

Chapter 2

Extraction/Fractionation Techniques for Proteins and Peptides and Protein Digestion

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2.1 Introduction

The use of proteomics approaches is a powerful tool in food science in terms of process optimization and monitoring, quality, traceability, safety, and nutritional assessment (Pedreschi et al. 2010). Proteins, together with peptides, are one of the major groups of food components, and they are found in many different organisms of both vegetal and animal origin. Peptides are also obtained during technological processes such as fermentation and storage of foods. Moreover, many experiments involve enzymatic hydrolysis of proteins from food resources such as milk, meat, fish, eggs, or plants to produce a variety of peptides (Minkiewicz et al. 2008).

The study of the food proteome at any specific time is extremely complex and diverse. The major limitations of proteome analysis are, in general, associated with the heterogeneity of proteins and peptides in terms of physicochemical properties and the vast differences in abundance. A typical proteomics workflow consists of (1) protein extraction, (2) protein or peptide separation and quantification, (3) protein identification, and (4) data analysis and interpretation (Carpentier et al. 2008). Sample preparation has a profound effect on the final outcome of protein and peptide separation and their subsequent analysis. These procedures need to be compatible with posterior analysis by two-dimensional electrophoresis (2DE) and/or liquid chromatography tandem mass spectrometry (LC-MS/MS). Therefore, sample preparation should include the steps needed to isolate and fractionate proteins and

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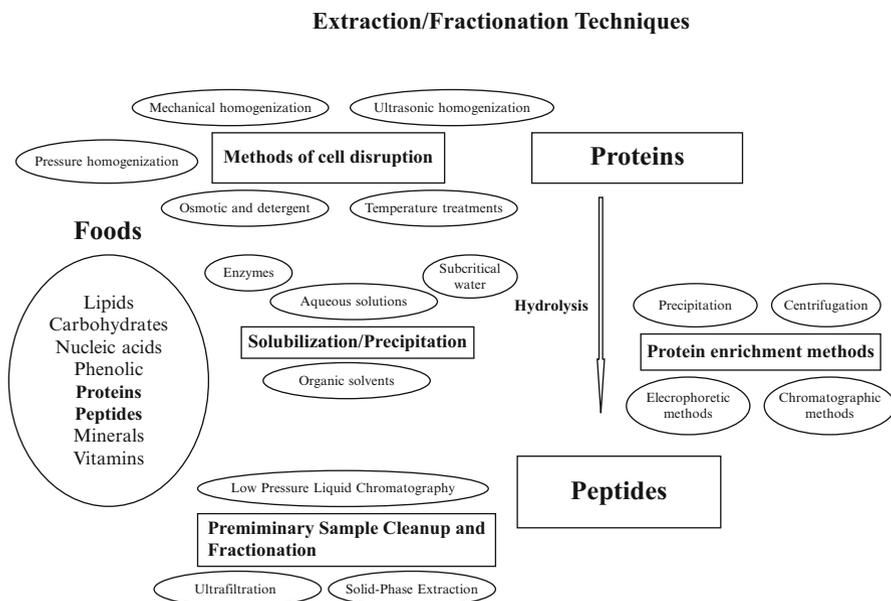


Fig. 2.1 Scheme illustrating integrated extraction and fractionation techniques for proteins and peptides employed on proteomics in foods

peptides ensuring an unbiased reliable map that gives an accurate representation of all proteins and peptides initially present in a particular food.

A wide variety of extraction and fractionation tools for proteins and peptides are available based on their physicochemical and structural characteristics such as solubility, hydrophobicity, molecular weight, isoelectric point (pI), and so on. Figure 2.1 shows an integrated view of extraction and fractionation techniques for proteins and peptides used in food proteomics studies. Generally, different technologies focused on cell disruption, solubilization/precipitation, and enrichment systems are needed to obtain the protein fraction of interest. Removal of interfering compounds (mainly lipids, nucleic acids, phenolic compounds, carbohydrates, proteolytic and oxidative enzymes, and pigments) is crucial. These procedures need to be optimized to minimize proteins' modifications and proteolysis, as well as to be compatible with subsequent analysis.

