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Bioactives in Fruit and Vegetables and their Extraction Processes : State of the Art and Perspectives

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Abstract

Fruit and vegetables are rich in bioactive compounds that contribute to prevention of a number of degenerative diseases. These bioactive compounds are also present, often in even higher concentrations, in the co-products from fruit and vegetable processing. This makes these co-products an attractive source for extraction of bioactives, or extraction bioactives an attractive valorisation of the co-products. There has been recently renewed interest in extraction methods, notably with process intensification using physical phenomena and the search for alternative solvents. This paper will review the main bioactives in fruit, vegetables and their co-products, the precautions to preserve these bioactives in the food processing chain, and the new developments in the extraction methods.

Keywords : micronutrients, functional properties, stability, process intensification, solvent

Introduction

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The increase in demand for naturalness from consumers and the trends towards plant-based foods has sparkled a renewed interest in fruit and vegetables and their co-products as sources of bioactive and functional components. Traditionally, most hydrocolloids (polysaccharides) as well as a number of colorants (anthocyans, carotenoids, betains) are extracted from plants. A high level ("five-a-day") of consumption of varied fruit and vegetables is associated with better health, and there has been much interest in identifying the molecules behind this effect. In recent years, this has progressed beyond the oversimplifying "antioxidant" hypothesis towards identification of mechanisms by which these molecules, or rather their metabolites, interact with specific targets in the Human organism (Dangles, 2012).

Concurrently there has been renewed interest in methods to obtain these bioactive components either for food uses, which are mostly relevant for molecules with functional properties (texture, colour, antioxidant, even aroma or taste), as food supplements, though their use as isolated molecules has proven disappointing, or as cosmetics. For economical reasons, preferred sources are the co-products from fruit and vegetable processing, as these are cheap and often highly concentrated in some bioactives. The ideal situation would be a complete use of the fruit and vegetable biomass in a biorefinery that would take into account food uses, high value bioactives as well as use of the remaining bulk material. A challenge is to conciliate the different aims and qualities of the products, as well as deal with availability issues.

Classical extraction techniques such as maceration or supercritical fluid have been supplemented with many "assisted" extraction techniques where an additional

physical phenomenon is used to intensify the process. There has been an explosive growth of publications on these topics since the year 2000. The physical phenomena range from ultrahigh pressure to void (DIC instantaneous controlled depressurization), from microwave to ultrasound or electric fields. Many of these techniques were first developed for analytical purposes in order to have true quantification of the bioactive molecules, and some have proven to be profitably upscalable, at least to pilot level. Newer challenges concern the solvents used in the extraction, as REACH comes into application, and costs of solvent recovery become more and more significant. Biosourced solvents, subcritical liquids or ionic liquids thus appear now more frequently in the scientific literature, and also pose specific challenges.

1 Bioactives in fruit and vegetables: chemical classes and activities

The very notion of "bioactive" or bioactive compounds can have different definitions in the scientific literature. It is generally associated with the existence of a positive effect on chronic pathologies, with different levels of proof (Amiot et al., 2012). In the most common meaning of the term, it does not cover vitamins, although proven to be indispensable for human health, dietary fibers, or compounds with demonstrated acute pharmacological or negative activities (notably alkaloids)". Indeed fruit and vegetables by definition have no detectable acute effect in a normal dietary pattern, at least in the Western world, while many drugs were originally identified in plants, giving rise to the large field of phytochemistry. Some chemical classes such as saponins are "borderline". The level of proof or activity needed for an health claim appropriate to a functional food or nutraceutical is therefore a tight balance.

In terms of plant physiology, most of these compounds are secondary metabolites, i.e. they are not necessary to the basic metabolism (energy production, protein synthesis…) of the plants. It is generally believed that their role *in planta* concerns interactions with the outer environment, where they may act as protection against UV radiation (flavonols), as deterrents against predators (glucosinolates, some polyphenols), as attractants for pollinisation (anthocyans).... The underlying chemical complexity explains that relatively few of these molecules have been studied for their action in preventing life-style diseases, and the relatively low levels of proof for many of them. This also may explain why most developments have been concentrated on a few classes that are either remarkably abundant (like the polyphenols or carotenoids) or remarkably active (glucosinolates, isoflavones).

