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Review

Eco-innovative technologies for extraction of proteins for human consumption from renewable protein sources of plant origin

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ABSTRACT

Background: The need for renewable and **sustainable sources of proteins** is growing. Diets containing more plant protein are increasing due to several reasons: the negative environmental impacts of animal protein production, the increasing vegetarianism and veganism trends, and inadequate consumer acceptance of food grade insects.

Scope and approach: This paper links the isolation of valuable proteins from sustainable sources – by-products from processing industry of plant origin and **eco-innovative technologies** which are emerging for this purpose (electrostatic separation, subcritical water **extraction**, reverse micelles extraction, aqueous two-phase systems extraction, enzyme-, microwave-, ultrasound-, pulsed electric energy- and high pressure-assisted extraction). In this way, not only the key challenges of modern food processing are met-the assurance of cost-effective, sustainable and environmentally friendly production, but also the concept of zero **food waste** seems more achievable.

Key findings and conclusions: A number of different techniques have emerged with high potential to assist protein extraction of preserved techno-functional properties, but they are still in the early stage of its industrial applications. In the EU, its industrial application may be hindered by legislative issues. The respective Novel Food Regulation classifies food obtained in a production process not used for food production before 15 May 1997, as "novel food'' and the regulatory status for each single case must be sought. On the other hand, the utilization of novel processing technologies is regulatory encouraged in EU due to their potential to reduce the environmental impact of food production, enhance food security and bring benefits to consumers.

1. Introduction: the need for renewable proteins

Proteins are the main constituents of agricultural raw materials with two main (complementary) functions: bio- and techno-function. Bio-functionality of proteins is related to their nutritional and physiological properties, while techno-functionality is related to their physico-chemical properties affecting appearance, texture and stability of food products (e.g. solubility, viscosity, foaming, emulsifying and gelling ability, fat absorption capacity) (Panyam & Kilara, 1996; Sforza, Tedeschi, & Wierenga, 2016; Tahergorabi & Hosseini, 2017).

One of the earliest indications of the importance of the increment of food protein resources to fight against the world malnutrition was given by Mortimer Louis Anson (1901–1968), a protein scientist, ascribing this problem to inadequate agricultural practices and processing technology (Chichester, Mrak, & Stewart, 1969). Ever since, protein–energy malnutrition together with micronutrient deficiency has been recognized as a severe health-related problem in developing countries. However, due to modern eating habits and inadequate levels of protein present in the diet, the prevalence of malnutrition in the developed countries is also not rare, but it has not been adequately treated (Grover & Ee, 2009).

Despite the negative environmental impacts of their production, demands for meat, dairy and fish products are still rising - world demand for proteins of animal origin is expected to double by 2050 (FAO, 2013). At the same time, a reversal trend towards diets containing more plant protein in Western countries, affected not only by growing vegetarianism and veganism, has been noticed. Due to the expectations of a world population of 9 billion by 2050, the increasing demand for

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food proteins can be met by utilization of proteins from novel sources which include insects, fungi and algae, as well as waste streams from food processing. The trend of inclusion of food grade insects in a diet is growing, but is not adequately perceived by Western Europeans (Belluco, Halloran, & Ricci, 2017; Megido et al., 2014; Verbeke, 2015). Fungi contain 45% of proteins (on a dry matter basis) of high biological value similar to milk proteins, of high digestibility and high satiating properties. The production of fungal proteins is characterized with low environmental impact, as it is the case with microalgal proteins containing 50–60% of proteins (Finnigan, Needham, & Abbott, 2017; Klamczynska & Mooney, 2017; Williamson et al., 2006). Unlike fungal proteins which are characterized with mild taste and low off-flavour, taste of algal proteins is perceived as savory and umami, limiting their utilization to certain types of food products (Nadathur & Carolan, 2017). The replacement of animal proteins with novel plant proteins from food by-products has been driven by sustainability assurance in the food production (Aiking, 2011). In that sense, several sources of proteins from different industrial waste materials can be identified, whereby they are inevitably used as animal feed livestock. The redirection of such protein sources from animal to human consumption has been requiring a serious engagement of education, science and technological development, in order to overcome the existing problems related to protein allergenicity, toxicity, off-flavour and the presence of high amount of fibers and antinutritional factors (e.g. tannins, phytic acid, glucosinolates, phytates, erucic acid, trypsin inhibitors) which may limit the use of some proteins (Nadathur, Wanasundara, & Scanlin, 2017; Rodrigues, Coelho, & Carvalho, 2012).

The recovered proteins from industry food waste could be reutilized in different ways: (1) as nutrients for food fortification and dietary supplements, (2) as techno-functional food ingredients due to their emulsifying, gelling, foaming, and water binding properties, (3) as biopolymer development material for variety of food, non-food and healthcare products and (4) for biorefinery purposes (Gupta & Nayak, 2015; Moure, Sineiro, Domínguez, & Parajo, 2006).

2. Food waste reduction strategies

Industrialization and global population growth leads to irreversible environmental damage. The demand for drastic reductions of environmental burdens, e.g. of greenhouse gases, implies a need for transformation to both "green" the economy and the society. The term eco-innovation is introduced in the literature (Rennings, 2000) addressing necessary changes towards sustainable development. Eco-innovation approach implies ''technological regime shifts'' where waste and by-products become a resource.

The recent concern about the issue of food waste has provoked the scientific and professional community to act towards discovering and putting into practice processes, technologies and management methods that will contribute to the reduction, reutilization and recycling of food waste and thus preserve the environment, ensure food security and support the sustainability of food systems (Galanakis, 2013; Parfitt, Barthel, & Macnaughton, 2010). In support of this, The European Parliament adopted a non-legislative resolution with the aim of halving food waste by 2025 drawing the public attention and raising awareness on this issue (European Union, 2012). One of the Sustainable Development Goals (SDGs) set to all nations was to halve food waste and reduce food loss by 2030. The justification of such initiatives lies in the fact that 12.5% of the global population for the time period of 2011–2013 were estimated to be suffering from chronic hunger, whereby the vast majority of hungry people live in developing regions with the prevalence of undernourishment estimated at 14.3% (FAO, 2013). Moreover, European Union has been striving to transit to a circular economy, in which waste and resource use are minimised, and waste is reused for creation of new value(s) to boost economy, innovation, growth and job creation. The exploitation of food waste as an alternative food source has been driven by the current human overpopulation and limited land resources threatening to reduce food supplies. Considering the fact that food waste has been recognized as an abundant and cheap source of valuable functional compounds such as antioxidants, dietary fibres, proteins, carbohydrates and colorants, it can be reprocessed and utilized in the production of new commercially valuable products either within the food chain or beyond (Galanakis, 2012; Lin et al., 2013; Luque & Clark, 2013).