This chapter describes the state-of-the-art of extraction and fractionation techniques for food proteins and peptides as a first step prior to proteome studies. The first part is dedicated to classical and novel extraction and fractionation techniques for food proteins, followed by a brief description on protein enzymatic digestion. The second part provides information about several extraction/fractionation techniques mainly used for food peptides.

2.2 Food Protein Extraction and Fractionation

2.2.1 Cell Disruption Methods

The preparation of any biological material as a sample for proteomic analysis requires homogenization. Plants are generally more problematic for protein extraction because tissues are rich in proteases and other interfering compounds (Wang et al. 2008a). Proteins are usually contained in protein bodies inside cell walls so cell disruption is required before they can be totally solubilized and extracted. The general procedure for sample preparation in this case strongly depends on the plant type, its fragment (leaf, fruit, seed, etc.), or even the stage of plant development. Generally, disruption of the cell wall and protein release is crucial for analytical success. Various chemical and physical techniques can be used to destroy the cell wall. These techniques can be grouped into five major categories: mechanical homogenization, ultrasound homogenization, pressure homogenization, temperature treatments, and osmotic and chemical lysis. A summary of these methods with their applications in different food matrices is shown in Table 2.1.

2.2.1.1 Mechanical Homogenization

Mechanical homogenization can be realized by at least two types of devices: so-called rotor–stator homogenizers and open blade mills. Rotor–stator homogenizers are one of the best homogenizing tools applied in laboratories. To homogenize dry samples using mechanical processing, open blade homogenizers, also called blenders, are used (Bodzón-Kulakowska et al. 2007). In the case of plant tissues, where cells are covered with strong cell walls, mechanical homogenization seems to be one of the best methods for disruption (Van Het Hof et al. 2000). Anderson and Guraya (2001) evaluated the use of colloid milling and homogenization to effect bran breakdown and extract rice protein. They demonstrated that the shearing actions of colloid milling and homogenization did not result in any significant denaturation of the proteins. Sometimes, a combination of mechanical homogenization with buffers is used. Examples of this are found in rice (Fukuda et al. 2003) and in olive tree seeds (Alche et al. 2006).

Wet-milling is a physicochemical separation of the components of grain, namely germ, bran, fiber, starch, and protein. Chemicals and enzymes can be added to the steeping water to facilitate the separation of grain components and increase starch recovery. Sulfur dioxide, sodium metabisulfite, sodium bisulfite, or sodium hydrogen sulfite, with variable effective concentrations, are typically added to solubilize the protein matrix enveloping the starch granules in the endosperm (De Mesa-Stonestreet et al. 2010).

Table 2.1 Examples of techniques used for food plant cell disruption

Type of disruption	Procedure	Food	Tissue	Reference	
Mechanical homogenization	Colloid milling and homogenization	Rice	Bran	Anderson and Guraya 2001	
	Centrifugal grinding and air dehulling	Pea, chickpea, lentil	Seed	Boye et al. 2010	
	Cool mortar with lysis buffer	Rice	Embryo	Fukuda et al. 2003	
	Cool mortar with Tris-HCl	Olive tree	Seed	Alche et al. 2006	
	Wet-milling with sulphur dioxide	Sorghum	Seed	De Mesa-Stonestreet et al. 2010	
Ultrasonic homogenization	Acoustic transducer	Soybean, rice	Root, sheath/hypocotyl, leaf	Toorchi et al. 2008	
	Ultrasonic generator	Rice	Bran	Chittapalo and Nookhorm 2009	
Pressure homogenization	High pressure homogenization	Peanut	Seed	Dong et al. 2011	
	Mortar and pestle with liquid N ₂	Rapeseed	Seed	Barbin et al. 2011	
Temperature treatments	Mortar and pestle with liquid N ₂	Tomato	Pollen	Sheoran et al. 2009	
		Olive	Leaf	Wang et al. 2003	
	Pulverization in dry ice and grinding in liquid N ₂	Apple, banana	Mesocarp/exocarp	Song et al. 2006	
		Peanut	Seed	Liang et al. 2006	
	Microwave, dry heating and parboiling	Maize	Endosperm	Méchin et al. 2007	
		Potato	Tuber	Delaplace et al. 2006	
	Wet-milling with temperature	Grape	Berry cluster	Vincent et al. 2006	
		Rice	Bran	Khan et al. 2011	
	Osmotic and chemical lysis	Hexadecyltrimethylammonium bromide (CTAB), sodium dodecyl sulphate (SDS) or isopropylalcohol (IPA)	Sorghum	Seed	De Mesa-Stonestreet et al. 2010
			Lactococcus lactis strains	-	Doolan and Wilkinson 2009

