Review

Enzyme-assisted extraction of bioactives from plants

Munish Puri[1,2], Deepika Sharma[2] and Colin J. Barrow[1]

[1] Centre for Biotechnology and Interdisciplinary Sciences (Biodeakin), Institute of Technology Research and Innovation (ITRI), Deakin University, Victoria 3217, Australia
[2] Fermentation and Protein Biotechnology Laboratory, Department of Biotechnology, Punjabi University, Punjab 147002, India

Demand for new and novel natural compounds has intensified the development of plant-derived compounds known as bioactives that either promote health or are toxic when ingested. Enhanced release of these bioactives from plant cells by cell disruption and extraction through the cell wall can be optimized using enzyme preparations either alone or in mixtures. However, the biotechnological application of enzymes is not currently exploited to its maximum potential within the food industry. Here, we discuss the use of environmentally friendly enzyme-assisted extraction of bioactive compounds from plant sources, particularly for food and nutraceutical purposes. In particular, we discuss an enzyme-assisted extraction of stevioside from *Stevia rebaudiana*, as an example of a process of potential value to the food industry.

Plant-based bioactives

Bioactives are metabolites synthesized by plants for self defence and other purposes and have the potential to be used by humans for a variety of applications. Essential and non-essential bioactives are present in a vast range of foods (such as fruits, vegetables and grains) and consumed as part of the human diet. Evidence is growing that use of bioactives might help to promote optimal health and reduce the risk of chronic diseases such as cancer, coronary heart disease, stroke and Alzheimer's disease [1,2]. Bioactives are obtained selectively from plants as specialty chemicals and can be used as nutraceuticals, processed foods to complement a balanced diet or as drug leads. Bioactive compounds in plants are typically present at low concentrations [3]. Unfortunately, solvent-based extraction of bioactives often suffers from low extraction yields, requires long extraction times and the final product often contains traces of organic solvents, which decrease the product quality [4]. Thus, the development of an effective and selective method for bioactive compound extraction is important.

Methods such as cold pressing, super-critical fluid and solvent extraction are used to extract bioactives from plants. However, the use of organic solvents for the recovery of natural products has several drawbacks, including safety hazards, high energy input, low product quality, environment risk and toxicological effects [5]. There is a need to develop optimized and comprehensive protocols for enhanced recovery of bioactives, particularly from plants where the cell wall can inhibit extraction efficiency.

Enzyme-based extraction of bioactive compounds from plants is a potential alternative to conventional solvent-based extraction methods. Enzymes are ideal catalysts to assist in the extraction, modification or synthesis of complex bioactive compounds of natural origin. Enzyme-based extraction is based on the inherent ability of enzymes to catalyze reactions with exquisite specificity, regioselectivity and an ability to function under mild processing conditions in aqueous solutions [6]. This method also offers the possibility of greener chemistry as pressure mounts on the food industry and even pharmaceutical companies to identify cleaner routes for the extraction of new compounds [7]. Enzymes have the ability to degrade or disrupt cell walls and membranes, thus enabling better release and more efficient extraction of bioactives [8].

Enzyme-assisted extraction methods are gaining more attention because of the need for eco-friendly extraction technologies. A quantitative characteristic of enzymatic processing in industry is represented in the literature by relatively few enzyme applications. These include laccase applied in bleaching in the pulp and paper industry [9], protease/ lipase applied in leather making [10], lipase applied in the production of skin care products [11], and phospholipase applied in degumming of soybean oil [12]. A particularly useful application of enzymes increases the effect of solvent pre-treatment and either reduces the amount of solvent needed for extraction or increases the yield of extractable compounds. Enzymes such as pectinases, cellulases and hemicellulases are widely used in juice processing and beer clarification to degrade cell walls and improve juice extractability. The disruption of the cell wall matrix also releases components such as phenolic compounds into the juice, thus improving product quality.

Enzyme-assisted extraction methods have been shown to achieve high extraction yields for compounds including polysaccharides, oils, natural pigments, flavours and medicinal compounds [13–17]. Recent studies on enzyme-assisted extraction have shown faster extraction, higher recovery, reduced solvent usage and lower energy consumption when compared to non-enzymatic methods. In this review, we provide a brief description of quantitative screening of enzyme applications, comparing the overall energy consumption of systems involving enzymatic processing to systems involving conventional chemical processing. We provide a brief description of enzyme-assisted

Corresponding author: Puri, M. (munish.puri@deakin.edu.au)

0167-7799/$ – see front matter doi:10.1016/j.tibtech.2011.06.014 Trends in Biotechnology, January 2012, Vol. 30, No. 1

extraction of bioactive components from plants and discuss recent progress in this field with particular reference to stevioside.

Bioactives extraction

Extraction is the most important step in isolating different types of bioactive compounds from plants. Ideally, extraction methods should be quantitative and time saving. There are numerous methods that have recently been reported for the extraction of bioactives and these methods are summarized below.

Chemical extraction processes

Chemical extraction largely depends on the type of solvents, energy input and agitation to improve the chemical solubility and efficiency of mass transfer. The chemical methods for bioactive extraction are widely used because they are well established and easy to perform. Mixtures of acetone and water have been used as good solvents for the extraction of antioxidants [18]. Lipophilic compounds are often extracted with non-polar organic solvents such as hexane or dichloromethane. Hydrophilic constituents including lignans are extracted with polar solvents such as acetone, methanol or ethanol. In some cases, the addition of polar solvents such as water to the sample can increase the recovery of more polar compounds such as lignan glycosides [19].

Subcritical water as an extraction solvent has been explored to extract polar bioactive components from herbs and foods. A recent study showed that 80% of oxygenates from savory and peppermint are extracted with subcritical water at 5.2 MPa and 140 °C. Optimal extraction conditions such as particle size (1 mm), temperature (40 °C), contact time, solvent–sage ratio (6:1) and ethanol–water ratio affect the extraction of the active compounds. These include rosmarinic acid (RA), carnosic compounds (CS) and essential oil from dried sage (*Salvia officinalis*). In this study, the highest yields (6.9% RA, 10.6% CS and 42% oil) of the three active compounds were obtained in 3 h [20].