3. The sources of proteins in industrial food waste of plant origin

3.1. Oil meals/press cakes

Oil meals/press cakes are the by-product of oil processing, incurred after oil extraction from the seeds of annual plants or oil-bearing fruits (O'Brien, 2009). Due to a protein content of 15–50%, oil meals remaining after oil extraction have been recognized as one of the most valuable sources for the recovery of proteins (O'Brien, 2009; Ramachandran, Singh, Larroche, Soccol, & Pandey, 2007; Saltveit, 2007).

Soybean, rapeseed, cottonseed, sunflower seed and peanut are considered to be the major oil crops worldwide, and the availability of their protein meals is proportionally pretty high (approximately 200 million of tonnes in 2015) (FAOSTAT, 2012). Significant oil-bearing crops are olive, palm and coconut, from which oil is extracted from fruit pulp, whose residues can be used for protein extraction (Roselló-Soto et al., 2015). Apart from coconut oil meal, coconut residue remaining after coconut milk extraction can be used for isolation of proteins (Rodsamran & Sothornvit, 2018). Besides them, the increasing use of alternative oil crops (sesame seed, grape seed, pumpkin seed, hemp seed, hazel nut, walnut) in the production of specialty oils have been recorded (Kochhar, 2011), which results in obtaining meals which in addition to high protein content have other value-added properties. The composition of oilseed meals and their protein content vary over a wide range depending on the type of oilseeds, the initial quality of oilseeds, pre-treatment method (e.g. dehulling, storage conditions, etc) and the processing method – method of oil extraction (Matthäus, 2012; Ramachandran et al., 2007). The degree to which the oil has been extracted from a starting raw material directly corresponds to the protein content of the remaining meal (Matthäus, 2012). The oilseeds can be processed either hulled or unhulled affecting thereby the protein content of the resulting meal. Generally, the dehulled meals are of higher protein and lower fibre content, while unhulled meals require fractionations into protein- and fibre rich fractions prior to their use for protein recovery. However, the oil extraction by mechanical pressing is more effective if a certain amount of hulls is left (min. 8%) because in that way the grip within the press is improved. Since the oilseed cakes can come in quite large pieces, the utilization of oilseed cakes for the recovery of proteins could require certain mechanical pre-treatment (crushing and fractionation) as well as careful storage due to the high oil content of cake ($>5\%$) (Matthäus, 2012; van Doosselaere, 2013). Utilization of oilseed meals originating from large scale oil seed processing is significantly impaired in comparison with the utilization of oilseed meals originating from small-scale oil-seed processing, because of the need for cake desolventizing after solvent extraction, which is not the case in the latter case. Heating used for solvent (e.g. hexane) removal from the defatted meal favours protein aggregation and tighter association between fibers and carbohydrates, aggravating not only the protein extractability, but also their techno-functional properties (e.g. solubility, foaming, and emulsifying properties) (Matthäus, 2012; Ochoa-Rivas, Nava-Valdez, Serna-Saldívar, & Chuck-Hernández, 2017).

3.2. By-products of cereal processing

By-products of cereal processing are suitable raw materials for recovery of proteins, with rice bran as the most important source (Hamada, 2000; Tang, Hettiarachchy, & Shellhammer, 2002; Tang,

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Hettiarachchy, Horax, & Eswaranandam, 2003; Wang, Hettiarachchy, Qi, Burks, & Siebenmorgen, 1999). Besides rice bran, several types of by-products have been designated as promising source of proteins, such as oat bran (Guan & Yao, 2008), wheat bran (Apprich et al., 2014), brewer's spent grains (Celus, Brijs, & Delcour, 2006; Cookman & Glatz, 2009), broken rice (Xia et al., 2012a), defatted wheat germ remaining after oil extraction (Zhu, Zhou, & Qian, 2006) etc.

Due to high nutritional value, reflected in well-balanced amino acid profile and hypoallergenic, hypocholesterolemic, hypolipidemic and anti-cancer effects, rice proteins have drawn a lot of attention of food scientists and nutritionists. In this regard, rice bran – emerging from dry milling process of rice, defatted rice bran – remaining after rice bran oil extraction, as well as broken rice - an unavoidable by-product during rice milling, have been recognized as suitable material for the extraction of proteins especially due to its availability, quantities and amino acid composition (Wang et al., 1999; Xia et al., 2012a; Amagliani, O'Regan, Kelly, & O'Mahony, 2017). Rice bran contains 12–20% of proteins. However, the utilization of this material, as well as material originating from other cereals, is limited due to very dense protein-starch agglomerates in the endosperm cell, which can be broken mechanically rather than applying hydrothermal treatments (Guraya & James, 2002; Kampen, 1991).

Wheat bran containing 13–18% of proteins can also be regarded as a possible source for protein extraction (Apprich et al., 2014; Balandrán-Quintana, Mercado-Ruiz, & Mendoza-Wilson, 2015; Prückler et al., 2014). Although being of high-quality, proteins in wheat bran are tightly enclosed within a polysaccharide matrix that prevents their easy digestibility, which ultimately results in waste of 15.5 million tons of usable proteins annually (Balandrán-Quintana et al., 2015). Therefore, the commercial exploitation of proteins from wheat bran is difficult due to the necessity of employment of strong alkaline conditions, involving the accumulation and subsequent disposal of environmentally hazardous reagents. However, wheat bran proteins can be utilized for the production of free amino acids, γ -aminobutyric acid (GABA), as well as bioactive peptides (Balandrán-Quintana et al., 2015; Shamloo, Eck, & Beta, 2015). Defatted wheat germ remaining after oil extraction contains more than 30% of proteins of well-balanced amino acid composition and the high amount of essential amino acids such as lysine, methionine and threonine, and can be used for the isolation of bioactive peptides (Ge, Sun, Ni, & Cai, 2000; Wang et al., 2018; Zhu, Wang, & Guo, 2015). Brewers' spent grain, the main by-product of the brewing industry, is also one of the promising sources of proteins (15–26%) (Connolly, Piggott, & FitzGerald, 2014). However, due to the interactions of proteins with other biopolymeric constituents, an impenetrable layer is formed on the spent grains during mashing, compromising protein extractability (Celus et al., 2006).

3.3. By-products of legume processing

High protein content in legumes makes them the second most important food raw material after cereals (Oomah, Patras, Rawson, Singh, & Compos-Vega, 2011). Consequently, the by-product of commercial legume processing could be utilized as rich sources of proteins. Commercial pulses milling yields approximately 25% of by-products consisting mainly of husk (up to 14%), powder (up to 12%), broken (up to 13%), shriveled and unprocessed seeds. However, the main problem in the utilization of legume by-products for the recovery of proteins is the fact that the legume processing is mostly performed in small-scale facilities and in a limited number of specialty or niche markets. Moreover, soybean processing in the production of soy milk and tofu results in soy pulp - a by-product called okara, containing a significant amount of residual proteins (about 25–33%). Due to large quantities of okara produced annually, disposal of such material is a serious problem unless it is revalorized (O'Toole, 1999; Mateos-Aparicio, Redondo-Cuenca, Villanueva-Suárez, Zapata-Revilla, & Tenorio-Sanz, 2010; Li, Qiao, & Lu, 2012).

