

Bioactive compounds in berries relevant to human health

Maurizio Battino, Jules Beekwilder, Beatrice Denoyes-Rothan, Margit Laimer, Gordon J McDougall, and Bruno Mezzetti

Berries contain powerful antioxidants, potential allergens, and other bioactive compounds. Genetic and environmental factors affect production and storage of such compounds. For this reason breeding and biotechnological approaches are currently used to control or to increase the content of specific health-related compounds in fruits. This work reviews the main bioactive compounds determining the nutritional quality of berries, the major factors affecting their content and activity, and the genetic options currently available to achieve new genotypes able to provide, under controlled cultivation conditions, berries with the proper balance of bioactive compounds for improving consumer health.

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ANTIOXIDANTS IN BERRY FRUITS

Comparison of the antioxidant capacities of different fruits provides a valid tool with which to rank their health benefits. Berries invariably rank high due to their powerful antioxidant content.¹ However, the total antioxidant capacity (TAC) results from the presence of several classes of compounds, such as vitamin C and polyphenols, which have a different impact on human health. Therefore, the TAC measurements of crude berry extracts do not reflect the entire picture, and the contribution of individual compounds must be examined. Individual compounds can be identified using mass spectrometry, while their antioxidant capacity can be assessed using the recently developed on-line high-performance liquid chromatography (HPLC) antioxidant analysis, which measures the antioxidant capacity of each individual compound eluted from an HPLC separation. The results obtained provide an overview of the phytochemical composition and the contribution of each compound to the antioxidant activity of the berry. For example, applying the method to raspberries revealed that about half of their antioxidant activity was due to the presence of

ellagitannins, a class of phenolic compounds predominantly found in fruit from the *Roseaceae* family, while more generally occurring antioxidants like vitamin C (20%) and anthocyanins (25%) had lower contributions.² In most other fruits, the contribution of vitamin C to TAC is much higher, e.g. 30–35% in some strawberry varieties³ where other specific compounds like pelargonidin-3-glucoside may also account for up to 25% of TAC.³ Consequently, different berries may have a unique impact on consumer health due to the different contributions of specific compounds to their TAC. Understanding the link between the antioxidant capacity of individual components and the bioactivities of different berries may direct the biotechnological improvement of new berry varieties.

Both genetic and environmental factors affect the production and accumulation of bioactive compounds (BC) in strawberry fruits. Differences in nutritional quality amongst strawberry cultivars are well known, even if only a few genotypes are well characterized for these important features. In addition to their nutritional quality, berries also display sensorial attributes (color, aroma, flavor) which are attractive and very important to the consumer and therefore must be maintained in

Affiliations: *M Battino* is with the Institute of Biochemistry, Università Politecnica delle Marche, Ancona, Italy. *J Beekwilder* is with the Plant Research International, Wageningen University, The Netherlands. *B Denoyes-Rothan* is with the INRA-Unité de Recherche sur les Espèces Fruitières et la Vigne, Villenave d'Ornon, France. *M Laimer* is with the Plant Biotechnology Unit, IAM and BOKU University, Vienna, Austria. *GJ McDougall* is with the Quality, Health and Nutrition Programme, Scottish Crop Research Institute Invergowrie, Dundee, United Kingdom. *B Mezzetti* is with the Department of Environmental and Crop Science (SAPROV), Università Politecnica delle Marche, Ancona, Italy.

Correspondence: *M Battino*, Institute of Biochemistry, Faculty of Medicine, Università Politecnica delle Marche, Via Ranieri 65, 60100 Ancona, Italy. E-mail: m.a.battino@univpm.it.

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successful new varieties. Breeding and biotechnological approaches are currently used to increase the content of specific BC in berries, since higher levels of micronutrients and phytochemicals may be important to support an increased antioxidant intake, especially in the case of low-level fruit consumption.

Until recently, breeding for these traits using classical genetic approaches was a long and difficult task, especially in berries for which a very limited number of genes had been studied. In the near future, breeding for berry varieties with enhanced nutritional and sensorial quality should be facilitated by the use of genomic tools recently developed in model plants (*Arabidopsis* and rice) and in crop species (especially the fleshy fruits, tomato and grape).

The aim of our work is to analyze the main BC determining the nutritional quality of berries, the major factors affecting their content and activity, as well as the genetic options available for the attainment of new genotypes that are able to provide berries with the proper balance of BC for improving consumer health.