2.2.1.2 Ultrasonic Homogenization

In recent years, some researchers have used the ultrasonic method for protein pellet homogenization. The energy is produced by an acoustic transducer coupled with the pellet in the microtube, which is carried out manually in small batches. Toorchi et al. (2008) have attempted to use a high-performance, single-tube sample preparation device (Covaris) for the noncontact disruption and uniform preparation of three different plant tissues from soybean (root, hypocotyl, and leaf) and rice (root, leaf sheath, and leaf).

Many researchers have investigated the advantages of ultrasonic-assisted extraction compared with the conventional method. Chittapalo and Noomhorm (2009) reported that protein yield increased using ultrasound and that this process can enhance existing extraction processes and enable new commercial extraction opportunities.

2.2.1.3 Pressure Homogenization

The use of high-pressure homogenization (HPH) for the extraction of food proteins has been investigated. Higher pressures (40 and 80 MPa) produced approximately double protein extraction compared to atmospheric pressure. Dong et al. (2011) have suggested that HPH treatment could increase the susceptibility of peanut proteins to proteolytic enzymes such as alcalase. The increase may be related to the denaturation, unfolding, or dissociation of the proteins into monomers, allowing the accessibility of enzyme to the binding sites. HPH revealed no alteration of protein solubility when compared with the raw protein with pH adjusted in rapeseed protein concentrates (Barbin et al. 2011).

2.2.1.4 Temperature Treatments

Temperature treatments include the use of freeze–thaw and heat treatments. Freeze–thawing uses the effect of ice crystal formation in the tissue during the freezing process. Lysis of the cells or tissues is usually achieved by flash-freezing the cells in liquid nitrogen and homogenizing in a mortar with a pestle. Examples of this process are found in the analysis of leaves (Wang et al. 2003), fruits (Song et al. 2006), and seeds (Liang et al. 2006; Méchin et al. 2007). Vincent et al. (2006) developed a very efficient cell disruption method for grape berry clusters, which were pulverized frozen with dry ice using a stainless steel blender.

The use of heat is common in protein processing. Heating protein solutions usually improves their solubility, emulsifying, and foaming properties, but it makes protein extraction more difficult as reported in rice bran (Tang et al. 2002; Khan et al. 2011). Another approach is the application of heat during the wet-milling process. Steeping experiments have been done on temperature and holding time on sorghum grain (De Mesa-Stonestreet et al. 2010).

2.2.1.5 Osmotic and Chemical Lysis

Cell permeabilization or cell lysis can be performed by osmotic shock or chemical treatment. The use of osmotic shock implies cell suspension in a gently shaken hypertonic solution. Chemical treatment can include antibiotics, chelating agents, detergents, and solvents capable of disintegrating the cells. This procedure relies on the selective interaction of the chosen chemicals with components of the membrane and allows proteins to seep through the cell wall. The application of two or more procedures combined with the cell-wall disruption is also reported (Klimek-Ochab et al. 2011). These procedures are used in cell cultures of bacteria, yeast, or fungi. Doolan and Wilkinson (2009) have compared the effects of various chemicals on cell permeability in *Lactococcus lactis* strains with the aim of selectively releasing important intracellular ripening enzymes. Their findings permit a better understanding of methods affecting cell permeability and can allow development of food-grade technologies for protein released from cells.

2.2.2 Protein Solubilization/Precipitation

Protein solubilization is considered one of the key steps in proteomic sample preparation procedures. It is generally employed to separate proteins in the sample selectively from different substances that may interfere in the proteomic assay (Berkelman and Stenstedt 1998). The solubilization/precipitation process strongly affects the quality of the final results and thus determines the success of the entire experiment. Taking into account the immense variety of proteins and the huge number of interfering contaminants present in food-derived extracts, simultaneous solubilization of all proteins remains a great challenge.