Traditionally, agro-industrial by-products and crop residues have been converted to animal feed. Apart from their high rate of reproduction, the monogastric animals are characterized by the best efficiency of nutrient transformation into high-quality animal protein, but the costs of this transformation are very high. Moreover, the demand for drastic reductions of greenhouse gases requests shifting away from meat production.

4. Emerging protein extraction technologies

The utilization of novel technologies for protein extraction can improve protein extraction yield, their techno-functional and nutritional properties. Moreover, they are rated as affordable, safe, effective, and ecologically-friendly alternatives enabling clean label status (Tiwari, 2015). In spite of numerous advantages, the application of novel technologies at industrial level is still limited (Liu, Gasmalla, Li, & Yang, 2016). The selection of the type of the process for protein extraction is dependent on the intended purpose of the resulting product, available resources and technical capabilities. For example, proteins obtained by alkali and enzymatic-extraction are applicable in the formulation of emulsion-based foodstuffs (Tirgar, Silcock, Carne, & Birch, 2017).

The resulting protein products are categorized on the basis of their protein content as *protein flours* (containing up to 65% of proteins), *protein concentrates* (containing up to 65–90% of proteins) and *isolates* (containing more than 90% of proteins) (Oreopoulou & Tzia, 2007). Despite the vast literature data about the production of different types of protein concentrates and isolates, only those originating from soybean (Ochoa-Rivas et al., 2017) and rapeseed/canola are commercialized, unlike the different types of protein flours which are widely marketed. Protein extraction techniques can be divided into dry and wet techniques.

4.1. Novel dry protein extraction techniques

Sieving and/or air classification techniques for the fractionation of protein-rich and fiber-rich fractions are widely covered in the literature (Banjac et al., 2017; Hansen, Skrede, Mydland, & Øverland, 2017; Pojić et al., 2014; Schutyser & van der Goot, 2011; Schutyser, Pelgrom, van der Goot, & Boom, 2015). Although they are able to yield fractions with retained protein functionality and proved to be more energy efficient in comparison to wet fractionation processes, the main disadvantage of these processes is impurity of resulting fractions and agglomeration of particles, especially when it is subjected to oilseed meals due to the residual lipid content. To overcome the existing drawbacks, novel dry fractionation process has been proposed: electrostatic separation. It is performed in two steps involving charging the particles followed by the separation of charged particles in an electric field (Hemery et al., 2011). Wang, Zhao, de Wit, Boom, and Schutyser (2016) and Tabtabaei, Vitelli, Rajabzadeh, and Legge (2017) utilized a tribo-electrostatic separation method for the fractionation of legume flour in order to obtain protein- and carbohydrate-enriched fractions, where it was found that protein enrichment by electrostatic separation is up to 15% higher than that obtained by air classification. Barakat, Jerome, and Rouau (2015) demonstrated the extraction of proteins from sunflower oil cake by electrostatic separation. In this process, the chemical integrity of carbohydrates and lignin from the starting material remain preserved, being of high importance for the concept of zero waste.

4.2. Novel wet protein extraction techniques

Protein extraction usually starts with solubilizing the protein rich source in a medium with the pH far from the isoelectric point, followed by their precipitation in medium with the pH close to the isoelectric point of the solubilized proteins. The other approach is to achieve protein solubilization using saline solutions followed by protein precipitation caused by salt removal by ultrafiltration and diafiltration. The pro-

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tein produced in this way has a micellar structure before being dried, with preserved native state (Hadnađev et al., 2017).

The variety of acid and alkaline protein extraction protocols have been reported so far depending on the type of plant protein source (Martínez-Maqueda, Hernández-Ledesma, Amigo, Miralles, & Gómez-Ruiz, 2013). In general, acid aided extraction appears less promising because of inefficient cell wall degradation by acid which prevents protein diffusion to the medium. Moreover, the applied acid pH is closer to the protein isoelectric point than that of the alkaline experiments; therefore, the protein has less net charge providing lower protein solubility (Sari, Bruins, & Sanders, 2013). Generally, alkaline extraction shows better results. Currently, only soybean protein has been commercially extracted under alkaline conditions (pH 8–9) because the process provides high protein yields with a low price (Nazareth, Deak, & Johnson, 2009). For the other potential sources of proteins, alkaline extraction doesn't provide such good yields and for that reason and due to extreme pH conditions which may cause protein denaturation as well as high consumption of acids, alkalis and water, alternative extraction methods have emerged. The combination of water and enzymes, water at subcritical conditions and extraction of proteins by reverse micelles is attracting more and more attention.

4.2.1. Enzyme-assisted extraction of proteins

The application of enzyme-assisted extraction of proteins is based on the disruption of the cell walls integrity caused by the action of specific enzymes degrading celluloses, hemicelluloses, and/or pectin - the major components of plant cell walls and fiber (Jung, Lamsal, Stepien, Johnson, & Murphy, 2006), as well as proteases to hydrolyze part of the protein to increase their solubility. By degradation of cell walls, the release of protein bodies is enabled. Enzyme-assisted protein extraction is characterized by long processing time, high operational costs, high energy consumption, irreversible carbohydrate-protein matrix disruption and the necessity of a careful adjustment of process parameters (pH and temperature). Still, it is considered a more mild extraction method with lower environmental impact in comparison to acid and alkaline assisted extraction (Sari et al., 2013). On the other hand, products obtained are of superior and preserved quality and more suitable for human consumption (Liu et al., 2016; Ochoa-Rivas et al., 2017).

Hanmoungjai, Pyle, and Niranjan (2002) demonstrated the effects of different commercial enzymes to assist enzymatic-water extraction of proteins from rice bran. Within this study the effects of different carbohydrates were tested, the effects of carbohydrase enzyme complex and the effects of proteases. The highest protein extraction yield was obtained by proteases, whilst degradation effects of carbohydrases did not affect the protein yield, but the amount of reducing sugars.

Rommi et al. (2014) demonstrated the effects of pectinolytic enzymes on the extraction yield of proteins from rapeseed press cakes from cold oil processing. The starting material, containing 36–40% protein, was effectively disintegrated due to the hydrolysis of pectic polysaccharides and glucans, which resulted in 1.7-fold higher protein yield in comparison to the treatment without enzyme. Hence, the amount of total protein extracted after enzyme treatment was 56% and 74%, depending on the type of press cake used (from intact and dehulled rapeseeds). In the subsequent study, Rommi et al. (2015) demonstrated the effects of enzyme-assisted aqueous extraction without pH adjustment in dilute conditions over alkaline extraction and isoelectric precipitation, which are commonly used for protein recovery from rapeseed press cake. It was shown that enzymatic hydrolysis of carbohydrates at pH = 6 resulted in proteins of better solubility and dispersion stability than that obtained from alkaline extracts by isoelectric precipitation, which were partially denatured. Enzyme-assisted extraction at pH 6 was feasible at relatively low solid content (ca. 10%), whereas the extraction of proteins from medium of higher solid content (20%) was more effective with alkaline conditions. The enzymatic treatment of defatted press cake for 2 h at 40% solid content and subsequent alkaline extraction for 1 h at 10% solid content resulted in the highest protein yield (53% of total protein).

