similarly, *Perilla frutescens* γ-TMT introduced seed-specifically into soybean also produced higher levels of α-tocopherol and β-tocopherol [204].

**Bioavailability and Bioefficacy as Determinants of Phytonutrient Effectiveness**

A final test of the true potential of a supplemental antioxidant or crop nutrients in human health benefits is in their accessibility, bioavailability and biological potency. The science related to absorption, metabolism and in vivo potency is still in its infancy. An important bearing on the antioxidant potential of polyphenols (or any other ‘potential’ antioxidant) is that: ‘the phenolic content itself is not a comprehensive index of antioxidant capacity; the chemical nature of phenols must be taken into account when evaluating the antioxidant capacity of polyphenol-rich beverages’ [110].

Bioavailability refers to the actual amount of an ingested nutrient that is available for its physiologic function, for instance, by measuring its blood plasma (serum) concentration [205]. Bioaccessibility of a nutrient is crucial in its effective bioavailability. In this context, important features include: the physical or chemical form in which a nutrient exists, whether processed and cooked, dose ingested and how much available in situ, its catabolism as
well as the metabolic state of an individual, and other (see [206]). Even when a phytonutrient in pure form is shown to have a strong antioxidant property in vitro, its positive effects on an individual’s health may be limited in vivo. A specific nutrient may directly interact with one or more organic components present in food, thereby stimulating or negating its absorption [207]. For example, folate bioavailability is increased in the presence of another nutrient, ascorbic acid, which helps stabilize folates during processing and absorption. In addition, ascorbic acid increases non-haem iron absorption by reducing iron to its soluble form (reviewed in [207]); in contrast, polyphenols form insoluble complexes with iron and thereby inhibit non-haem iron absorption.

Fat intake (up to 5 g per day) helps absorption of \(\beta\)-carotene [208], while dietary fibre can reduce carotenoid absorption and result in reduced plasma \(\beta\)-carotene concentration. Citrus pectin and \(\beta\)-carotene given as part of a diet resulted in a 42% decrease in plasma \(\beta\)-carotene, possibly because of dietary fibre interactions with bile acids affecting lipid metabolism [209].

Compared with a pure carotenoid-supplemented diet, intake of vegetables led to increased plasma levels of \(\beta\)-carotene and lutein by 14 and 67%, respectively. However, this did not cause any significant effect on the antioxidant activity of plasma or on the susceptibility of LDL to oxidation \textit{ex vivo} [210]. Fruit intake was more effective than the intake of green leafy vegetables in increasing the serum levels of \(\beta\)-carotene, respectively, 0.52 \(\mu\)mol/l versus 0.14 \(\mu\)mol/l, indicating that the higher bioavailability of \(\beta\)-carotene from fruit than green leafy vegetables may be related to the low complexity of food matrix in the former [211].

Food processing helps to change the plant matrix and thereby influence bioavailability of phytonutrients. The plant inositol hexaphosphate, commonly called phytate or phytic acid, interferes with the uptake of essential minerals from foods including decreasing the absorption of iron. Processing of foods rich in phytates prior to consumption increases absorption of essential minerals [212]. It was shown that thermal processing is an effective way to hydrolyse phytates and improve iron absorption [213]. Consumption of cooked carrot or spinach increased by three-fold the plasma \(\beta\)-carotene levels compared with intake in their raw forms [214]. Thermal processing of foods is known to enhance absorption of carotenoids, particularly \(\beta\)-carotene and lycopene, this is likely as a result of breakdown of cell walls and protein–carotenoid complexes [208, 215, 216]. Likewise, intake of paste instead of a fresh tomato greatly increased plasma lycopene levels [217, 218]. However, lycopene was found easily bioavailable from fresh-frozen watermelon as well as canned tomato juice [219]. Carotenoids are hydrophobic in nature with limited solubility in aqueous milieu. It was shown that when delivered through lipophilic micelles or after solubilizing in organic solvent tetrahydrofuran, lycopene content increased equally from both forms in a concentration- and time-dependent manner in cells physiologically relevant to cardiovascular system [220].