Derris indica seeds are a rich source of lipids. When soxhlet extracted with n-hexane for 12 h, ground seed material (2 mm particle size) was found to contain 56% crude seed oil high in linoleic acid content, which makes the oil nutritionally valuable [21]. The phenolic compounds from coffee industry byproducts (coffee pulp, husk, silver skin and spent coffee) were extracted using a mixture of isoproponal and water [22]. Examples of organic solvents used for the separation of bioactives based on their polarity are given in Table 1.

Physical extraction processes

Pressurized hot water extraction (PHE) methods offered higher phenolic compound recovery from *Salvia officinalis* when compared with ultrasound-assisted extraction (UAE), hydro-distillation and maceration with 70% ethanol. The use of methanol during UAE produced the lowest recovery with results not statistically different from maceration with 70% ethanol. Potential exists for combining ultrasound as an adjunct with the other extraction procedures to improve efficiency and yield [23]. Polysaccharides and polyphenol were also extracted from kiwi fruit (*Actinidia deliciosa*) using different concentration of ethanol in water. Ethanol (96%, v/v) extracted the maximum amounts of pectic polysaccharides (estimated as uronic acid content 1.7%) from fruit skin [24].

Microwave and ultrasound treatments have been investigated to extract pigments from strawberries. Optimal extraction was achieved using microwaves at 624 W, with a treatment time of 60 s, together with ultrasonic processing for 40 s and a ratio of material to extraction solvent of 1:6 [25]. In another example, microwave-assisted extraction (MAE) procedures were used to extract water soluble polysaccharides (WSP) from kiwi and cherry fruits. MAE was performed with 100 W of microwave power of 100 W and at 140 °C. In this study, extracted WSP yields were lower than yields obtained from boiling water extraction [26].

Sonication has been used for extraction of anolignan from *Terminalia sericea*. The roots were dried in an oven (50 °C, 7 days) and subjected to sonication for 1 h before overnight extraction with ethyl acetate on an orbital shaker. The extract was concentrated to powder form with a yield of 0.021 w/w [27].

Supercritical carbon dioxide extraction (SC-CO_2) is a promising and alternative process for concentrating flavonoids from spearmint (*Mentha spicata*) leaves with high recovery. Flavonoid compounds were extracted from spearmint using SC-CO_2. The highest extraction yield (60.57 mg/g) was obtained at 200 bar, 60 °C for 60 min. The composition of the extracted yields was greatly impacted by the operating conditions. Optimized extraction conditions (200 bar, 60 °C and 60 min) yielded a high concentration (0.657 mg/g) of luteolin among all other detected flavonoid compounds [28].

Although there are advantages and disadvantages for different bioactive extraction methods, there are general limitations to both the chemical and physical extraction methods. These general limitations include: (i) the raw material requires treatment prior to extraction; (ii) the chemicals and solvents used normally cannot be recycled, thereby increasing cost and requiring removal of hazardous waste; (iii) the methods are nonspecific and introduce batch-to-batch variation; (iv) the methods of extraction cause variation in product quality, such as a 'bitter' aftertaste due to presence of remnants of solvents; (v) solvents such as hot water cannot always penetrate into the sample core (due to cellulose fibrils in the plant tissue), resulting in low extraction efficiency. These drawbacks can be partially

Table 1. Solvents used for the extraction of bioactive compounds from plants

Polarity of solvents	Solvent used	Product	Refs
Apolar	Cyclohexane, hexane, toluene, benzene, ether, chloroform, ethyl acetate	Alkaloids, terpenoids, coumarins, fatty acids, flavanoids, terpenoids	[19,20,25]
Polar	Acetone, acetonitrile, butanol, propanol, ethanol, methane	Flavanols, lectins, alkaloids, quassinoids, flavones, polyphenols, tannins, saponins	[16–18,21]

overcome by the use of enzymatic steps within the extraction process.

Enzyme-assisted extraction processes

The successful application of enzymes for the extraction of a variety of products, including the extraction of carotenoids from marigold flower or tomato skin [15,29], vanillin from vanilla green pods [30], polysaccharide from sterculia [13], oil from grape seed [14] and polyphenols [17], indicates that enzymes can also be useful for the extraction of bioactive compounds from other plant sources. Enzyme-based extractions are the subject of continuing research and have the potential to be commercially attractive.

Enzymes have been used particularly for the treatment of plant material prior to conventional methods for extraction. Various enzymes such as cellulases, pectinases and hemicellulase are often required to disrupt the structural integrity of the plant cell wall, thereby enhancing the extraction of bioactives from plants. These enzymes hydrolyze cell wall components thereby increasing cell wall permeability, which results in higher extraction yields of bioactives. Enzymes can be derived from bacteria, fungi, animal organs or vegetable/fruit extracts. To use enzymes most effectively for extraction applications, it is important to understand their catalytic property and mode of action, optimal operational conditions and which enzyme or enzyme combination is appropriate for the plant material selected.

Enzymes have been used to increase flavonoid release from plant material while minimizing the use of solvents and heat [31]. One example of the use of an enzyme system is in the processing of pectic polysaccharide for enhancing extraction of an antioxidant [32]. The enzyme at 0.1% w/w increased extraction from 1.7 to 7.4 g/kg of raw material dry weight. A second example showed improved yield of lycopene extraction from tomatoes. Enzyme-aided extraction of lycopene from tomato tissues using cellulases and pectinases under optimized conditions resulted in a significant increase (206%) in lycopene yield versus control experiments [33]. Similarly, lycopene-assisted pancreatin digestion of tomato skin provided a 2.5-fold increase in yield. A digestion step prior to extraction by solvents was necessary to efficiently extract lycopene from the raw material [29]. As another example, cellulose, pectin and hemicellulose in grapefruit peel waste can be hydrolyzed by pectinase and cellulase enzymes into monomer sugars, which can then be used by microorganisms to produce ethanol and other fermentation products [34]. Currently, cellulase is introduced at the liquefaction step to improve the saccharification process (depolymerize hemicelluloses) in the treatment of sugarcane bagasse to produce bioethanol. The cellulases improved saccharification (~81 g/l total sugars), which significantly increased ethanol production [35]. We have recently observed that enzymes can be used to disrupt the pectin–cellulose complex in citrus peel and enhance flavonoid (naringin) production [36]. A list of some products of industrial importance [14,34–48] obtained using enzyme-assisted extraction in recent years is presented in Table 2.