BERRY POLYPHENOLS: BIOACTIVITIES, BIOAVAILABILITY, AND HUMAN HEALTH EFFECTS

Considerable research has been directed at the potential health benefits of eating berries. As well as being a good source of vitamin C, dietary fiber, and minerals, berries contain high levels of natural polyphenol components that act as potent antioxidants. Berry extracts, rich in polyphenols, have a range of biological effects that can have beneficial outcomes on human health.

Cardiovascular health

Berry extracts have cardioprotective effects in model studies.⁴ They are very effective inhibitors of low density lipoprotein oxidation, a key step in the development of atherosclerosis. They have beneficial effects on platelet aggregation. At low levels, they provide protection of nitric oxide levels in arterial systems. Nitric oxide is crucial for the maintenance of flexible blood vessels and thereby important in controlling blood pressure.

Control of cancer growth

Berry extracts inhibit the growth of cultured cancer cells and certain berries are considerably more effective than others (Figure 1). The most active components in raspberry were the ellagitannins, but these components break down readily and the resultant products, including ellagic acid, may be the actual active components.⁵ Continuing joint research between the Scottish Crop Research Institute and the University of Ulster has shown that berry extracts can inhibit the initiation, progression, and invasiveness of colon cancer cells.

Control of blood glucose levels

Berry extracts inhibit starch digestive enzymes. Inhibition of α -glucosidase is already an accepted means of controlling post-meal glucose levels in patients suffering from non-insulin-dependent diabetes mellitus. Different berry components are responsible for the inhibition of α -glucosidase and α -amylase, which suggests considerable potentiation of effects on blood glucose levels.⁶ These

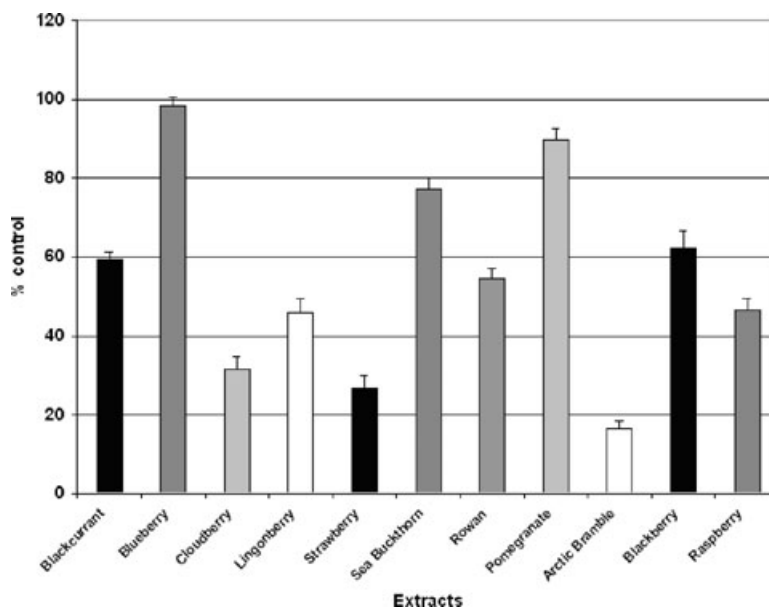


Figure 1 Inhibition of growth of HeLa cancer cells by berry extracts.

effects are unrelated to the antioxidant potential of the polyphenols. Similar effects on lipid digestion have been noted.⁷ Future research will extend the range of bioactivities of berry components and seek to understand their mechanisms of action and their bioavailability in humans.

ALLERGENS IN BERRIES: AN ISSUE FOR RISK ASSESSMENT

Reports on allergenicity of berries such as the strawberry, raspberry, blackberry, and blueberry are still rare. Whether this is related to a general low allergenicity, the small amounts consumed, or the restricted timeframe of consumption still remains to be answered. Low exposure to certain allergens might be the reason for the limited problems recorded so far. However, with ongoing encouragement of berry consumption this situation might change. Berries are not only consumed fresh but are also eaten as common ingredients in different food products, either as the main or an added component.

Fruit proteins with high primary-sequence similarity also display homologous tertiary structures, resulting in similar epitopes to immunoglobulin E molecules (IgE) and consequently in cross-reactivity, even across related taxa.⁸ Most plant allergens belong to only a few protein families associated with stress and defense mechanisms, indicating that conserved structures and biological activities may play a central role in determining or promoting allergenic properties.