Each food sample requires a specific protocol that needs to be optimized to minimize proteolysis and modification of proteins (Bodzon-Kulakowska et al. 2007). For animal tissues, which have higher protein yields, various protein solubilization buffers, including the use of chaotropic agents, detergents, reducing agents, buffers, and ampholites are used (Pedreschi et al. 2010). The proper use of these additives avoids protein modifications, aggregation, or precipitation that may result in the occurrence of artifacts and the subsequent lowering of protein yield (Gorg et al. 2004).

2.2.2.1 Organic Solvents

The main organic solvents and additives used to extract proteins from food sources are shown in Table 2.2. Many studies performed in the last few years aimed to compare different protein solubilization methods suitable for proteomic analysis (Jiang et al. 2004; Natarajan et al. 2005; He and Wang 2008). The most common

Table 2.2 Examples of organic solvents and additives used to extract proteins from food sources

Solvent (s)	Food	Tissue	Reference
Acetic acid/urea/cetyltrimethylammonium bromide	Rice	Bran	Hamada 1997
Aqueous ethanol	Distiller's grain	Grain	Cookman and Glatz 2009
Aqueous isopropanol	Soybean	Seed	Natarajan et al. 2009
	Rapeseed	Seed	Barbin et al. 2011
Ethanol	<i>Saccharina japonica</i>	–	Kim et al. 2011
Glacial acetic acid	Sorghum	–	de Mesa-Stonestreet et al. 2010
Phenol	Tomato	Pollen grain	Sheoran et al. 2009
	Potato	Tuber	Delaplace et al. 2006
	<i>Aloe vera</i>	Leaf	He and Wang 2008
	Soybean	Seed	Natarajan et al. 2005
Phenol/ammonium acetate	Barley	Root	Hurkman and Tanaka 1986
	Avocado/tomato/orange/banana	Fruit	Saravanan and Rose 2004
	Banana	Leaf	Carpentier et al. 2007
	Grape	Fruit	Vincent et al. 2006
	Pear	Fruit	Pedreschi et al. 2007
	Apple/strawberry	Fruit	Zheng et al. 2007
	Coniferous	Seed	Zhen and Shi 2011
Phenol/methanol-ammonium acetate	Banana/apple/potato	Tissues	Carpentier et al. 2005
	Coniferous	Seed	Zhen and Shi 2011
Sodium dodecyl sulphate/acetone	Potato	Tuber	Delaplace et al. 2006
	Apple/banana	Tissue	Song et al. 2006
Sodium dodecyl sulphate/TCA/acetone	Apple/banana	Tissue	Song et al. 2006
TCA	Bean	Anther	Wu and Wang 1984
TCA/acetone	Citrus	Leaf	Maserti et al. 2007
	Soybean	Seed	Natarajan et al. 2006
	Soybean	Leaf	Xu et al. 2006
	Coniferous	Seed	Zhen and Shi 2011
	Tomato	Pollen grain	Sheoran et al. 2009
	<i>Aloe vera</i>	Leaf	He and Wang 2008
	Apple/banana	Tissues	Song et al. 2006
	Olive	Leaf	Wang et al. 2003
	Bamboo/grape/lemon	Leaf	Wang et al. 2006
	Apple/orange/tomato	Fruit	Wang et al. 2006
Thiourea/urea	Soybean	Seed	Natarajan et al. 2005
	Apple/banana	Tissues	Song et al. 2006
Tris–HCl buffer	Tomato	Pollen grain	Sheoran et al. 2009

method used for the extraction of plant proteins is trichloroacetic acid (TCA)/acetone precipitation as proposed by Damerval et al. (1986). This method has been used to extract proteins from different tissues of cereals, legumes, and fruits. The extreme pH

and negative charge of TCA and the addition of acetone realizes an immediate denaturation of the protein, along with precipitation, thereby instantly arresting the activity of proteolytic and other modifying enzymes. However, a disadvantage of TCA-precipitated proteins is that they are difficult to redissolve (Nandakumar et al. 2003). Sample solubility can be improved by using an appropriate mixture of chaotropic agents (urea or thiourea), and new efficient detergents (such as sodium dodecyl sulphate, SDS). In the last decade, the phenol extraction procedure has been widely used because of its high clean-up capacity. In contrast to its strong solvent action on proteins, phenol has little predisposition to dissolve polysaccharides and nucleic acids. However, phenol shows the disadvantages of being more time consuming than other sample precipitation procedures and of being toxic.