In food processing, pectic enzymes are employed industrially for the extraction, clarification and concentration of fruit juices [49], extraction of pectin [43], extraction of oils [37], flavours and pigments from plant materials [14,16]. The enzymes most frequently used for oil extraction are

Table 2. List of bioactive compounds of industrial importance obtained by enzyme-assisted extraction from plants

Product type	Product	Source	Enzyme used	Maximum yield (%)	Refs
Oils and carotenoids	Oil	Grape seed	Cellulase, protease, xylase and pectinase	17.5	[12]
	Carotenoids	Marigold flower	Viscozyme, Pectinex, neutrase, corolase and HT-proteolytic	97	[13]
	Volatile oil	Mandarin peel	Xylan-degrading enzymes	15	[37]
	Carotene	Carrot pomace	Pectinex Ultra SP-L	0.0064	[38]
	Lycopene	Tomato	Pancreatin	2.5-fold	[29]
		Tomato	Cellulase and pectinase	206	[33]
	Capsaicin	Chilli	Cellulase, hemicellulase and pectinase	n.d.[a]	[39]
	Colourant	Pitaya	Pectinolytic, hemicellulolytic and cellulolytic enzymes	83.5	[40]
	Anthocyanin	Grape skin	Pectinex BE3-L	n.d.[a]	[52]
Glycosides	Sugar	Grapefruit peel waste	Cellulase and pectinase	0.6377	[34]
	Oligosaccharide	Rice bran	Cellulase	39.9	[59]
	Inulin	Jerusalem artichoke	Inulinase	n.d.[a]	[41]
	Starch	Cassava	Pectinase enzyme	45.6	[42]
	Pectin	Pumpkin	Xylase, cellulose, β-glucosidase, endopolygalacturonase and pectinesterase	14.0	[43]
Others	Vanillin	Vanilla green pods	β-glucosidase and pectinase	14–21	[30]
	Flavonoid (naringin)	Kinnow peel	Recombinant rhamnosidase	n.d.[a]	[36]
	Phenolics	Citrus peel	Celluzyme MX	65.5	[44]
	Proteins	Lentils and white beans	Glucoamylases	50.3	[45]
	Polyphenols	Grape pomace	Pectinolytic	98.1	[46]
	Catechins	Tea beverage	Pepsin	80	[47]
	Lignans	Flax	Cellulase and glycosidase	40.75 mg/g	[61]
	Soluble fibre	Carrot pomace	Cellulase-rich crude preparation	77.3	[48]

[a]Abbreviation: n.d., not defined.

cellulase, α-amylase and pectinase. Enzyme incorporation in oil extraction processes produces a high content of antioxidant compounds in olive oil [50], defatted meal of evening primrose and borage oil [51]. Enzyme (pectinases and β-glucanases) usage further improved oil yield by 15% compared with the control, which corresponds to an oil yield increase of about 2 kg olive oil per 100 kg of olives [50]. The quality of oils obtained by enzyme treatment is relatively good as compared with hexane-extracted oils. Thus, enzyme-assisted cold pressing (EACP) is an ideal alternative for oilseed extraction because of its nontoxic and nonflammable properties.

An increase of phenolic compounds (25.90–39.72%) and sugars (12–14 g/l) have recently been observed after enzyme-assisted extraction from citrus peel and grape pomace [44,46]. Enzyme application improved the extraction of total phenolic content from 32.33 to 61.90%. In another example, more pigment (anthocyanin) was extracted during the vinification process when enzymes were applied on grapes skin [52]. Defatted grape seed meal is high in phenolic antioxidants. Enzyme-assisted oil extraction gave a 59.4% yield improvement when compared with a non-enzymatic oil extraction process [53].

Enzymes also increase the yield of extraction of polyphenols and anthocyanins from blackcurrant juice. Commercial pectinolytic enzymes decreased particle size from 500 to 1000 μm to <125 μm and increased the phenolics yields from 1.6- to 5-fold in pomace [54]. The effect of *Thermobifida fusca* cellulase on apple peel produced an improvement in the yield of phenols and reduced sugar

Box 1. An overview of stevioside and methods of its extraction

Stevioside, a high intensity non-nutritive sweetener, is extracted from the leaves of *Stevia rebaudiana*, a sweet plant native to north-eastern Paraguay. It is a white crystalline water soluble powder, which is 200 to 300 times sweeter than sucrose [66]. The chemical structure of stevioside is given in Figure I.

Stevioside is present intracellularly in plant leaves and its expression levels are higher in mature tissues compared to young rapidly growing tissues [66]. Its content varies between 4 and 20% of the dry weight of the leaves depending on the cultivar and growing conditions. The advantages of stevioside as a dietary supplement include its high stability, non-calorific nature and protection of dental health [67]. Decreased sugar intake due to the use of stevioside opens the possibility for its use by diabetic [68], anti-amnesic [69] and phenylketonuria patients and obese persons [70]. The steviol glycosides are used to sweeten a number of foods in Asia and South America [71]. *Stevia* leaves are used to prepare a sweetened tea in a number of countries [72]. Maximum use levels of *Stevia* glycosides are provided in Table 3 [73].