Table 1 summarizes the classes of compounds that are most commonly called "bioactives" and which can be found in fruits or vegetables *sensu largo*, i.e. including herbs and spices, but not medicinal plants. Some of the classes are very specific of a given botanical family, for example the capsaicinoids (Capsicum) or glucosinolates (Brassica) while others are very widespread, like the phenolic acids or flavonols.

Table 1: The main chemical classes of bioactive compounds in fruit and vegetables (from Renard et al., 2012)

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Many of these compounds have antioxidant properties. It is now generally recognized that the health effects of bioactives are mediated by specific interactions of their circulating metabolites with cellular targets (Dangles, 2012). However the antioxidant properties may be relevant when focussing on stabilisation of food or cosmetics, for replacement of synthetic antioxidants such as butylhydroxytoluene or prevention of oxidation of polyunsaturated fatty acids in the gut (Brewer, 2011).

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2 Co-products from fruit & vegetable processing and their stabilization

Many of the secondary metabolites are concentrated in the outer, least palatable, parts of fruit and vegetables and therefore are present in high concentrations in coproducts from fruit and vegetable processing (Martins & Ferreira, 2017; Banerjee et al., 2017; Baiano et al., 2016; Strati & Oreopoulou, 2014). However these high concentrations are relative: polyphenols may reach from 10 to 50 g/kg dry weight in pomaces from fruit juice extraction, while carotenoids stay under 10 g/kg even in tomato by-products (Lavelli & Torresani, 2011) dietary fibers represent over 500 g/kg (Kolodziejczyk et al., 2009; Garcia et al., 2009).

A major limiting factor is that of an eventual co-product resource, as the question of bioactives can not be addressed from the target property but from the availability. We do not ask ourselves "from what can I extract this molecule of interest" but "what can I do with the co-product I have". Another point to be considered is the high variability of the resource: concentrations of the target compounds are likely to vary, sometimes over a factor 10, depending on the exact variety (and its maturity stage) (Garcia et al., 2009).

The alternative is establishment of a specific production chain, which demands a demonstrated and sufficient final value of the bioactive. Indeed, there are often alternatives using specific plants or other parts of plants that make more sense economically than relying on fruit and vegetables. For example lutein is extracted from *Tagetes* flowers and alfalfa, β-caroten from specific varieties of carrot with high concentrations, phloridzin is much more abundant in apple leaves than apple fruit (Gaucher et al., 2013), ….

In practical terms, this means that research on bioactives from fruit and vegetables is still very much "opportunity-driven" i.e. focussed on products that meet conditions of availability and potential interest. Availability of a co-product is in turn linked to the existence of a production chain. The most studied sources are thus pomace from grapes (wine) (Barba et al., 2016), tomato (pulp & concentrate), apples (juice), berries (juice), peels from Citrus (juice) or mango (puree or dried mangoes), sugarbeet pulp from sugar refining, waste water from olive processing, peels from onion or salad. Dietary fibers and polyphenols are the main chemical classes of interest, only tomato co-products being abundant and rich in carotenoids.

The treatments that take place for the initial product preparation (for example preparation of cloudy or clear juice, Kolodziejczyk et al., 2009) as well as those used for stabilisation of the co-product will also impact its composition and potential as source of bioactive. Two phenomena must be taken into account when envisioning extraction of polyphenols from e.g. apple pomace: one is that the molecules present in the pomace may not be not those of the original fruit, as there can be extensive enzymic oxidation during the juice extraction and pomace processing (Herrera-Ramirez et al., 2012) as well as chemical degradation, and the other is that some polyphenols, notably the condensed tannins, form strong adducts with the cell wall material (Renard et al., 2017) that may become un-extractable. Whether the pomace was initially treated to inhibit enzymes, the mode and temperature of drying, its final water content and storage temperature all impact the final composition (Herrera-

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Ramirez et al., 2012; Lavelli & Corti, 2011; Lavelli & Torresani, 2011)Rahja et al., 2014; Yu et al., 2013). Carotenoids, in contrast to polyphenols, appear less stable at the lower aw values (Lavelli & Torresani, 2011).

A major difficulty in going from a laboratory demonstration to actual industrial production lies in the seasonal availability of fruit and vegetable and also of their coproducts. For example, industrial tomatoes are harvested and processed over a period of about 2 months. Therefore their pomace, although remarkably high in lycopene, is only available fresh during this short period. The implementation of coproducts, which are prone to microbial waste, may require immediate processing or a stabilization step, most often by drying, which may lead to some loss of bioactive by thermal degradation of the more fragile molecules or interfere with the extraction procedure itself (Rahja et al., 2014).