Enzyme-assisted extractions enable simultaneous extraction of proteins and residual oil from oilseed meals (Niu et al., 2012). Niu et al. (2012) demonstrated the effects of protein extraction by aqueous enzymatic process from rapeseed cake. It was found that a concentration of enzymes of 1% (Viscozyme and Alcalase) on a water-to-cake ratio of 6:1 for 80 min of incubation time yielded 82.10% of proteins. Tirgar et al. (2017) showed that enzymatic and enzymatic-solvent extractions yielded higher protein content from flaxseed meal in comparison to that obtained by alkali extraction, and with the better emulsifying properties (emulsion capacity, emulsifying activity and stability). Chirinos, Aquino, Pedreschi, and Campos (2017) demonstrated the superiority of enzyme-assisted (Alcalase) extraction of proteins from sacha inchi (*Plukenetia ubilis* L.) kernel meal which yielded 44.7% of proteins with a low hydrolysis degree over alkaline extraction which yielded 29.7% of proteins. The optimal enzyme-assisted extraction conditions which yielded proteins of hydrolysis degree of 7.8% were: 5.6% enzyme concentration, 40.4 min extraction, solvent/meal 50/1 (v/w) ratio), pH 9.0 and temperature of 50 °C.

Sari et al. (2013) demonstrated the increase in protein extraction yield of 90% for soybean and 50–80% for rapeseed meals by the utilization of serine, endo-and exoproteases in comparison to protein extraction yield of 80% for soybean and 15–30% for rapeseed meal without enzyme addition. The response surface optimization of cellulolytic enzyme-assisted extraction of oat bran protein was carried out by Guan and Yao (2008). The optimized enzymatic pretreatment method (Viscozyme L) concentration 30 FBG/10 g of oat bran, pH 4.6, incubation time 2.8 h and temperature 44 °C) extracted significantly more protein (56.2%) from oat bran than did the alkaline (pH 9.5) method (14.8%).

4.2.2. Subcritical water extraction

Subcritical water (SW) extraction is a technique using hot water between 100 and 374 °C under high pressure to maintain its liquid state. The increase in the water temperature to 250 °C causes its relative dielectric constant to decrease from around 80 to near 27, which is similar to that of acetone at ambient temperature, allowing it to dissolve hydrophobic substances (Herrero, Cifuentes, & Ibañez, 2006). Biomaterials, such as proteins and carbohydrates, could be hydrolyzed in subcritical water without additional catalysts because the dissociation constant of water to hydrogen and hydroxyl ions is orders of magnitude greater than that in ambient water, so the water acts as an acid or base catalyst in chemical reactions (Haghighat, Ota, Kimura, & Adachi, 2006).

In the case of heat-denatured soy meals, soy protein extraction yield was significantly increased using enzyme-assisted subcritical water treatment (59.3%) in comparison to the yield obtained by conventional alkaline extraction and acid precipitation (16.4%) (Lu, Chen, Wang, Yang, & Qi, 2016). Improvement in protein solubility by SW treatment has been explained by the disruption of large insoluble protein aggregates into smaller soluble protein (Wang, Wang, & Johnson, 2006) and by balance of hydrophobic and hydrophilic sites in proteins' molecular surface derived from protein unfolding and accompanying structural rearrangement upon SW condition (Wang et al., 2012, 2011; Xia et al., 2012b). According to Lu et al. (2016), lower dielectric constant of water maintained in the range above 100 °C may have caused high dissolving power of SW.

When defatted rice bran was extracted with water and subcritical water at 50–250 °C the highest protein yield was achieved at 200 °C (Wiboonsirikul et al., 2007). Referring to the authors, solubility of the rice bran protein in water was generally low due to strong aggregation through a hydrophobic interaction and extensive association with the cell wall. The solubility increase at higher temperatures results from hydrolysis of the protein and cell walls.

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4.2.3. Reverse micelles extraction

Reverse micelles (RM) extraction applies reverse micelles - nanometer sized aggregates of surfactant molecules containing inner cores of water molecules in nonpolar solvents. The polar water pools inside reverse micelles can solubilize hydrophilic biomolecules, such as proteins (Bu et al., 2014). Reverse micelles form a three-phase system including water–surfactant–organic solvent so the biomolecules inside the polar water pools are protected from denaturation by organic solvents. The procedure of protein extraction from plant sources by reverse micelles consists of two steps: a forward extraction and a backward extraction. In the forward extraction process, proteins solubilize into the reverse micellar solution from plant sources, while the solubilized proteins recover from the reverse micellar solution in the backward extraction process.

The solubility of proteins and the efficiency of reverse micelles extraction is highly affected by the electrostatic interaction between reverse micelle and protein, pH change, W_0 (the molar ratio of water to surfactant), ionic strength and the nature and concentration of target protein, and reverse micelle composition (Zhao, Zhang, Liu, Zhang, & Ao, 2018).

Ionic surfactants as amphiphilic molecules are often used to form reverse micelles for protein solubilization, such as anionic di-2-ethylhexyl sodium sulfosuccinate (AOT). When using them, electrostatic interactions between the ionic surfactant molecules and the counter charge of the protein molecules are considered as the main driving force in forward extraction processes. Therefore, pH and ionic strength that mainly affect the charge numbers of proteins are dominant factors for the extraction process. Unfortunately, back extraction of proteins is not a simple reversible process of forward extraction in view of dynamics and thermodynamics (Liu, Dong, & Sun, 2008). Traditional backward extraction method applies changes in pH and ionic strength in a fresh aqueous phase to recover the proteins from micellar solution.

Sun, Zhu, and Zhou (2008; 2009) proposed novel method for the extraction of defatted wheat germ proteins (DWGP). They applied the reverse micellar system formed of AOT, isooctane and KCl for a forward extraction and a novel backward extraction method. First, forward extraction was optimized on the basis of single-factor experiments and the highest forward extraction efficiency of DWGP was obtained with AOT concentration 0.06 g mL^{-1}, pH 8, KCl concentration 0.1 mol/L, time 30 min, the amounts of DWGF 0.500 g, W_0 25 and temperature 36 °C. Under these conditions, the forward extraction efficiency of DWGP achieved 37% (Sun et al., 2008). A novel backward extraction method was proposed by the same group of authors (Sun, Zhu, & Zhou, 2009). Briefly, isooctane was recovered by vaporization firstly. Then the remaining residue was dissolved in a small amount of KCl solution. The recovery of DWGP was easily performed by the ternary liquid system (acetone: deionized water: isooctane = 15:5:1) precipitation, while most of AOT remained in the ternary liquid system. DWGP was washed with 65% ethanol solution to further remove any residual AOT.