There are a number of patents on the chemical-based extraction of natural compounds. Most of the reported processes use coagulating and organic solvents. Some of the selected processes utilize chromatographic separation and chelating agents followed by solvent extraction [74]. Most of the extraction methods involve four key processes: aqueous or solvent extraction, ion exchange, precipitation or coagulation by filtration, then crystallization and drying. The extraction is carried out with a mixture of butanol or isobutanol and a less polar solvent, such as benzene, chloroform or hexane. Selective adsorptions on zeolites X and A have been studied subsequent to *S. rebaudiana* extraction for extract clarification. *Stevia* extract in contact with the zeolite CaX showed highest clarification [75].

Methanol gave improved (5.2%) extraction yield compared with water (4.7%) when used in PHE for the isolation of stevioside from *S. rebaudiana* within the temperature range of 110–160 °C. However, water represents a greener alternative to methanol, therefore it can be a preferable solvent even with slightly lower yields. The glycoside composition of extract from *S. rebaudiana* leaves was optimized (36.6 mg/g) using SC-CO_2 [76]. Pressurized hot water extraction (PHWE) and MAE showed that stevioside (13.90 and 21.37 mg/g) and rebaudioside A could be extracted at elevated temperature using water without the addition of organic modifier or solvent [77]. On using UAE, the yield (43.62%) of extracts increased by a factor of 1.5 over classical extraction procedures. The optimal extraction conditions were an extraction temperature of 68 °C, a sonic power of 60 W and an extraction time of 32 min [78].

A variety of extraction methods has been used for the extraction of stevioside [79]. However, most methods involve using solvents such as chloroform–methanol or propylene glycol followed by decolorization, coagulation and crystallization, resulting in low yields. Enzyme-assisted extraction can be used to improve yields of stevioside and also for improved extraction of a variety of bioactives from various natural sources.

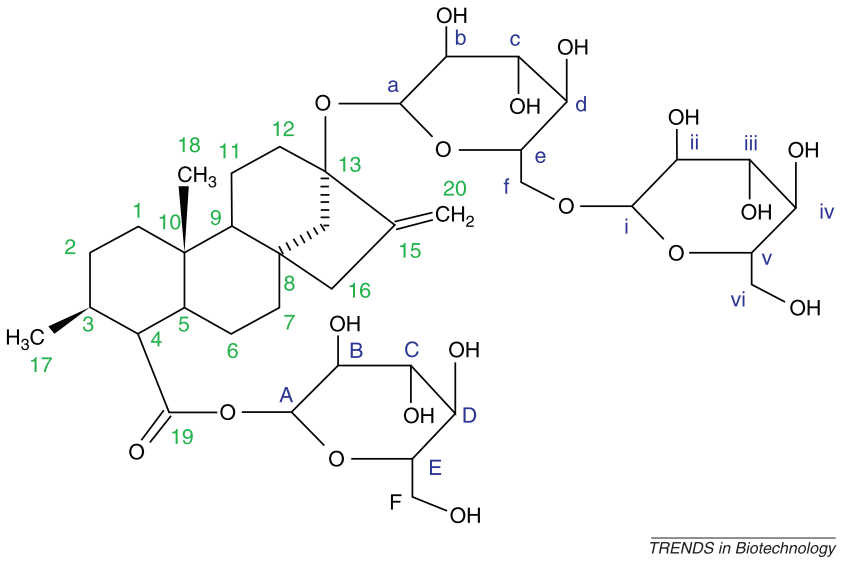

Figure I. Chemical structure of stevioside based on NMR analysis [80].

Table 3. Maximum stevioside levels permitted in various foods [73]

Food type	Stevioside level (mg/kg)
Beverages	500
Desserts	500
Yogurt	500
Cold confectionery	500
Sauces	1000
Pickles	1000
Delicacies	1000
Sweetcorn	200
Bread	160
Biscuits	300

production and antioxidant capacity. Approximately 60 mg of reducing sugar equivalent was produced per g of apple peel when treated with cellulase enzyme compared with only 20 mg using a non-enzymatic extraction method [55]. The most important parameters for assisted aqueous extraction from five different citrus peels have been determined to be the condition of the peels, temperature of extraction, type of enzymes, enzyme concentration and citrus species [44]. A process for enzyme-assisted extraction of polyphenols from grape pomace has recently been developed to pilot–plant scale. The introduction of a120 min enzymatic step during treatment of pomace resulted in 65.8% increase in anthocyanins yield. Economic feasibility of the process was enhanced by minimizing the enzyme concentration required for efficient extraction [56]. Similar results were observed for extracting antioxidants from blackcurrant pomace with a commercial cellulase enzyme [57].

Enzymes are normally applied in red wine clarification. Improvement in chromatic (colour) and sensory characteristics of enzyme-treated wine in comparison with control wine is normally observed [58]. In addition, an enzyme-assisted extraction method proved to be more suitable for recovery of catechins (~100% yield) from various milk tea beverages instead of acid precipitation (~74% yield) [47].

A cellulase enzyme was employed to improve the extraction of oligosaccharides from defatted rice bran. The enzyme was effective in breaking down the fibrous matrix in rice bran, facilitating the subsequent release of oligosaccharides. The extraction yield increased from 13.4% (control) to 39.9% with 2% cellulase [59]. Similarly, pectinase from *Aspergillus awamori* was demonstrated to improve protopectin extraction from pumpkin. A 3 h enzyme hydrolysis improved protopectin yield (14%) over an acid-based extraction (7%) process [43]. A commercial cellulase improved extraction of flavonoids from *Ginko biloba* leaves. Enzyme from *Penicillium decumbens* resulted in far better degradation of powdered dried leaves than *Trichoderma reesei* cellulase and *Aspergillus niger* pectinase. The extraction yield under optimized conditions was 28.3 mg/g dry weight, which was 102% higher than extraction without enzymes [60]. Extraction of lignans (secoisolariciresinol) from flax (*Linum usitatissimum*) hulls and whole seeds was improved by using cellulase and β-glucosidase. Both enzyme preparations proved to be effective for extracting lignin. Under optimized conditions, the highest yield of lignin was 40.75 mg/g in hulls and 15.20 mg/g in whole seeds, representing an increased yield compared to previous published methods [61]. We have recently observed the feasibility of enzyme-assisted extraction of stevioside (a glycoside sweetener) from *Stevia rebaudiana*, which provides a higher yield than conventional solvent extraction methods (Box 1). Response surface methodology (RSM) optimized the enzyme-assisted extraction conditions to maximize extraction yield. The results demonstrated that enzymatic-assisted extraction is highly efficient and a viable alternative to conventional solvent extraction of stevioside (M. Puri *et al.*, unpublished).