Bioavailability of different phytonutrients can widely differ depending upon the chemistry of the nutrient, its modification, site of absorption and the presence or absence of interfering compounds in a diet. A summary of 97 studies on the bioavailability of polyphenols provided as pure compound, plant extract, or whole food/beverage indicated that polyphenol bioavailability differs between polyphenols. Gallic acid and isoflavones were recognized as the most well-absorbed polyphenols, while catechins, flavonones and quercetin glycoside came next. Proanthocyanidines and anthocyanins were among the poorly absorbed polyphenols [221].

Conclusions and Outlook

Studies showing that antioxidants in traditionally bred fruits provide protection against diet-related diseases and improve human health have now been corroborated by at least two different studies that employed tomato fruit whose antioxidant capacity was significantly enhanced by genetic engineering. High anthocyanin transgenic tomato fed to cancer-susceptible Trp53 knock-out mice, used as a tumorigenesis model, led to a significant extension of their life span [170]. Similarly, the health effects of flavonoid-rich transgenic tomato were evaluated in human C-reactive protein transgenic (CRPtg) mice, a model that expresses markers of cardiovascular risks [222]. These mice were fed a diet that had tomato quantity supplied at a level achievable in human diet. The transgenic tomato diet significantly reduced basal human CRP concentration compared with that achieved with just the wild-type tomato. Previous experiments conducted with diets from traditional, non-genetically engineered, germplasm led to diverse conclusions: diet-based increase in serum antioxidant activity was not correlated with a delay in the onset of disease of transgenic mice, a model for familial amyotrophic lateral sclerosis – a neurodegenerative disease [223], or when oxidative stress and plasma oxidative stability (\textit{ex vivo}) caused by fish oil in smokers was found partially offset by fruit and vegetable-enriched diets, no corresponding increase was found in the plasma antioxidant (carotenoids and vitamins A, C or E) levels [224]. However, it is known (see above) that the levels of various antioxidants in traditionally bred horticultural crops are not at par with the RDA set for each potential nutrient. Therefore, more experiments in the future with animal models or human subjects should rely on and make use of new germplasm where the antioxidant levels have been elevated to suggested RDA. Such studies should incorporate well thought out experimental designs and include also non-genetically engineered produce to throw light into any risks of genetically engineered fruits to human health [225]. Furthermore, differences in the
genetic background of a model animal employed in such research studies can show dramatic variability [226, 227]. In this context, the study showing polymorphic nature of AREs present in the human genome is relevant [21]. SNPs of ARE may modulate NRF2-mediated responses as well as the gene × antioxidant interactions. This may explain the wide variations observed in preclinical and clinical studies on responsiveness of human health including cancer risk and tumour to food components.

The knowledge of plant metabolism, signalling pathways, physiology and the genome together with the advancement in molecular and transformation techniques has led to the development of genetically engineered, value-added plants. Such crops with higher phytonutrient and antioxidant capacity will create a niche market of specialty produce, more so when their usefulness in improving the health benefits including protection against a particular disease is demonstrated. It is important to generate unambiguous data on their phytonutrient nature and content, antioxidant capacity, bioavailability and sustainability. To derive maximum health benefits, we need knowledge about what dose of an antioxidant is sufficient to provide prevention or cure against a disease, and in what form is a value-added fruit bioavailable – fresh or processed.

Antioxidant capacity of horticultural crops is affected by agronomy and there is need for development of improved agronomical practices to preserve their nutritional quality. Growth environments created by using a plastic or black polyethylene were found to uniquely interact with the tomato genotypes, in particular with transgenic tomatoes. Likely, new knowledge on the behaviour of transgenes in eco-friendly growth environments will be revealed in follow-up studies.

Integration of transgenic fruit crop with alternative agriculture will reduce the total farming costs because of reduced input of chemicals, while still maintaining higher productivity and high nutritional quality fruit [229].

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