
FOOD
ENGINEERING & INGREDIENTS
Texturising dairy proteins for food applications
Enterocins: safe and natural food preservatives
Nanocomposites in food packaging
Limiting mycotoxins in stored cereals
The case for menu labelling
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FOOD ENGINEERING & INGREDIENTS

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8 Texturisation of dairy proteins for food applications

The structures of dairy proteins can be changed to improve their physical and nutritional functionality over the unmodified forms of whey proteins. Tortilla are just one of the many products incorporating such texturised proteins.

13 Enterocins: safe and natural food preservatives

Enterocins have shown their potential as food preservatives either through the addition of enterocin-producing cultures or through the use of preparations of enterocins produced *ex situ*.

27 Limiting mycotoxins in stored cereals

Naturally produced toxic secondary metabolites, mycotoxins are synthesised by certain fungi, and can have a significant impact on human and animal health. High quality cereals should be free of contamination by residues of any kind, but particularly mycotoxins. Strategies are available to help minimise contamination by natural fungal toxins.

18 Balancing the risks and benefits of red meat

Close inspection of studies purporting to show that red meat contributes to cardiovascular disease and colorectal cancer reveals that the study design is often flawed and the consistency of data is poor. The benefits as well as the risks of red meat consumption should be carefully evaluated.

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Bioactives from broccoli by-products

by R Dominguez-Perles, C Garcia-Viguera and DA Moreno

The widely accepted association of a regular intake of broccoli with health benefits has been responsible for an increase in broccoli production worldwide. The vegetable is mainly consumed as florets, i.e. the head inflorescences, which represent only a quarter of the above-ground biomass of the plant. There is a great potential for using broccoli by-products as a source of health-promoting bioactives and nutrients for dietary supplements or drugs. Since the bioactive compounds are also found in the parts of the plant that are not usually consumed, the exploitation of what is currently agrowaste as a source of bioactive ingredients would be of great benefit for the food industry.

Broccoli consumption and health

Several epidemiological studies have clearly demonstrated that the dietary intake of broccoli (*Brassica oleracea* var. *Italica*), contributes significantly to the prevention of several chronic diseases, cancers and cardiovascular pathologies, as well as generally improving gut health. The extent of the beneficial health effect is closely dose-related to the portion and number of servings consumed [1,2] The "healthy food" attributes of broccoli have been well documented and, in this context, many broccoli components have been characterised. In particular, the nitrogen/sulphur-containing compounds (glucosinolates and isothiocyanates), phenolic compounds (hydroxycinnamic acid derivatives and flavonoids), carotenoids, and essential nutrients (vitamins A, B, C, K, E, etc., and minerals K, Na, S, P, Fe, Se, Zn, etc.) have been identified in edible parts of broccoli and their levels quantitated [1, 2].

The glucosinolates in general, and glucoraphanin in particular (which is hydrolysed in the plant to sulforaphane, SFN) have attracted particular attention as being involved in the anti-cancer properties of broccoli; it is thought that the chemoprevention of diseases by glucosinolates is due to their ability to induce detoxification of enzymes [3]. The high content of natural antioxidants such as vitamin C, carotenoids, and phenolic compounds in broccoli limits the creation under stress of reactive oxygen species (ROS) [3, 4]. Likewise, broccoli contains a high level of essential minerals and microelements, which are involved as cofactors in many physiological reactions important for the maintenance of general well-being and metabolic body balance [4].

Broccoli consumption has also been associated with the prevention of heart disease via regulation of the serum level of homocysteine, a known independent risk factor in the development of coronary diseases. The anti-inflammatory effect of broccoli consumption has been attributed to SFN, which *in vitro* reduces the secretion of pro-inflammatory molecules from macrophages and inhibits the activation of central transcription factor (e.g. NF-$\kappa\beta$) in inflammatory processes and cancer. The dietary fibre content of broccoli has also been reported to improve digestive transit [3, 4].

The question of precisely how such bioactive components exert their effect, and the role of diets containing such compounds, has clear implications for human health and is thus the stimulus for intense worldwide biological and clinical research, with currently no fewer than 20 clinical trials ongoing [5].

Broccoli by-products as a source of bioactive ingredients

The vast majority of information on the phytochemical impact of broccoli comes from the edible inflorescences that are currently marketed and consumed. However, the florets only represent a small percentage of the total above-ground biomass whereas the remainder, e.g. leaves, stalks, secondary heads or non-commercial heads, may represent from 65 to 95% of the plant, existing in the form of so-called harvest remains or by-products [Figure 1]. This makes broccoli an environmentally-costly agrifood that generates tons of agrowaste of no current use.