The traditional one-factor-at-a-time approach to process enzyme-assisted extraction optimization is time consuming and can ignore the interactions among various factors. RSM enables evaluation of several process parameters such as time, temperature, enzyme type and concentration. It is a powerful and efficient mathematical approach [62] that has been successfully applied for developing, improving and optimizing biochemical and biotechnological processes related to food systems, including production of pectic polysaccharide [13], enzymes [63] and phenolic antioxidants from fruits [64].

Benefits of enzyme-assisted extraction

The application of enzymes for complete extraction of bioactives without the use of solvents is an attractive proposition. Enzyme pretreatment of raw material normally results in a reduction in extraction time, minimizes usage of solvents and provides increased yield and quality of product [7,16]. Prior knowledge of the cell wall composition of the raw materials helps in the selection of an enzyme or enzymes useful for pretreatment. Decreased solvent use during extraction is particularly important for both regulatory and environmental reasons, providing a 'greener' option than traditional non-enzymatic extraction.

Enzyme-assisted extraction of bioactive compounds from plants has potential commercial and technical limitations: (i) the cost of enzymes is relatively expensive for processing large volumes of raw material; (ii) currently available enzyme preparations cannot completely hydrolyze plant cell walls, limiting extraction yields of compounds, including the extraction of stevioside; (iii) enzyme-assisted extraction can be difficult to scale up to industrial scale because enzymes behave differently as environmental conditions such as the percentage of dissolved oxygen, temperature and nutrient availability vary. However, if the above limitations can be overcome, then enzyme-based extraction could provide an opportunity to not only increase extraction yields, but also to enhance product quality by enabling the use of milder processing conditions such as lower extraction temperatures.

Process development for enzyme-assisted extraction

Unlike other non-thermal processes, such as high hydrostatic pressure (HP), compressed carbon dioxide (cCO_2), SC-CO_2 and high electric field pulses (HELP), enzyme-assisted extraction can readily be tested on the laboratory scale. Enzymes can be selected for specific functionalities as well as for optimum process conditions, such as temperature and concentration. Although enzymes normally function at

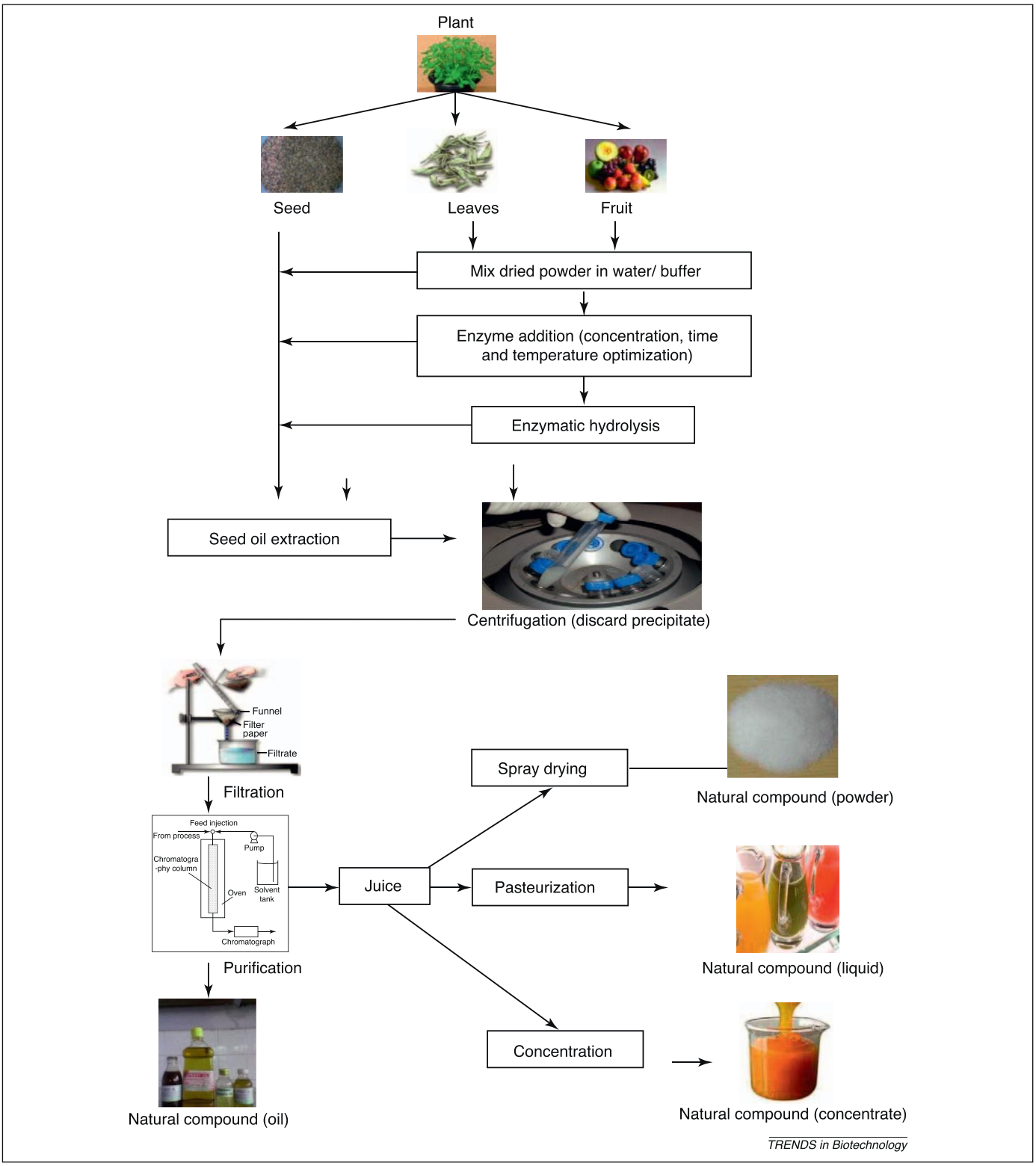

Figure 1. Enzyme-assisted extraction of bioactive compounds from a plant source. Images were taken, with permission, from the Minerva database, http://www.cognitivesolutions.com and http://www.21food.com (Food & Beverage online).