Clinical reactivity depends on a variety of factors, but a valid interpretation of the phenomena depends strongly on the availability of sensitive, patient-independent detection tools. In vitro detection tools allow for the analysis of the presence, expression, and cross-reactivity of some berry allergens, and these data are confirmed with patient sera along with the relevant clinical history.

So far, four major allergens have been reported in the model apple plant and included in the official allergen list of the World Health Organization Allergen Nomenclature Subcommittee (<http://www.allergen.org>): Mal d 1 (PRP-10), homologous to Bet v 1 (17.5 kD), Mal d 2 (PRP-5), a thaumatin-like protein (TLP) (23 kD), Mal d 3 (PRP-14), a lipid transfer protein (LTP) (9 kD), and Mal d 4, a profilin homologous to Bet v 2 (14 kD).⁹

Karlsson et al.¹⁰ reported the presence of Bet v 1 homologues in strawberries. In fact, strawberries contain proteins homologous to Bet v 6, Mal d 1, and Mal d 3, as confirmed by IgE-binding assays and skin-prick tests.¹¹ Inhalation of frozen raspberry powder caused occupational asthma.¹² Allergenic proteins were characterized from fruit and pollen extracts of raspberry (i.e. Rub i 1, Rub i 3, and Rub i chiIII). In Southern blot analyses, DNA

homologous fragments to Mal d 1 and Mal d 3 could be detected in genomic DNA of the strawberry, raspberry, blueberry, and cranberry.¹¹

BREEDING AND BIOTECHNOLOGY FOR IMPROVEMENT OF BERRY NUTRITIONAL QUALITY AND CONSUMER ACCEPTANCE

Breeding and biotechnological approaches are currently used to increase the levels of specific BC of plants, but changes in plant metabolism are still not easy to address, e.g. the vitamin C biosynthetic pathway has only recently been determined. Rigorous and unprejudiced evaluation requires a defined set of criteria, tools, and methods, particularly when breeding and biotechnological programs aim to produce new varieties with improved nutritional value, combined with high production efficiency and berry quality. To develop new genotypes and commercial cultivars, the availability of new sources of quality attributes and nutritional attributes should be explored. In strawberries, wild species such as *F. virginiana glauca*, *F. vesca*, and some *F. x ananassa* proved to be good donor sources for BC (Figure 2), but in raspberries, the introduction of wild germplasm has, so far, not improved the nutritional quality of berries. Genes and regulatory sequences responsible for the desired improvements in fruit quality are of limited availability, and identification of these genes will require major efforts.

Furthermore, the public's attitude toward genetically modified (GM) crops is, at least in Europe, still rather skeptical, and EU regulations are very restrictive. The entry of GM strawberries into the EU market is effectively blocked. To increase the acceptance of GM strawberries by consumers, transgenic strawberries could be produced following the "clean gene" approach, i.e., transgenic plants would be free of foreign, undesired coding and regulatory DNA sequences.¹³ In addition to these criteria, only strawberry DNA sequences (as genes- and promoters-of-interest) should be considered for GM of strawberries.

Precise methods are required to assess the "substantial equivalence" (Table 1) of transgenic and conventionally bred berries.¹⁴ The methods available for assessing berry TAC,^{15–17} combined with polyphenol concentrations and other nutritional and quality parameters (sugars, total acidity, and fruit color) are seen as appropriate tools to develop a fast and reliable program for screening large breeding populations, for the identification of new cultivars with improved nutritional quality, as well as optimized yields under changing climatic and cultivation conditions,^{18,19} and as a first step in assessing "substantial equivalence" in transgenic plants produced for other aims.