The level of the bioactive S-N containing compounds such as glucosinolates has been shown to be even higher in stalks than in leaves [Table 1]. The same is true for the phenolic compounds with leaves containing between two and four times greater amounts of phenolic compounds than inflorescences. Even more striking, the Vitamin C level in stalks is greater than that in the leaves (~12%) or in the inflorescences (~38%). However, the level of minerals and vitamins in leaves and stalks is the same as in the inflorescences [1, 4, 6].

Factors affecting the content of bioactive compounds

The nutritional composition of broccoli is dependent on pre-harvest factors, including genetic (cultivar), physiological (organ and age) and environmental (agronomy, temperature, photoperiod, season, abiotic stress, etc.) ones [Figure 2]. The genetics of current commercially used cultivars is of particular importance. The variability in antioxidant capacity and in the concentration of different compounds present in different broccoli cultivars also suggests that it could be interesting to develop new varieties with optimal bioactive content and an improved capacity to adapt to adverse growth conditions.

The age of the plants at harvest has also been cited as a critical factor affecting the phytochemical content; the higher level of sulforaphane in the stalks of harvest-remains compared to the leaves has been attributed to this. The separate physiological role of the various plant organs is mirrored by the different levels of glucosinolates and nutrients in the organs [2, 4]. It has been shown that the levels of glucosinolate in broccoli by-products are more affected by environmental factors than those in the inflorescences, possibly because of their mobilisation from the roots under adverse growth conditions. There was more variation in the level of phenolic compounds in leaves and stalks than in flowering heads, and the same is true for vitamin C and minerals.

The nature of agricultural fertilisers used also appears to be critical for the phytochemical content in broccoli. In general, higher levels of glucosinolates and phenolic compounds are observed under 'sulphur' fertilisation. A combined 'sulphur/nitrogen' fertilisation system benefits broccoli growth, enhancing the synthesis of glucosinolates. Fertilisation however does not seem to affect the vitamin C content, which, on the contrary, appears to be strongly affected by air temperatures, with high temperatures reducing the vitamin C content. The level of aliphatic and indole glucosinolates is

The edible part of broccoli is the flowery-shaped head (inflorescence), which is made up of many individual florets. The non-edible part of the plant, i.e. the stalks, leaves etc., accounts for more of the total above-ground biomass of the plant than the edible part. The non-edible part contains large quntities of of bioactive compounds that have health-giving properties.

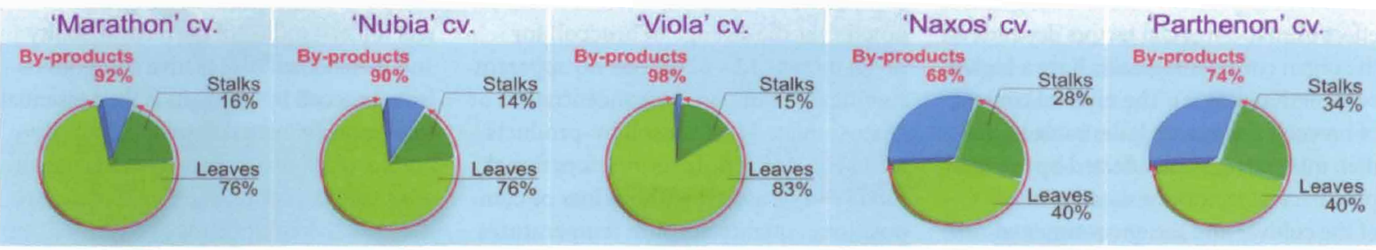

Figure 1. Fresh weight of florets (blue), leaves (light green), and stalks (dark green) [2, 4, 6]. The relative contribution of each organ to the total aboveground biomass of broccoli varies according to cultivars (genetic variability). In all cases, the amount of by-products at harvest is significantly greater than the edible part of broccoli.