The alcoholic extraction process after dehulling and conventional deoiling has a high efficiency of protein recovery. Aqueous alcohols (ethanol, isopropyl alcohol, butanol) are widely used on a commercial scale to remove phenolics, oligosaccharides, or inhibitors from defatted meals and seeds (Moure et al. 2006). However, as a result of the extraction with these alcoholic solvents, protein structures can be coagulated and therefore show reduced functional properties. To avoid these problems and to obtain protein concentrates or isolates with good functionality and suitable as food ingredients, mechanical and thermal treatments are applied (Moure et al. 2006; Barbin et al. 2011). Recently, extractions with different organic solvents, such as n-hexane, 2-methyl pentane, diethyl ether, acetone, 2-propanol, and ethanol were compared regarding effectiveness, suitability, and protein solubility of the full-fat and defatted lupin (Bader et al. 2011).

2.2.2.2 Aqueous Solutions

In recent years, because of the growing environmental concerns over the use of organic solvents to extract oil/protein from oil-bearing food materials, aqueous extraction is gaining attention. Water is also operationally advantageous over alcohols because it is nonflammable and neither explosive nor toxic. Commercially, the production of protein concentrates (48–70% protein) or isolates (85–90% protein) consists of an aqueous solubilization of protein and carbohydrates at acid, neutral, or alkaline pH and the selective recovery of the solubilized protein, separation, and, optionally, washing and neutralization before drying. The protein extraction yield and properties are influenced by the type of extraction process and by different factors such as pH, salts concentration, the ionic strength of the medium, net charge, and electrostatic repulsions (Tan et al. 2011).

A number of acid and alkaline protein extraction protocols have been published from various plant and animal tissues. In the last decade, different studies have focused on evaluating the effect of extraction methods on the functional and rheological properties of proteins recovered from by-products of the meat and fish industry (Liang and Hultin 2003; Chaijan et al. 2006; Hrynets et al. 2010, 2011; Moayedi et al. 2010; Omana et al. 2010). In the case of plant proteins, the ideal extraction method is particularly challenging due to the metabolic and structural characteristics

of plant tissues, including the cell wall matrix. The majority of alkaline extraction protocols are based on the so-called Osborne method (Osborne 1924), but each method is optimized according to the aim of the study and the type of vegetal protein source. Recent studies report the use of mainly sodium and calcium salts to extract proteins from different vegetal foods (Ghaly and Alkoaik 2010; Horax et al. 2010; Lestari et al. 2010; Karaca et al. 2011; Nadal et al. 2011). These extraction methods are simple because the agents required are easily available. However, as a result of the degradation at high pH conditions, the protein yield is generally low. Also, the protein quality can be altered by alkaline processing due to undesirable reactions involving racemization of amino acids, formation of toxic compounds such as lysinoalanine, reduction of digestibility, loss of essential amino acids, and decrease in nutritive value. Furthermore, the remaining alkali needs to be washed thoroughly from the final product, leading to generation of a large amount of wastewater (Sereewatthanawut et al. 2008). To optimize protein precipitation recovery different strategies have been developed. Use of additives, such as TCA or carboxymethylcellulose is generally accepted (Massoura et al. 1998). Extraction and further formation of protein micelles have also been proposed (Krause et al. 2002; Murray 2003; Ser et al. 2008; Green et al. 2010). This method has been demonstrated to reduce the concentration of problematic antinutritional or toxic factors, including the glucosinolates and their degradation products during canola protein extraction (Tan et al. 2011).