3 Recent developments in extraction methods

3.1 Conventional methods

The more classical methods for extraction of bioactives rely on maceration (with more or less intense stirring) in a solvent of appropriate polarity (Galanakis, 2012). A first improvement is increasing the extraction temperature for better dissolution and lower solvent viscosity, as is done e.g. in heated reflux extraction (which also takes advantage of concentration equilibria) or in a Soxhlet apparatus (which further has the advantage of separating the soluble from the insoluble fractions).

Supercritical fluids, and among them mostly carbon dioxide, have been used industrially in the food and cosmetics industry. Supercritical carbon dioxide has a low critical temperature and pressure (31°C, 7.39 MPa), which are of interest for labile molecules, and its polarity can be increased by adding co-solvents (e.g. ethanol). It is GRAS and non explosive, relatively cheap, and the extracted compounds can be recovered by evaporation of the gas. It is commonly used for extraction of essential oils and has a good potential for carotenoids.

Enzymic pretreatment have also been used in improving extraction yields, because it has long been recognised that plant cell walls are one of the limiting factors. However pretreatments with cellulases or pectinases demand long incubations (typically a few hours) in aqueous media.

Table 2: Conventional and emerging technologies for extraction of plant bioactives

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3.2 Emerging technologies

More recent developments concern the use of non-thermal concepts to facilitate extraction without risking overheating of the matrix, and decreasing energy use. Recent reviews describe application of conventional and emerging technologies to extraction of various classes of bioactives (Ameer et al., 2017; Lu et al., 2017; Poojary et al., 2016; Gil-Chavez et al., 2013; Wijngaard et al., 2012). All aim to destroy cell integrity by elimination of the cell membranes or cell walls. The most studied applications since 2005, as identified from scientific publication trends, concern microwave and ultrasound-assisted extraction (Mandal & Tandey, 2016).

Microwaves are electromagnetic waves, generally used at 2.45 GHz, which interact with polar molecules (typically water, ethanol…) and generate heat (Zhang et al., 2011). In microwave-assisted extraction the moisture inside the cell is heated and its evaporation increases the porosity of the biological matrix, which in turns allows better penetration of a solvent. The elevated temperature also generally increases solubility and improves yield. Its main advantage is reduction of the extraction time and solvent use. Elevated temperatures may still result in some degradation of the more labile molecules.

Ultrasound (> 20 KHz) improves extraction through acoustic cavitation: above certain energy levels the acoustic waves interact with the solvent and dissolved gas by creating free bubbles that can expend to a maximum size and violently collapse, generating locally extreme heat and pressures (Tiwari, 2015). Due to this cavitation phenomenon the cell walls can be ruptured, providing channels for solvent access, and mass transfer is improved. The small size of the bubbles means that the heat generated upon collapse can be dissipated rapidly and bulk temperature increase can stay limited. However liquid-solid separation may be hindered as ultrasound can lead to swelling and disintegration of the plant material.

Among electrotechnologies, different pulse protocols and intensities of electric fields have been used to generate cell disintegration and thus enhance extraction of intracellular compounds. The most studied is Pulsed Electric Fields, in which the sample is submitted to very short periods (several nanoseconds to several milliseconds) of an intense electric field. This causes the formation of pores in the cell membranes (electroporation) which helps in solvent diffusion and facilitates mass transfer (Rastogi, 2003; Puertolas et al., 2012)). PEF is also used to increase juice yields, i.e. non selective extraction of intracellular fluids.

Pressurized liquid extraction (or accelerated solvent extraction) relies on increased temperature and pressure in a solvent that is kept below its boiling point to enhance mass transfer and modify surface equilibria in solid – liquid extraction (Mustafa & Turner, 2011). Pressure is used primarily to maintain the solvent n the liquid phase, though it may also facilitate solvent entrance in the pores of the matrix. It is commonly used in analytical quantification of bioactives but also pesticides. The elevated temperature also modulates the polarity of the solvent and thus its extraction selectivity.

Negative pressure cavitation is a patented technology that aims to produce cavitation by depression to generate an intense erosion of solid particles, and increase turbulence and mass transfer from the solid matrix to the solvent (Roohinejat et al., 2016).