Nutrients and Units	Broccoli flower clusters	Broccoli leaves	Broccoli stalks	Source (reference)
Protein (N*6.25) (g)	~2.12	~2.98	~3.40	[1]
Total lipids (g)	~0.25	~0.35	~0.40	
Monoinsaturated (g)	~0.02	~0.02	~0.03	
Poliinsaturated (g)	~0.12	~0.17	~0.19	
Carbohydrates (g)	~3.72	~5.24	~5.97	
MINERALS				
Calcium (mg)	~34.00	~48.00	~55.00	[1]
Iron (mg)	~0.62	~0.88	~1.00	
Magnesium (mg)	~18.00	~25.00	~29.00	
Phosphorus (mg)	~47.00	~66.00	~75.00	
Potassium (mg)	~231.00	~325.00	~371.00	
Sodium (mg)	~19.00	~27.00	~31.00	
Zn (mg)	~0.28	~0.40	~0.46	
Copper (mg)	~0.03	~0.05	~0.05	
Manganese (mg)	~0.16	~0.23	~0.26	
Selenium (μg)	~2.10	~3.00	~3.40	
VITAMINS				
Vitamin C (mg)	~66.2	~93.2	~106.20	[1]
Thiamin (mg)	~0.05	~0.07	~0.07	
Riboflavin (mg)	~0.08	~0.12	~0.14	
Niacin (mg)	~0.45	~0.64	~0.73	
Pantothenic acid (mg)	~0.38	~0.54	~0.61	
Vitamin B-6 (mg)	~0.11	~0.16	~0.18	
Folate (μg)	~50.00	~71.00	~81.00	
Vitamin A (IU)	~2130	~16000	~456	
Bioactive phytochemicals in broccoli				
GLUCOSINOLATES				
Total	~3.00 - ~17.70*	~2.09 - ~3.67***	~3.27 – ~4.50***	
Aliphatic glucosinolates	~1.30 - ~4.00*	~0.80 - ~1.70***	~2.43 - ~2.48***	
Indole glucosinolates	~1.80 - ~13.80*	~1.25 - ~1.81***	~0.61 - ~1.11***	
PHENOLIC COMPOUNDS				[2, 4, 6]
Total	~15.8 - ~176.4**	~99.38 - ~135.64***	~9.78 - ~11.74***	
Chlorogenic acid derivatives	~15.80 - ~65.40**	~42.13 - ~112.44***	~6.56 - ~9.66***	
Flavonoids	~14.5 - ~38.20**	~11.28 – ~30.59***	~0.11 - ~0.24***	
Sinapic acid derivatives	~25.40 - ~82.50**	~9.85 - ~26.55***	~1.14 - ~1.44***	

*μmol g-1 dw, **mg Kg-1 fw, ***mg g-1dw

Table 1. Nutritive (amounts in 100 g of fresh weight) and bioactive composition of broccoli.

not influenced by either genetic or environmental factors, whereas the synthesis of aliphatic glucosinolates is mainly regulated by genetic factors (~60%) with environmental factors having only a minimal effect. On the other hand the profile of indole glucosinolates, is mainly influenced by environmental factors rather than the genotype [2, 4, 6].

As for antioxidant compounds, they have been shown to be more dependent on the cultivar than on environmental factors. For example, the vitamin C content is clearly affected by the cultivar grown. However the precise evaluation of the effect of environmental factors depends on the organ considered (stalks have a higher content than leaves). The mineral content of broccoli also makes it desirable in the diet; minerals are also affected by pre- and post-harvest factors. In short, control of the cultivar and the environmental conditions could significantly improve the nutritive value of broccoli [2, 4].

Post-harvest factors are also critical to obtain broccoli foods with high levels of healthy phytochemical contents and the use of appropriate storage conditions prior to sale and consumption is critical to ensure quality. Parameters such as temperature and relative humidity should be controlled so the use of modified and/or controlled atmospheres can be very useful in this respect [Figure 2]. Both broccoli inflorescences and by-products are perishable; storage at < 4 °C and 90% relative humidity is ideal to avoid degradation of the bioactive components. However, it should be noted that the storage of broccoli for seven days at 12 - 22 °C has no apparent significant effect on the concentration of glucosinolate [4]. Broccoli by-products for use in new products and functional foods can be stored without loss of compositional quality at room temperatures if high humidity and exposure to light is avoided.

The use of controlled atmospheres has been shown to be a suitable alternative for storing broccoli inflorescences, and can double the retail period compared to regular storage conditions. Such an approach can be useful whenever the pre-distribution temperature is a problem but may significantly increase the process costs of broccoli by-products. Increased glucosinolate content has been reported in broccoli-based foods packed under controlled atmospheres. Low temperature storage at cold or freezing conditions can also reduce the levels of phytochemicals in the stored material.

Thermal processing

Thermal processing of broccoli, e.g. by blanching or boiling, usually reduces the glucosinolate content significantly whereas the effect on the levels of phenolic compounds and vitamin C depends on which precise thermal treatment has been applied [3, 4]. The reduction of the level of bioactive compounds in broccoli during thermal processing has been attributed to the lack of an internal structure that facilitates the diffusion of the phytochemicals. The involvement of enzymatic reactions in the decrease of bioactive components during thermal processing has been excluded since the thermal treatment inactivates the enzymes responsible. Overall, broccoli is still considered as a good source of phytochemicals and essential nutrients even after different thermal processes in domestic and industrial conditions [3, 4].

Bioavailability

The bioavailability of broccoli phytochemicals is critical for human consumption to ensure that the health-promoting attributes of broccoli are fully utilised in novel functional foods and ingredients. A real understanding of the bioavailability and metabolism of bioactive compounds from broccoli by-products is thus essential to support industrial development. The development of new matrices must ensure adequate absorption and distribution of the bioactive compounds so that they can exert the positive effect described in many *in vitro* and *in vivo* studies.