In the case of soybean flour, much higher protein yield (72.40%) was obtained by (AOT)/hexane reverse micelles system than by aqueous extraction (61.53%) (Zhao, Zhu, & Chen, 2015). Zhao et al. (2018) found soybean proteins obtained through AOT reverse micelles (RMs) significantly higher than that of alkaline extraction and isoelectric precipitation (AEIP) in protein solubility index, oil absorption capacities, foaming capacity and stability as well as emulsification capacity and stability. Furthermore, they found soybean proteins obtained through RM treatment significantly higher in nutritional and biological properties than those obtained by alkaline extraction and isoelectric precipitation. According to them, the reverse micelles are highly selective and suitable for the large-scale continuous processing.

Sugar surfactants are a relatively new class of non-toxic and environmentally friendly surfactants, comprised of a sugar head group and alkyl chain. Some of these occur in nature (as certain sugar esters, for example), others can be synthesized chemically or enzymatically (Imamura et al., 2014; Jin et al., 2013; Neta et al., 2012). However, the application of sugar-based surfactants in protein extraction is still limited. Chen, Dong, and Guo (2017) tested reverse micelles from di(N-dodecylglucosylammonium) (GA) and di(N-dodecyllactosylammonium) (LA) sugar surfactants (with dicarboxylate as counter ion) to extract bovine serum albumin. They observed that under optimum condition, the maximum forward extraction efficiency was ca. 86% with GA, while only around 50% with LA, and almost all proteins solubilized in reverse micelles prepared from GA could be recovered into aqueous phase, while the recovery of proteins from the reverse micelles of LA was lower.

There are many reports on RM extraction (Sun et al., 2009, 2008; Zhao et al., 2015) which confirm that this method of extraction has many advantages because the surfactant and organic solvents could be used repeatedly by recovery, representing low costs. Secondly, since the environment of polar core in RM approaches to the physiological environments, it is possible to solubilize the proteins in RM without damaging their native conformation and maintain their activity. Although it is claimed that RM is convenient and easy to scale up, reverse micelle extraction is still on the lab scale.

4.2.4. Aqueous two-phase systems extraction

Aqueous two-phase systems (ATPSs) are formed when two polymers, one polymer and one salt, or two salts are mixed at appropriate concentrations or at a particular temperature and have been proposed as substitute and environmentally friendly protein extraction method (Cheng, Chen, Shu, & Wang, 2008; Dallora, Klemz, & Filho, 2007; Zainal-Abidin, Hayyan, Hayyan, & Jayakumar, 2017). Zeng et al. (2013) first reported that the extraction of proteins by an ionic liquid aqueous two-phase system based on the guanidine ionic liquid and hydrogen phosphate is possible. The results showed that under the optimum conditions, the extraction efficiency could reach up to 99.6% and that the conformation of the protein was not changed after the extraction.

Deep eutectic solvents (DESs) have appeared as a new generation of "green" solvents, which are formed by mixing substituted quaternary ammonium salts and hydrogen bond donors such as amines, alcohols and acids. Xu, Wang, Huang, Li, and Wen (2015) found green deep eutectic solvent-based aqueous two-phase system promising for protein extraction. In their work, they tested the efficiency of choline chloride (ChCl)-based DESs to extract bovine serum albumin (BSA), and ChCl-glycerol was selected as the suitable extraction solvent. Their experimental results showed 98.16% of the BSA extracted into the DES-rich phase in a single-step extraction under the optimized conditions. Furthermore, a high extraction efficiency of 94.36% was achieved when the same conditions were applied to the extraction of trypsin. They also pointed out that conformation of BSA was not changed during the extraction process. Referring to the conductivity, dynamic light scattering and transmission electron microscopy results, Xu et al. (2015) suggested that the formation of DES–protein aggregates plays a significant role in the separation process. Hence, the superiority of nontoxic DES in combination with their high protein extraction efficiency makes them promising for potential applications in bio-separation. Li et al. (2016a) established deep eutectic solvent aqueous two-phase systems for the extraction of proteins on the basis of betaine–urea used as extractant. By using model system (pure proteins), they demonstrated the protein extraction efficiency up to 99.82% under the optimum conditions, which were determined in single factor experiments. These optimal conditions were: salt concentration of 0.75 g ml^{-1}, the mass of DES of 1.4 g, the separation time of 12 min, the amount of protein of 15 mg, the temperature of 30 °C, while the extraction efficiency was not sensitive to the change of pH value. Simultaneously, the efficacy of the proposed extraction process was checked by using real sample which increased with the decreasing of salt concentration up to 32.66% for the salt concentration of 0.5 g ml^{-1}. More importantly, such designed ex-

traction process did not change the conformation of proteins. Dai, van Spronsen, Witkamp, Verpoorte, and Choi (2013) discovered *natural deep eutectic solvents* (NADES): liquids originating from plant primary metabolites in solid state mixed in a certain ratio. NADES is divided into four groups: ionic liquids with an acid and a base, sugar based NADES with only neutral compounds, sugar based NADES with bases and sugar based NADES with acids. Despite high viscosity, the NADES are still liquid at room temperature and even at low temperature. Their viscosity decreases significantly with the addition of small amounts of water, with preserving their characteristics. Macromolecules such as DNA, proteins and polysaccharides are soluble in NADES. Their high solubilizing capacity is related to their supramolecular structure and broad polarity range. The nontoxic and environmentally friendly NADES makes them perfect candidate to be tested as new *green technology* media for protein extraction.

4.3. Novel assisting cell disruption techniques

Both dry and wet protein extraction techniques require cell disruption as initial phase to allow protein release from protein bodies inside plant cells. Classically, cell disruption is performed by mechanical methods (e.g. grinding, milling) or by thermal and chemical treatments. However, due to high sensitivity of proteins to heat or solvent use, novel processing technologies have emerged and used for cell disruption demonstrating more efficient yield, extraction time, costs and environmental impact (e.g. microwave-assisted extraction, ultrasound-assisted extraction, supercritical fluid extraction, pressurized liquid extraction, pulsed electric field) (Zhu, Sun, & Zhou, 2009; Tosh & Yada, 2010; Liu et al., 2012; Kadam, Tiwari, & O'Donnell, 2013; Tiwari, 2015; Chéreau et al., 2016; Cravotto & Binello, 2016; Ochoa-Rivas et al., 2017).

Although certain number of novel technologies in food industry has been emerging, ultrasound (US) and microwave (MW) have been singled out as the most convenient ones from the economic, processing and energy efficacy point of view. Still, their utilization to assist protein extraction from vegetable sources has scarcely been reported (Ochoa-Rivas et al., 2017; Tiwari, 2015).

4.3.1. Microwave-assisted extraction of proteins

Microwave technology uses electromagnetic waves of frequency in the range 300 MHz - 300 GHz. The energy of this radiation disrupts hydrogen bonds, enables the migration of dissolved ions, increases the porosity of the biological matrix, which results in increased penetration of solvent into the matrix and facilitates the extraction of compound of interest (Kadam et al., 2013).