an optimal temperature, they can still be used over a range of temperatures, providing flexibility for both cost and product quality. Substrate particle size reduction prior to enzymatic treatment provides better accessibility of the enzyme to the cell to increase extraction yields significantly. In enzyme-assisted aqueous extraction, the enzymes can rupture the polysaccharide–protein colloid in the cell wall creating an emulsion that interferes with extraction. Therefore, non-aqueous systems are preferable for some materials because they minimize the formation of polysaccharide–protein colloid emulsions [65]. Enzyme-assisted extraction methodology for the extraction of bioactive components from various plant sources is summarized in Figure 1. However, the parameters impacting enzyme-assisted release of

bioactives need to be optimized for each specific process. These parameters include pH, time, temperature and enzyme concentration.

Concluding remarks

The exploitation of enzymes in industry for extracting plant bioactives for their application in food is a promising field. Success in this area requires interdisciplinary research from food technologists, food chemists, nutritionists and toxicologists. Investigating the stability and interactions of enzymes with other food ingredients during processing and storage is an important area of research. Also, a more in-depth understanding of the polysaccharide structure of the plant substrate and the use of specific enzymes for improved hydrolysis would help the enzyme to reach the active site. Synthesis of new enzymes and purification of enzymatic mixtures would also help in improving the level of released bioactives.

The application of enzymes for sweetener extraction is a relatively new area, which requires more research to establish its viability at a commercial scale. The application of enzymes for the extraction of natural compounds, particularly in the absence of solvents, is an attractive proposal. Tailored enzymes, either through screening available biodiversity, genetic engineering approaches, or a combination of both, are needed to further improve extraction techniques. A market exists for ecofriendly extraction methods for the production of a variety of bioactives. The enzyme-assisted extraction of natural compounds can save processing time and energy, and potentially provide a more reproducible extraction process at the commercial scale. Future investigations are needed to expand the currently available enzymatic processes, in particular to further enhance the yields of bioactive compounds.

Acknowledgements

We acknowledge funding from the Centre for Biotechnology (Biodeakin), ITRI, Deakin University, Australia and Department of Biotechnology, Punjabi University, India. Authors are thankful to Anne Jones for reproducing a figure in this article.