2.2.2.3 Aqueous Enzymatic Extraction

An alternative approach combining aqueous and enzymatic extraction is attracting attention. Studies using this extraction process are shown in Table 2.3. Enzymes can aid in the extraction of proteins in several ways. Carbohydrases, which can attack the cell wall components, may increase protein yield by liberating more protein from the matrix source (Ansharullah et al. 1997; Wang et al. 1999; Tang et al. 2002). A combination of cell wall-hydrolyzing enzymes (i.e., Viscozyme L) has been used to cleave linkages within the polysaccharide matrix effectively and hence, liberate more intracellular protein from oat bran (Guan and Yao 2008). In the last few years, different proteases, alone or in combination, have been used to partially hydrolyze proteins to peptides, increasing their solubility and making them more easily extractable. Recently, De Moura and co-workers (2011) developed a two-stage countercurrent aqueous enzymatic extraction process for soybean, significantly reducing the amount of water used. They achieved slightly higher oil and protein extraction yields than those from standard single-stage aqueous enzymatic extraction.

Aqueous enzymatic protein extraction has been defined as an environmentally friendly, safe, and cheap alternative to extract oil and protein simultaneously (Latif and Anwar 2009). Moreover, this process avoids serious damage to the proteins produced by the refining steps, improving their nutritional and functional properties (Domínguez et al. 1994; Moure et al. 2000). However, and although the enzymatic extraction process produces no toxic chemicals, it shows some disadvantages, such

Table 2.3 Aqueous-enzymatic extraction processes used to obtain protein isolates or concentrates from food sources

Enzyme	Food	Tissue/sample	Protein extracted (%)	Reference
Alcalase™	Rice	Bran	81.0	Hamada 2000
Alcalase 2.4 L	Rapeseed	Seed	66.7	Zhang et al. 2007
	Peanut	Seed	82.5	Wang et al. 2008b
	Peanut	Roasted seed	80.1	Zhang et al. 2011
Alcalase + Protamex (1:3)	Tea	Leave pulp	47.8	Shen et al. 2008
Alkaline protease	Rice	Broken rice	75.5	Hou et al. 2010
Flavourzyme	Rice	Bran	88.0	Hamada 2000
Glucosylase	Lentil	Bean	–	Bildstein et al. 2008
Neutrase 1.5MG	Coconut	Meat	83.0	Sant'Anna et al. 2003
Olivex+Celluclast	<i>Guevinia avellana</i>	Pressed cakes	85.8	Moure et al. 2002
Papain	Rice	Broken rice	46.3	Hou et al. 2010
Pectinase + Protease P	Rice	Bran	80.0	Tang et al. 2003
Phytase	Rice	Bran	80.0	Wang et al. 1999
Protex 6 L	Distiller's grain	Grain	90.0	Cookman and Glatz 2009
	Lupin	Seed	77.2	Jung 2009
	Soybean	Seed	84.6	Jung 2009
	Soybean	Flakes	96.0	De Moura et al. 2011
Protex 7 L	Sesame	Seed	87.1	Latif and Anwar 2011
	<i>Moringa oleifera</i>	Seed	75.4	Latif and Anwar 2009
Viscozyme L	Coconut	Meat	83.0	Sant'Anna et al. 2003
	Oat	Bran	56.2	Guan and Yao 2008
	Rice	Bran	37.0	Tang et al. 2002
Viscozyme L + Cellulast 1.5 L		Bran	53.0	Ansharullah et al. 1997
Xylanase	Rice	Bran	82.0	Wang et al. 1999
Xylanase + Phytase amylase	Rice	Bran	75.0	Wang et al. 1999

as the long time required and the high cost of enzymes that makes this strategy uneconomical. The use of immobilized enzyme in protein extraction may reduce the overall cost by allowing the reuse of enzymes.

2.2.2.4 Subcritical Water

Recent studies demonstrate the use of water at subcritical conditions as an environmentally friendly reaction medium to extract proteins from different food sources. Subcritical water is water that maintains its liquid state in the temperature range of 100–374°C under pressurized conditions. Its unique properties, such as a lower relative dielectric constant and a higher ion product than ambient water, make subcritical water a promising extraction solvent for various compounds, including proteins (Hata et al. 2008). Ho et al. (2007) used pressurized low-polarity water to extract proteins from defatted flaxseed meal. A number of studies have demonstrated the ability of water at subcritical conditions to extract proteins from rice bran and soybean meal with high protein yields and good functional properties (Watchararujit et al. 2008; Fabian and Ju 2011).