Phongthai, Lim, and Rawdkuen (2016) demonstrated the utilization of microwave-assisted extraction of proteins from rice bran and proved its superiority over the extraction without using microwave treatment in terms of protein yield, digestibility and techno-functional properties. Ochoa-Rivas et al. (2017) demonstrated that microwaves (at 725 W for 8 min) as applied for the extraction of proteins from defatted peanut flour from oil industry yielded the extraction rate of 55% (100% purity), which in total resulted in 77% more protein in comparison to the extraction without using microwave treatment used as a control. Use of microwaves did not affect primary, but secondary protein structure causing the improvement of certain functional properties, such as fat absorption index, water absorption index, foam activity, emulsifying activity, and *in vitro* protein digestibility. On the other hand, certain functional properties were impaired such as water and nitrogen solubility index and foam stability. However, the alteration of secondary protein structure and protein configuration is also related to the possible change in epitopes, which will no longer be recognized by IgE antibodies and consequently will not be able to activate immune response (Shriver & Yang, 2011). It has been confirmed by Li, Zhu, Zhou, Peng, and Guo (2016b) who decreased the allergenicity of soy protein isolate for infant formula for 24.7% by microwaving due to the alteration of secondary structure. Moreover, microwave treatment was demonstrated to be effective in decreasing heat stable and heat labile antinutrients, as demonstrated by Kala and Mohan (2012) and Hefnawy (2011) (e.g. phytic acid, hydrogen cyanide, total oxalate, trypsin inhibitor activity, oligosaccharides and phytohaemagglutinating activity).

4.3.2. Ultrasound-assisted extraction of proteins

Ultrasound technology (US) uses sound waves at a frequency of 20 kHz, inducing cavitation phenomenon which increases the porosity of matrix by inducing the formation of micro-fissures and channels that improve permeation of solvent into the matrix (Karki et al., 2009; Tiwari, 2015). The application of US to assist extraction has multiple advantages which are reflected in more effective mixing, faster energy transfer, selective extraction, reduced thermal gradients and extraction temperature, reduced equipment size, faster response to process extraction control, quicker start up and increased production (Cravotto & Binello, 2016).

However, if applied to assist the extraction of proteins the drawbacks of US must be noted, which are reflected in the possible changes in protein structure, protein denaturation and functional properties (decreasing emulsification and foaming capability), especially in the case of high-power and prolonged time of sonication. Moreover, the modification of amino acids with sulfhydryl and phenolic residues is also possible resulting in the formation of new covalent linkages between proteins (Karki et al., 2009; Mawson, Gamage, Terefe, & Knoerzer, 2011; Yang et al., 2017). Yang et al. (2017) showed that ultrasonic pretreatment did not significantly increase the degree of hydrolysis of defatted wheat germ protein. After ultrasound pretreatment the changes in protein structure were recorded, and proved that ultrasound treatment looses the protein structure and influence the exposure of hydrophobic amino acid residues of protein.

Ultrasound-assisted extraction has been used to recover valuable proteins from several food industry byproducts. Zhu et al. (2009) demonstrated the feasibility of ultrasound pretreatment to increase the protein extraction from defatted wheat germ by reverse micelles from 37% to 57% of proteins, with final protein extraction efficiency of 45.6% (Zhu et al., 2009). By applying ultrasound pretreatment of brewer's spent grain, Tang et al. (2010) enhanced the yield of extracted protein at room temperature (P = 88.2 W/100 mL, solid:liquid ratio of 2:100, 81.4 min). By application of ultrasound to assist alkaline extraction of proteins from peanut flour, Ochoa-Rivas et al. (2017) obtained an increase of 136% of proteins (86% purity) (100% amplitude at 24 KHz and 15 min), where the assistance of ultrasound to alkaline treatment gave decrease in water solubility index, nitrogen solubility index, foam stability, emulsifying activity and *in vitro* digestibility, while water absorption index and foam activity were better in comparison to the control extraction without using ultrasound treatment. Preece, Hooshyar, Krijgsman, Fryer, and Zuidam (2017a) demonstrated the feasibility of ultrasound at different scales (lab- and pilot-) to assist the protein extraction from okara. Preece et al. (2017a) demonstrated the significant improvement of the protein extraction yield up to 4.2% by the ultrasonic treatment of okara solution on pilot plant scale. Moreover, it was shown that the okara concentration and okara flow rate had the higher contribution to the protein extraction yield than that of ultrasound treatment. The lowest flow rate resulted in the highest protein extraction yield due to the increased residence time within the ultrasonic cell and the increased contact time between the extraction medium (alkali water) and protein. The lowest okara concentration resulted in the highest protein extraction yield due to the increased quantity of available solvent per unit of protein. However, pilot-scale sonication was not enough to destroy all the cells and enable the releasing of protein bodies indicating a reduced effect of pilot-scale sonication. On the other hand, during lab-scale okara sonication, the samples experienced higher energy intensity which consequently resulted in a greater impact of ultrasound treatment and in a higher protein extraction yield (up to 40% after 15 min treatment). Preece, Hooshyar,

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Krijgsman, Fryer, and Zuidam (2017b) demonstrated that ultrasound treatment increased the protein extraction yield from soy slurry by 10% after 1 min of treatment, which was attributed to improved solubility rather than cell disruption.

Recent research has indicated that non-thermal treatments, like that of an ultrasonication, have the potential to alter the allergenicity of several foods (Li et al., 2016b; Shriver & Yang, 2011). Li et al. (2016b) succeeded in decreasing the allergenicity of soybean protein isolate by 18.9% using high-intensity ultrasound.

4.3.3. Pulsed electric energy-assisted extraction of proteins

Different types of pulsed electric energy (PEE) technology have emerged for the intensification of separation, extraction, pressing, freezing, diffusion and drying in different agri-food applications. It uses short duration of electric pulses (from several nanoseconds to several milliseconds) of high-pulse amplitude (from 100 to 300 V/cm to 10–50 kV/cm) to induce the structural changes of matrix of interest (Vorobiev & Lebovka, 2016).

Among different PEE techniques, pulsed electric fields (PEF), pulsed ohmic heating (POH) and high-voltage electrical discharges (HVED) have emerged as the most interesting ones to be applied in the food industry (Vorobiev & Lebovka, 2016). By acting of PEF the rupture of cell membranes occurs, enabling a cold diffusion of intracellular material (Sarkis et al., 2015). PEF is commonly designated as non-thermal treatment, which enables undesirable changes in biological material, being of special interest when extracting proteins.