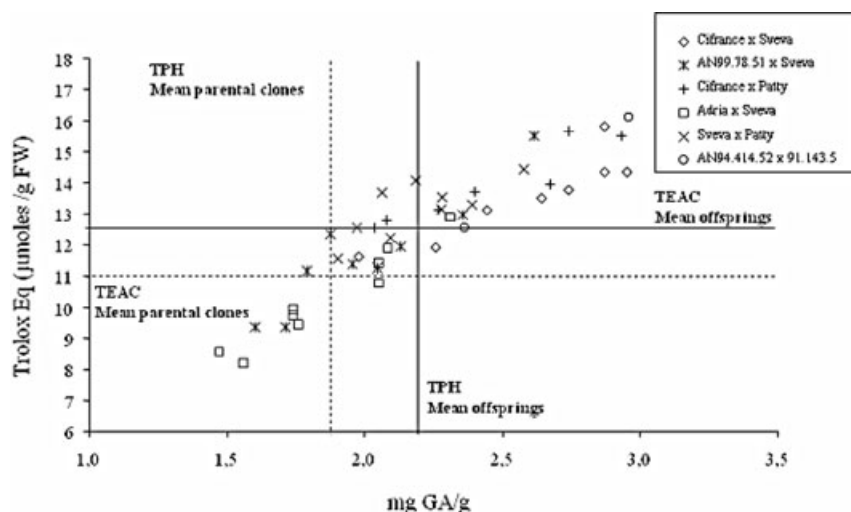


Figure 2 Variation in TAC, measured as trolox equivalent antioxidant capacity (TEAC) and total phenols (TPH) detected in progenies originated from cross-combinations between parental lines of *F. x ananassa* cultivars and selections of a F1 selection from *F. virginiana glauca* (AN94.914.52).

GENOMICS OF BERRIES: TOWARDS IMPROVEMENT OF NUTRITIONAL QUALITY

Genetic maps for strawberries and raspberries have been obtained recently.^{20–22} Several quantitative trait loci controlling traits involved in nutritional quality such as vitamin C have been mapped in strawberries.²³ This approach will also benefit from the current development of tools for large-scale analysis of fruit metabolites (metabolome), which has already been applied to the strawberry.²³ In parallel, the rapid development of comparative genetic maps between different plant species, including berries, will allow the localization of candidate genes previously identified in model plant species on genetic maps from berry species. High-throughput technologies for plant genotyping, such as those currently developed for the detection of single nucleotide polymorphisms, will accelerate the fine mapping of quantitative trait loci and ultimately the positional cloning of the

genes responsible for traits involved in berry nutritional quality.

Reverse genetic approaches aiming at identifying candidate genes and at analyzing their function *in planta* have also been used for berries, mainly for the strawberry. One of the first indications of the function of a gene is to know where, at what stage of fruit development, and in what genotype, this gene is expressed. Towards this end, various transcriptome approaches have been developed in berries, including expressed sequence tag sequencing (<http://www.ncbi.nlm.nih.gov/>), differential display reverse transcription-polymerase chain reaction) analyses²⁴ and microarray analyses in the strawberry.²⁵ These studies identified several candidate genes involved in berry quality.^{26,27} Among them were candidate genes later shown to control anthocyanin and flavonoid synthesis²⁸ as well as ascorbic acid synthesis.²⁹ At the same time, tools for the efficient and stable transformation of various berry species have been developed,³⁰ thereby allowing the

Table 1 Example of a first level of evaluation of substantial equivalence of GM fruit with total acidity (TA), solid solubles (SS), TAC as ferric reducing antioxidant potential (FRAP) and total phenols (TPH) of control and a line of *F. x ananassa* selection AN93.231.53, transgenic for the ovule-auxin expression *DefH9-iaaM* gene, and of *F. x ananassa* cv. Calypso, transgenic for the *rolC* gene.

Line	TA*	SS (°Brix)	FRAP†	TPH‡
AN93.231.53	13.9 ± 0.2a	8.5 ± 0.2a	13.49 ± 0.2 ^{ns}	2.23 ± 0.0 ^{ns}
<i>DefH9-iaaM</i> – Line 1	13.1 ± 0.2b	7.7 ± 0.1b	13.44 ± 0.2 ^{ns}	2.53 ± 0.0 ^{ns}
Calypso	12.9 ± 0.9 ^{ns}	9.2 ± 0.4 ^{ns}	13.26 ± 0.6b	2.26 ± 0.1b
<i>rolC</i> – Line A	11.5 ± 0.7 ^{ns}	8.2 ± 0.3 ^{ns}	12.26 ± 0.4b	2.23 ± 0.0b
<i>rolC</i> – Line B	11.7 ± 0.9 ^{ns}	8.1 ± 0.1 ^{ns}	14.33 ± 0.7b	2.41 ± 0.1b
<i>rolC</i> – Line F	12.3 ± 0.8 ^{ns}	8.8 ± 0.4 ^{ns}	16.29 ± 0.8a	3.02 ± 0.1a

* mEq NaOH/100 g FW.

† µmoles TroloxEq/g FW.

‡ mg GAE/g FW.

functional analysis in the fruit context of target genes involved in fruit nutritional quality, e.g. by RNA interference.³¹

CONCLUSION

The health aspects of berries are evident. The challenge is to increase our understanding of the mechanisms involved, and to translate them into novel, healthier fruits.

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Declaration of interest. The authors have no interests to declare.

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