The intake of excessive amounts of glucosinolate-rich foods could however, in theory, give rise to undesirable effects. Further safety studies are necessary to establish the optimal dietary intake levels or recommended daily amounts so that the health-promoting effects can be obtained without any risk of undesirable side effects.

The potential of broccoli by-products for the industry

The use of broccoli by-products has so far been restricted to the production of some fibre for animal foodstuff and feeding purposes or for the extraction of glucosinolate. Nowadays, however, the potential use of broccoli as a cost-effective source of ingredients for the food and drug industry is receiving much more attention from the scientific community [2].

There is increasing interest in the bio-conversion of broccoli by-products into high-added-value products and this has resulted in the development of methods for the determination and isolation of SFN as a single ingredient. Such methods for the conversion of glucoraphanin to SFN must be efficient and equally applicable to both fresh and lyophilised broccoli by-products.

The use of broccoli agro-wastes, i.e. harvest remains, as animal feed for goats and sheep can partly reduce the environmental effects of the annual accumulations in large production sites such as those found in the Murcia region of south east Spain. However, this animal feed use is still insufficient to deal with all the by-products generated from this highly important and economically relevant activity in Spain, which is the main EU producer of broccoli.

The exploitation of the excess broccoli biomass for either energy production or as feedstock could increase over the next few years as the need for increased food production to feed future generations expands. Total substitution of the fossil fuel sources currently used for energy production by biomass would require double the amount that is available today. In order to avoid conflict between the use of biomass for food or for energy it will be necessary to develop strategies for using agrifood biomass and by-products more efficiently.

One of the most significant handicaps of the agrifood sector in general is the environmental impact of the by-products generated. This applies not only to broccoli but also to by-products from other agricultural products, such as guava leaves and cereals [7], tomato seeds [8] and cocoa fibres [9]. Efficient use of agrowastes or by-products is however still limited due to the lack of appropriate industrial procedures sufficiently developed to be economically feasible. However, the demand for food for the current population and for future generations could provide the stimulus for the agricultural and food production sectors to develop just such efficient, inexpensive and environmentally friendly methods for biowaste treatment. It is becoming more and more important to transform bioburden into bioactives. A good example is the use of broccoli by-products as a source of glucosinolates, phenolics, vitamins and minerals.

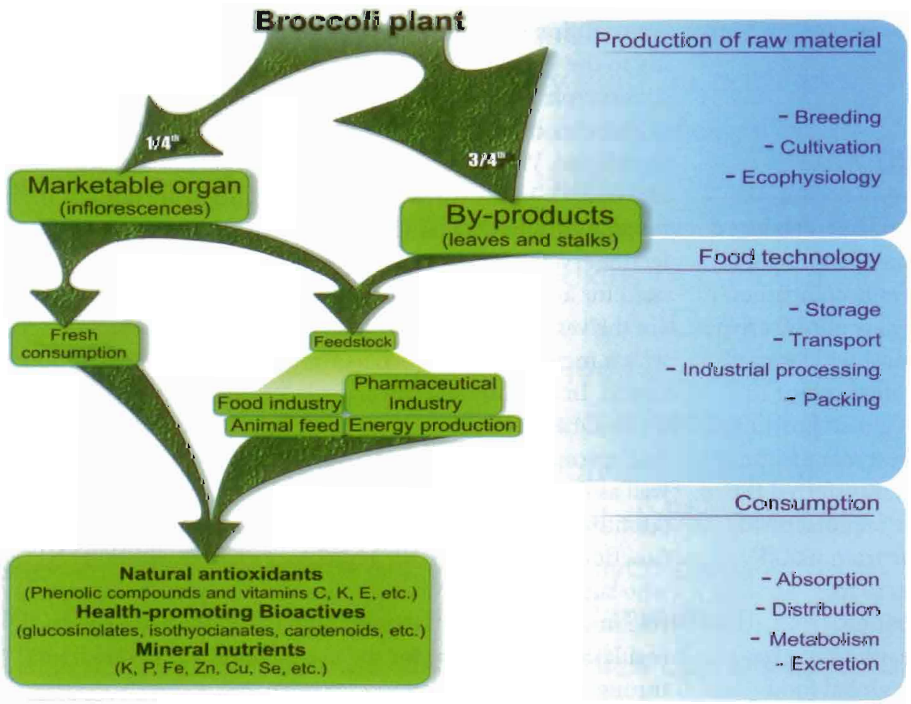

Figure 2. Processing broccoli and the potential for innovative use of byproducts.

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