Application of PEE (PEF and HVED) as a pretreatment for the extraction of proteins from sesame cake resulted in the reduced amount of organic solvents, time and temperature needed for the extraction has been demonstrated. Proteins were extracted at 40 °C in the first 20 min of process, indicating the relevance of this technique for industrial use. Literature on the use of PEF to assist extraction of proteins from vegetal sources is scarce. However, there are a lot of papers demonstrating the extraction of other bioactive compounds e.g. polyphenols (Sarkis et al., 2015). One study demonstrated its applicability to assist proteins extraction from rapeseed biomass (stems and leaves), and has been found that only high electric field strength (20 kV/cm) statistically enhanced proteins yield (up to about 80%) from rapeseed leaves (Yu, Bals, Grimi, & Vorobiev, 2015).

The reports on the assistance of PEF to protein extraction are available for algae (e.g. *Nanochloropsis*, *Chlorella*) (Coustets & Teissié, 2016), and olive kernel (Roselló-Soto et al., 2015). Roselló-Soto et al. (2015) demonstrated the superiority of HVED treatment over ultrasound and pulsed electric field in terms of energy input and effective treatment time engaged for protein extraction from olive kernels.

PEF treatment is not able to significantly affect the secondary structure of proteins and therefore not able to affect the allergenicity of proteins (Johnson et al. (2010).

Ohmic heating uses the electrical resistance of foods to directly convert electricity to heat. As it generates rapid and uniform heat in the entire volume between the electrodes, ohmic heating has emerged as an alternative to thermal methods of pasteurizing and sterilizing (Jaeger et al., 2016). Apart from heating, applied electric field under ohmic heating causes electroporation of cell membranes, a significant increase in their electrical conductivity and permeability which positively influence extraction rates of different biomolecules (Nair et al., 2014). Due to electroporation, the potential applications of this technique in extraction technologies are very wide especially for very sticky (viscous) materials or fluids containing solid particles (Gavahian & Farahnaky, 2018; Jaeger et al., 2016; Yodsuwan, Kamonpatana, Chisti, & Sirisansaneeyakul, 2018). To the best of our knowledge, there is no available data in the literature on the application of ohmic heating for the extraction of proteins from food sources except that applied as a pre-treatment in solvent extraction of oil from rice bran (Nair et al., 2014).

4.3.4. High hydrostatic pressure-assisted extraction of proteins

The most common application of high hydrostatic pressure processing (HHP) in the food industry is large scale microbial cell disruption, emulsification and meat tenderization. HHP extraction assistance has been restricted to certain bioactive compounds rather than proteins (Sikes & Warner, 2016; Preece, Hooshyar, Krijgsman, Fryer, & Zuidam, 2017c). The study describing the effects of HHP on the extraction of proteins from processing by-products is limited to the study of Preece et al. (2017c) who applied HHP treatments based on hydrodynamic cavitation (50–125 MPa) to soy slurry and okara, and found improved extraction yields of protein up to 82% with a single pass of soy slurry at 100 MPa, wherein a particle size reduction and disruption of intact cells occurred. However, by application of multiple iterations of HHP, decreased separation efficiency and reduced extraction yield was observed due to cell wall swelling, increase in particle sizes and increase in dynamic viscosity.

On the other hand, HHP technology has turned out to be applicable in the alteration of protein allergenicity due to a reversible or irreversible structural modification in proteins it causes (Huang, Hsu, Yang, & Wang, 2014). Li, Zhu, Zhou, and Peng (2012) demonstrated the reduction of the allergenicity of soy protein isolate (SPI) for 48.6% in comparison to native SPI upon pressurization at 300 MPa for 15 min, while pressurization at 200–300 MPa for 5–15 min, induced the increase in free SH content and hydrophobicity of SPI. Hu et al. (2011) demonstrated the decrease of antigenicity of peanut allergen Ara h 2 due to high-pressure microfluidisation at 60, 90, 120, 150 and 180 MPa. Li et al. (2016b) succeeded to decrease the allergenicity of soybean protein isolate for 29.8% and 46.6% by high-pressure homogenization and high hydrostatic pressure, respectively. Peñas, Gomez, Frias, Baeza, & Vidal-Valverde (2011) showed the industrial relevance of HHP for the production of hypoallergenic soybean products.

Eco-innovative approach to the protein extraction from renewable sources of plant origin is presented in Fig. 1.

As an overview of renewable sources and technological solutions to the protein extraction, Table 1 containing quantitative data for the feedstock, protein content and obtained protein yield.

5. Conclusion: the current status of the utilization of food waste and novel processing technologies for protein extraction

Eco-innovative extraction technologies reviewed have been emerged mostly as an alternative for conventional technologies with the main aim to enable the production of safe, nutritious and chemical-free proteins of preserved techno-functional properties. Although these technologies have been investigated extensively for the extraction of proteins, the majority of them are still in its infancy for their commercial adoption. The majority of the presented examples and obtained results refer to the experiments carried out at laboratory scale. Although some of the authors emphasize great scalability of proposed processes, exclusively on the basis of lab experiments it is hard to estimate if the global process will be cost-effective and economically viable. However, their great potential lies in the fact that they are able to alter the allergenicity of proteins. In contrast to wet extraction technique, great deal of dry extraction is already on pilot scale. Given the absence of solvents, catalyst and external heating, compatibility with downstream enzymatic processes, a competitive energy consumption and possibility to obtain lignin, protein, and carbohydrate fractions through a continuous dry process at the same time, it seems that dry extraction techniques at this point have the greatest potential to be commercially applied.

The commercial exploitation of eco-innovative extraction technologies reviewed in this paper is limited due to lack of industrial-scale equipment of high capacity and easy implementation within the existing processing lines, and above all high energy consumption. The technological developments conducted in the last years, has driven the suc-

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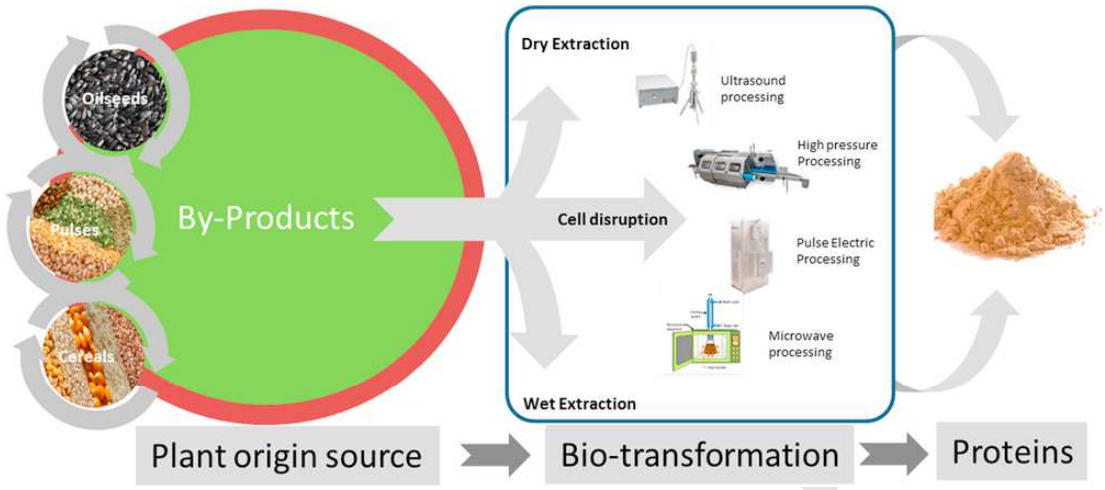


Fig. 1. Eco-innovative technologies for protein extraction.

cessful transfer of the PEF and HHP technology for large scale industrial applications, while for the others it is still challenging to have the equipment responding to the food industry needs (Versteeg, 2016).