Another potential pretreatment is high pressure homogeneization (Xi, 2017; Corrales et al., 009), a wet milling process where plant particles are disintegrated by high intensity mechanical stresses as a consequence of the liquid flow through an homogeneization chamber at high pressures (50-500 MPa). It can be used on wet samples.

Another method which has been proposed for sample disintegration prior to extraction is instant controlled pressure drop (DIC) (Mounir et al., 2014). In DIC, the sample is subjected for a short time to saturated steam then to a sudden pressure trop at low pressures. This causes instantaneous vaporisation of water, resulting in cell wall expansion and a rapid cooling. The instantaneous vaporization allows recovery of essential oils, while polyphenols or carotenoids are more easily extracted from the remaining fine powder. Similar results may be obtained by intense grinding, provided care is taken to prevent temperature increase during grinding.

3.3 Alternative solvents

The solvent choice is a concern as it impacts selectivity, removal and disposal method, costs and safety. The REACH directive has notably been an incentive for "green" solvents in replacement to *n*-hexane. For example, among alcohols ethanol is often proposed due to its low boiling point and GRAS status, but may not be efficient for less polar molecules such as carotenoids. Water is also often tested, alone or in mixture with ethanol or acetone, in spite of its high energy requirements for evaporation. More apolar solvents, mostly terpenes (limonene, alpha-pinene) have also been proposed (Filly et al., 2015). Another option is the use of subcritical solvents such as liquefied gases (e.g. *n*-butane), which may ally low polarity and

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facile solvent elimination by decompression at or close to room temperature (Rapinel et al., in press). However safety issues must be addressed for these new solvents.

Recently ionic liquids have been proposed for extraction of bioactives. Ionic liquids *senso strictu* are organic salts in a liquid state. Although they are viscous, their polarity can be adjusted across a wide range of hydrophobicity / hydrophilicity, and some of them are distillable in conditions compatible with recovery of bioactives (Almeida et al., 2014; Lu et al., 2013)). The most recent trend in this field is the use of "natural deep eutectic solvents" (NADES), which are combinations of natural components such as sugars, organic acids or aminoacids, with tailored solvent properties (Dai et al., 2013; Radosevic et al., 2016). However solvent removal becomes more complex.

Conclusion

A lot of experimental work has been devoted in the last 10 – 20 years to development of "alternative" technologies for extraction of bioactives from plants, including fruit and vegetables. It is time that this body of experimental data, which mostly relies on statistical optimisation of yields, gives place to a more mechanistic oriented research. In addition, post-extraction treatments (elimination of the solvent, purification…) are the poor relatives in this research.

A number of technical issues have to be addressed before and after extraction: will the raw material be stabilised or only extracted fresh, i.e. over a short period? If it is stabilized, how do to it with the least loss of bioactive? Is a purification step needed? Most of these molecules are only present in low concentrations, therefore their extraction will still leave large amounts of waste: can that waste in turn become a coproduct? This requires anticipation and conceiving the whole chain in an integrated manner for valorisation of all the biomass.

Two main conditions must be met for the extraction of bioactives from fruit and vegetables to be economically interesting:

- The activity or functionality of the molecules must have a market potential;
- It must be present in an amount compatible with its market in a cheap co-

product – or the market potential must be sufficient for specific production. Colour or antioxidant capacity are properties relatively easy to assess, which may explain why they constitute the main fields of application for extracts from fruit and vegetables. Having sufficient data for an health claim or nutraceutical use is a totally different proposition as the cost of demonstrating the claim becomes high.

All of this may explain why, in spite of much research, few fruit and vegetable coproducts are actually used at industrial scale for production of bioactives (Galanakis, 2012): anthocyans from grape skin, (oenological) tannins from grape seeds, lycopene from tomato waste (Strati & Oreopoulou, 2014), polyphenols from olive mill waste water and flavonoids from citrus peel. Production of oil from grape seeds or kernels of plums, apricots and peaches, dietary fibers from vegetable wastes and pectin extraction (from citrus peel, apple pomace or sugar-beet pulp) may also be counted in this valorization of fruit and vegetable co-products. The few successful examples

need to be carefully analysed, not from a technological point of view but from a socioeconomic and legislative point of view to identify the factors of their success.

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