References

1 Biesalski, H.K. *et al.* (2009) Bioactive compounds: definition and assessment of activity. *Nutrition* 25, 1202–1205
2 Denny, A.R. and Buttriss, J.L. (2007) *Plant Foods and Health: Focus on Plant Bioactives*, EuroFIR Synthesis Report No. 4, EuroFIR Project Management Office/British Nutrition Foundation, London
3 Stafford, A.M. (2002) Plant cell culture as a source of bioactive small molecules. *Curr. Opin. Drug Discov. Dev.* 5, 296–303
4 Yang, B. *et al.* (2011) Extraction and pharmacological properties of bioactive compounds from (*Dimocarpus longan* Lour.) longan fruit: a review. *Food Res. Int.* 44, 1837–1842
5 Teo, C.C. *et al.* (2010) Pressurized hot water extraction. *J. Chromatogr. A* 1217, 2484–2494
6 Gardossi, L. *et al.* (2009) Guidelines for reporting of biocatalytic reactions. *Trends Biotechnol.* 28, 171–180
7 Meyer, A.S. (2010) Enzyme technology for precision functional food ingredients processes. *Ann. N. Y. Acad. Sci.* 1190, 126–132
8 Pinelo, M. *et al.* (2006) Upgrading of grape skins: significance of plant cell-wall structural components and extraction techniques for phenol release. *Trends Food Sci. Technol.* 17, 579–590
9 Fu, G.Z. *et al.* (2005) Preliminary assessment of the environmental benefits of enzyme bleach boosting for pulp and paper making. *Int. J. Life Cycle Assess.* 10, 136–142
10 Nielsen, P.H. *et al.* (2007) Environmental assessment of digestibility improvement factors applied in animal production: use of Ronozyme® WX CT xylanase in Danish pig production. *Int. J. Life Cycle Assess.* 13, 49–56
11 Veit, T. (2004) Biocatalysis for the production of cosmetic ingredients. *Eng. Life Sci.* 4, 508–511
12 deMaria, L. *et al.* (2007) Phospholipases and their industrial applications: mini review. *Appl. Microbiol. Biotechnol.* 74, 290–300
13 Wu, Y. *et al.* (2005) Optimization of extraction process of crude polysaccharides from boat-fruited sterculia seeds by response surface methodology. *Food Chem.* 105, 1599–1605
14 Passos, C.P. *et al.* (2009) Enhancement of grape seed oil extraction using a cell wall degrading enzyme cocktail. *Food Chem.* 115, 48–53
15 Barzana, E. *et al.* (2002) Enzyme-mediated solvent extraction of carotenoids from marigold flower (*Tagetes erecta*). *J. Agric. Food Chem.* 50, 4491–4496
16 Sowbhagya, H.B. and Chitra, V.N. (2010) Enzyme-assisted extraction of flavorings and colorants from plant materials. *Crit. Rev. Food Sci. Nutr.* 50, 146–161
17 Yang, Y-C. *et al.* (2010) Optimisation of microwave-assisted enzymatic extraction of corilagin and geraniin from *Geranium sibiricum* Linne and evaluation of antioxidant activity. *Food Chem.* 122, 373–380
18 Awika, J.M. *et al.* (2003) Screening methods to measure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products. *J. Agric. Food Chem.* 51, 6657–6662
19 Cacace, J.E. and Mazza, G. (2006) Pressurized low polarity water extraction of lignans from whole flaxseed. *J. Food Eng.* 77, 1087–1095
20 Durling, N.E. *et al.* (2007) Extraction of phenolics and essential oil from dried sage (*Salvia officinalis*) using ethanol–water mixtures. *Food Chem.* 101, 1417–1424
21 Ramadan, M.F. *et al.* (2009) Chromatographic analysis for fatty acids and lipid-soluble bioactives of *Derris indica* crude seed oil. *J. Chromatogr.* 70, 103–108
22 Murthy, P.S. and Naid, M.M. (2010) Recovery of phenolic antioxidants and functional compounds from coffee industry by-products. *Food Bioprocess. Technol.* DOI: 10.1007/s11947-010-0363-z
23 Ollanketo, M. *et al.* (2002) Extraction of sage (*Salvia officinalis*) by pressurized hot water and conventional methods: antioxidant activity of the extracts. *Euro. Food Res. Technol.* 215, 158–163
24 Sun-Waterhouse, D. *et al.* (2009) Evaluation of the extraction efficiency for polyphenol extracts from by-products of green kiwifruit juicing. *Int. J. Food Sci. Technol.* 44, 2644–2652
25 Cai, J. *et al.* (2003) Study on extraction technology of strawberry pigments and its physicochemical properties. *Food Fermen. Ind.* 29, 69–73
26 Fan, H. *et al.* (2009) Effects of microwave extraction on characteristics of polysaccharides from cherry, kiwi and wolfberry. *Trans. Chin. Soc. Agric. Eng.* 25, 355–360
27 Ibrahim, M.S. *et al.* (2006) Anolignan B: a bioactive compound from the roots of *Terminalia sericea*. *J. Ethnopharmacol.* 103, 135–138
28 Bimakr, M. *et al.* (2009) Supercritical carbon dioxide (SC-CO2) extraction of bioactive flavonoid compounds from spearmint (*Mentha spicata*) leaves. *Euro. J. Sci. Res.* 33, 679–690
29 Dehghan-Shoar, Z. *et al.* (2011) Lycopene extraction from extruded products containing tomato skin. *Int. J. Food Sci. Technol.* 46, 365–371
30 Ruiz-Teran, F. *et al.* (2001) Enzymatic extraction and transformation of glucovanillin to vanillin from vanilla green pods. *J. Agric. Food Chem.* 49, 5207–5209
31 Kaur, A. *et al.* (2010) Hydrolysis of citrus peel naringin by recombinant α-L-rhamnosidase from *Clostridium stercorarium*. *J. Chem. Technol. Biotechnol.* 85, 1419–1422
32 Gan, C.Y. and Latiff, A.A. (2010) Extraction of antioxidant pectic-polysaccharide from mangosteen (*Garcinia mangostana*) rind: optimization using response surface methodology. *Carbohydr. Polym.* 83, 600–607
33 Choudhari, S.M. and Ananthanarayan, L. (2007) Enzyme aided extraction of lycopene from tomato tissues. *Food Chem.* 102, 77–81
34 Wilkins, M.R. *et al.* (2007) Hydrolysis of grapefruit peel waste with cellulase and pectinase enzymes. *Bioresour. Technol.* 98, 1596–1601
35 Geddes, C.C. *et al.* (2011) Simplified process for ethanol production from sugarcane bagasse using hydrolysate resistant *Escherichia coli* strain MM 160. *Bioresour. Technol.* 102, 2702–2711
36 Puri, M. *et al.* (2011) Molecular characterization and enzymatic hydrolysis of naringin extracted from kinnow peel waste. *Int. J. Biol. Macromol.* 48, 58–62