Food processed by new technologies is already considered in EU regulations. According to the Novel Food Regulation (EU) 2015/2283, "novel foods" include foods obtained in a production process not used for food production within the Union before 15 May 1997, which significantly may affect the food composition, food structure, nutritional value, metabolism or level of undesirable substances. Thus, a new production process applied in food production does not automatically mean the food becomes "novel". It is the responsibility of the party who wants to market the food to seek clarification on the regulatory status. On the other hand, the utilization of novel processing technologies is regulatory encouraged in EU due to their potential to reduce the environmental impact of food production, enhance food security and bring benefits to consumers (). Moreover, the legislation which should be considered in the exploitation of food by-products and food waste is the regulation on novel foods and novel food ingredients (Regulation (EC) No. 258/97) (Waldron, 2007). Nevertheless, the feedstock availability for protein extraction should be taken into account as it is regionally dependent and not evenly distributed worldwide.

Uncited references

Endres, 2001; European Union, 2012; Peñas et al., 2011.

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Table 1
On overview of different techniques used for protein extraction from by-products from food industry.

Extraction method	Feedstock	Starting protein content	Resulting protein content	Protein recovery	Disruption technique	Comments
Feedstock from cereal processing						
Electrostatic separation	Wheat bran	15.4% d.m.	Max. 19.5% d.m.	Max. 64.1% (relatively to the separation yields)	Ambient and cryogenic milling	Better separation by three successive grindings at ambient temperature, than that by one under cryogenic conditions
Alkaline extraction	Rice bran	14.13 ± 0.07%	71.27%	22.07%	Microwave (1000 W, extraction time of 90 s, solid-liquid ratio 0.89 g/10 ml)	Increased protein yield by ~1.54-fold
Alkaline extraction	Rice bran	14%		~65%	Enzyme (Alcalase at pH 9)	Marginal increase in protein yield observed
Water extraction	Defatted rice bran	16.8%		~30%	Subcritical water treatment (200 °C, 5 min)	n/a
Reverse micelles	Defatted wheat germ	37%	57%	45.6%	Ultrasound (power 363 W, 24 min, pulse mode 2.4:2 s)	Great potential for scaling-up
Reverse micellar system	Defatted wheat germ	31.48%	87.47%	~30%	Hammer milling	Great potential for scaling-up
Alkaline extraction	Defatted oat bran	17.6%	81.7%	56.2%	Enzyme (Viscozyme L at pH 4.6)	Significantly higher protein recovery (alkaline method 14.8%)
Alkaline extraction	Brewer's spent grain	7.6%	10.42%	~100%	Ultrasound (power 88.2 W/100 ml, 81.4 min, solid-liquid ratio 2.0 g/100 ml)	Need for removing large volumes of water
Feedstock from legume processing						
Electrostatic separation	Lupine flour	40.5% d.m.	Max 65% d.m.	Fraction yield 6%; 10% of protein in the flour was recovered	Pin + impact milling	Custom-build bench scale
Single-stage tribo-electrostatic separation	Bean flour	25.4% d.m.	39.4–42.9%	~45%	Pin-milling	A vertical laboratory-scale triboelectric separator used
Two stage tribo-electrostatic separation			~38%	~60%		
Feedstock from edible oil processing						
Electrostatic separation (10 000 V)	Sunflower oil cake	30.8%	48.9%	93%	Knife + impact milling (ambient t°, 12 000 rpm)	The absence of solvents, catalyst and external heating, compatibility with downstream enzymatic processes, low energy consumption, simultaneous extraction of lignin, proteins, and carbohydrates
Alkaline extraction	Peanut flour	54.86%	~93%	~55%	Microwave (725 W, 8 min)	A synergic effect of MAE and US was not observed
			~86%	~ 68%	Ultrasound assisted (24 kHz, 100% amplitude, 15 min)	
Alkaline extraction	Rapeseed meal	26.3%	58%	50–80%	Enzymes (Protex 5L, Protex P and Protex 40XL at alkaline pH 9.5–11)	Enzymes improved protein extraction from rapeseed and microalgae, but not from soybean meal
	Soybean meal	36.4%	61%	90%		
	Microalgae meal	37.3%	60%	50-80%		

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Table 1 (*Continued*)

Extraction method	Feedstock	Starting protein content	Resulting protein content	Protein recovery	Disruption technique	Comments
Water extraction	Defatted rapeseed press cake	39%	37.8%	40–41%	Enzyme (Pectinase at pH 6)	Better solubility and dispersion stability of proteins obtained
Alkaline extraction			57.3%	53%		
Water extraction	Intact rapeseed press cakes	35.9%	32.3%	56%	Enzyme (Pectinex Ultra SP-L at pH6)	n/a
	Dehulled rapeseed press cakes	40.1%	31.4%	74%		
Water extraction	Dehulled cold-pressed double-low rapeseed cake			71.89%	Enzymes (combination of Viscozyme L and Alcalase)	n/a
Alkaline extraction	Cold-pressed flaxseed meal	34.13%	65.08%		Enzyme (cellulose at pH 5)	Poor techno-functional properties of proteins with solvent extraction
Solvent extraction/ precipitation (ethanol)			86.80%		Enzyme (cellulose at pH 5)	
Alkaline extraction	Sacha inchi kernel cake	~59%		44.7%	Enzyme (alcalase at pH 9)	~1.5 fold higher protein recovery
Alkaline extraction	Heat-denatured soy meals		~81%	~50%	Enzyme (protease at pH 4.5) and subcritical water treatment	Better emulsifying ability and physical stability of emulsion obtained
Reverse micellar system	Soybean flour	37.53%	83.6%	72.40%		Improved thermal, textural and some physical-chemical properties of soy proteins
Other feedstocks						
Alkaline extraction	Soybean	Soy slurry 5.2%		Soy slurry 81%	High pressure homogenization (50–125 MPa)	A single pass at 100 MPa, made higher impact on slurry than on okara treatment
		Okara 5.4%		Okara 90%		
Alkaline extraction	Soy flakes	Lab scale 60.6%	Pilot scale 92.8%	Lab scale 62.4%	Enzyme (Indi Age Super L; enzyme; pH 7.0)	No synergistic effect with enzyme combinations
		Pilot scale 53.5%		Pilot scale 40.7%		
Alkaline extraction	Coconut milk press cake	5.19%		43.15%	Hammer mill	1 million tonnes/year

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