37 Mishra, D. *et al.* (2005) Aqueous enzymatic extraction of oil from mandarin peels. *J. Oleo Sci.* 54, 355–359
38 Stoll, T. *et al.* (2003) Process for the recovery of a carotene rich functional food ingredient from carrot pomace by enzymatic liquification. *Innov. Food Sci. Emerg. Technol.* 4, 415–423
39 Sampathu, S.R. *et al.* (2006) A process for making chilli oleoresin of improved quality, US Patent No. 7097867
40 Schweiggert, R.M. *et al.* (2009) Development and optimization of low temperature enzyme-assisted liquefaction for the production of colouring foodstuff from purple pitaya (*Hylocereus* sp.). *Eur. Food Res. Technol.* 230, 269–280
41 Singh, R.S. *et al.* (2006) Production of inulinase from *Kluyveromyces marxianus* YS-1 using root extract of *Asparagus racemosus*. *Proc. Biochem.* 41, 1703–1707
42 Dzogbefia, V.P. *et al.* (2008) Evaluation of locally produced *Saccharomyces cerevisiae* pectinase enzyme for industrial extraction of starch from cassava in Ghana. *Sci. Res. Essay* 3, 365–369
43 Ptichkina, N.M. *et al.* (2008) Pectin extraction from pumpkin with the aid of microbial enzymes. *Food Hydrocoll.* 22, 192–195
44 Li, B.B. *et al.* (2006) Extraction of phenolics from citrus peels: II. Enzyme-assisted extraction method. *Sep. Purif. Technol.* 48, 189–196
45 Bildstein, M. *et al.* (2008) An enzyme based extraction process for the purification and enrichment of vegetable proteins to be applied in bakery products. *Eur. Food Res. Technol.* 228, 177–186
46 Kammerer, D. *et al.* (2005) A novel process for the recovery of polyphenols from grape (*Vitis vinifera*) pomace. *J. Food Sci.* 70, 157–163
47 Ferruzzi, M.G. and Green, R.J. (2006) Analysis of catechins from milk–tea beverages by enzyme assisted extraction followed by high performance liquid chromatography. *Food Chem.* 99, 484–491
48 Yoon, K.Y. *et al.* (2005) Enzymatic production of a soluble-fibre hydrolyzate from carrot pomace and its sugar composition. *Food Chem.* 92, 151–157
49 Nakkeeran, E. *et al.* (2011) *Aspergillus carbonarius* polygalacturonase purified by integrated membrane process and affinity precipitation for apple juice production. *Bioresour. Technol.* 102, 3293–3297
50 García, A. *et al.* (2001) Improvement of phenolic compounds content in virgin olive oils by using enzymes during malaxation. *J. Food Eng.* 48, 189–194
51 Soto, C. *et al.* (2008) Antioxidant content of oil and defatted meal obtained from borage seeds by an enzymatic-aided cold pressing process. *Proc. Biochem.* 43, 696–699
52 Muñoz, O. *et al.* (2004) Effects of enzymatic treatment on anthocyanic pigments from grapes skin from Chilean wine. *Food Chem.* 87, 487–490
53 Tobar, P. *et al.* (2005) Winery solid residue revalorization into oil and antioxidant with nutraceutical properties by an enzyme assisted process. *Water Sci. Technol.* 51, 47–52
54 Landbo, A.K. and Meyer, A.S. (2001) Enzyme-assisted extraction of antioxidative phenols from black currant juice press residues (*Ribes nigrum*). *J. Agric. Food Chem.* 49, 3169–3177
55 Kim, Y.J. *et al.* (2005) Phenolic extraction from apple peel by cellulases from *Thermobifida fusca*. *J. Agric. Food Chem.* 53, 9560–9565
56 Maier, T. *et al.* (2008) Optimization of a process for enzyme-assisted pigment extraction from grape (*Vitis vinifera* L.) pomace. *Eur. Food Res. Technol.* 227, 267–275
57 Kapasakalidis, P.G. *et al.* (2009) Effect of a cellulase treatment on extraction of antioxidant phenols from black currant (*Ribes nigrum*) pomace. *J. Agric. Food Chem.* 57, 4342–4351
58 Bautista-ortín, A.B. *et al.* (2005) Improving colour extraction and stability in red wines: the use of maceration enzymes and enological tannins. *Int. J. Food Sci. Technol.* 40, 867–878
59 Patindol, J. *et al.* (2007) Cellulase-assisted extraction of oligosaccharides from defatted rice bran. *J. Food Sci.* 72, C517–C521
60 Chen, S. *et al.* (2011) Enzyme-assisted extraction of flavonoids from *Ginkgo biloba* leaves: improvement effect of flavonol transglycosylation catalysed by *Penicillium decumbens* cellulase. *Enz. Microbiol. Technol.* 48, 100–105
61 Renouard, S. *et al.* (2010) Cellulase-assisted release of secoisolariciresinol from extracts of flax (*Linum usitatissimum*) hulls and whole seeds. *Food Chem.* 122, 679–687
62 Myers, R.H. and Montgomery, D.C., eds (2002) *Response Surface Methodology: Process and Product Optimization Using Designed Experiments* (3rd edn), p. 824, Wiley
63 Puri, M. *et al.* (2010) Response surface optimization of medium components for naringinase production from *Staphylococcus xylosus* MAK2. *Appl. Biochem. Biotechnol.* 19, 162–181
64 Pompeu, D.R. *et al.* (2009) Optimisation of the solvent extraction of phenolic antioxidants from fruits of *Euterpeu oleracea* using response surface methodology. *Bioresour. Technol.* 100, 6076–6082
65 Concha, J.C. *et al.* (2004) Enzymatic pretreatment on rose-hip oil extraction: hydrolysis and pressing conditions. *J. Am. Oil Chem. Soc.* 81, 549–552
66 Geuns, J.M.C. (2003) Stevioside. *Phytochemistry* 64, 913–921
67 deSlavutzky, S.M.B. (2010) Stevia and sucrose effect on plaque formation. *J. Consumer Protec. Food Safety* 5, 213–216
68 Herrnaz-Lopez, M. *et al.* (2010) Stevia is a source for alternative sweeteners: potential medicinal effects. *Agro Food Indus. Hi-Tech.* 21, 3842
69 Sharma, D. *et al.* (2010) Anti-amnesic effect of Stevioside in scopolamine treated rats. *Indian J. Pharmacol.* 42, 164–167
70 Chatsudthipong, V. and Muanprasat, C. (2009) Stevioside and related compounds: therapeutic benefits beyond sweetness. *Pharmacol. Ther.* 121, 41–54
71 Clos, J.F. *et al.* (2006) Photostability of rebaudioside A and stevioside in beverages. *J. Agric. Food Chem.* 56, 8507–8513
72 Kroyer, G. (2010) Stevioside and stevia-sweetener in food: application, stability and interaction with food ingredients. *J. Consumer Protec. Food Safety* 5, 225–229
73 Wallin, H. (2004) *Steviol Glycosides: Chemical and Technical Assessment*, JECFA (FAO) 1–5
74 Kumar, S. (1986) Methods for recovery of stevioside, US Patent No. 4599403.
75 Giovanetto, R.H. (1990) Method for the recovery of steviosides from plant raw material, US Patent No. 4892938.
76 Erkucuk, A. *et al.* (2009) Supercritical CO_2 extraction of glycosides from *Stevia rebaudiana* leaves: identification and optimization. *J. Supercrit. Fluids* 51, 29–35
77 Teo, C.C. *et al.* (2009) Validation of green-solvent extraction combined with chromatographic chemical fingerprint to evaluate quality of *Stevia rebaudiana* Bertoni. *J. Sep. Sci.* 32, 613–622
78 Liu, J. *et al.* (2010) Ultrasonic assisted extraction of total carbohydrates from *Stevia rebaudiana* and identification of extracts. *Food Bioprod. Process* 88, 215–221
79 Puri, M. *et al.* (2011) Downstream processing of stevioside and its potential applications. *Biotechnol. Adv.* 29, 781–791
80 Puri, M. and Sharma, D. (2011) Anti-bacterial activity of stevioside towards food-borne pathogenic bacteria. *Eng. Life Sci.* 11, 326–329