2.2.3 Protein Enrichments Methods

Once the protein fraction has been isolated from the rest of the constituents and the interference substances have been eliminated, there are still some other steps that are needed prior to the analysis of the sample by mass spectrometry (MS). Despite the last technological developments, no single analytical method exists covering the protein concentration range present in a specific sample. Sometimes the total protein content is very low or the objective is the determination of minor proteins with post-translational modifications (e.g., phosphorylation).

In many cases the methods described in this part are comparable (if not the same) to those previously seen in this chapter during protein extraction. However, this section is focused more on those steps to be applied once the protein fraction has been separated from other interfering components. As a matter of fact, the purpose of fractionation and enrichment methods is to obtain distinguishable fractions and increase the concentration of the proteins of interest.

2.2.3.1 Centrifugation

The use of centrifugation is one of the simplest methods used for isolation and enrichment/fractionation of proteins. Centrifugation can be used for different purposes. It can be a first step to separate different cell substructures where our proteins of interest are locally concentrated, for instance, mitochondria, membrane, or nucleus. This process involves multiple centrifugation steps and, as a result, the

cellular homogenate is separated into different layers based on the molecular weight, size, and shape of each component. Afterwards, solubilization steps, as explained above, and enrichment and fractionation steps should be carried out to isolate the protein fraction from the selected layer prior to MS analysis.

Apart from its use separating crude mixtures of cell components, centrifugation is also commonly used to fractionate a protein mixture into different fractions. The separation takes place based on the coefficient of sedimentation of the proteins. This coefficient is usually expressed in *Svedberg units* (S), and the smaller the S value, the slower a molecule moves in a centrifugal field. Separation will depend on the mass, the shape, and the protein density. Numerous examples are found in the literature using the differential coefficient of sedimentation of the proteins to carry out fractionation (Sharma et al. 2010; Jiang et al. 2011). The efficiency of this fractionation step can be enhanced using gradient centrifugation, where the centrifuge tube is filled with a solution of sucrose, forming a density gradient.

2.2.3.2 Precipitation

It is recognized that among the different precipitants the most widespread is ammonium sulphate (Bodzon-Kulakowska et al. 2007). The addition of high amounts of this salt or other such as sodium chloride into a protein solution provokes an increase of protein interactions followed by protein aggregation and finally precipitation. This is known as a salting-out process and, as the salt concentration needed for protein precipitation varies from one protein to another, it allows selective protein separation. An alternative salting-out method using decreasing solutions of salt can also be used to enrich previously precipitated protein fractions. This salting-out approach has been used to separate the main storage soybean proteins, glycinin and β -conglycinin (Deak et al. 2006).

Another type of protein enrichment is immunoprecipitation, based upon the binding of the antigen to its specific antibody to form the antigen–antibody complex. In general it offers high recoveries of the proteins and it is widely used for food allergens (Pastorello and Trambaioli 2001).

2.2.3.3 Electrophoretic Methods

Electrophoresis separates mixtures of proteins based on charge, charge/mass ratio, size, or shape. This technique is mainly used as an analytical and preparative tool, especially one-dimensional separation, often employed as a pre-fractionating technique (Guttman et al. 2004; Jorgenson and Evans 2004). Often, laboratories dedicate one-dimensional gel electrophoresis (1DE) to evaluate the outcome of protein purification preceding the analysis by (2DE) (Chen et al. 2007).

Electrophoretic pre-fractionation methods include electrokinetic methodologies performed in free solution, essentially all relying on isoelectric focusing (IEF) steps. Purification using IEF is especially advantageous when protein activity must be maintained. Bioactivity is maintained because the proteins remain in solution in their native conformation. Based on the IEF principle, different instruments have been developed such as the Rotofor, a multicompartamental device with focusing chambers that allows the fractionation of volumes of sample (12–60 mL) containing micrograms to grams of protein (Hey et al. 2008). Another well-known device is the so-called “Off-Gel IEF” (Keidel et al. 2011). Upon application of an electric field perpendicularly to the liquid chamber, the current lines penetrate into the chamber and extract charged proteins from the solution into the IEF gel. In its multicompartamental format, the protein fractions are separated by ranges of pI depending on their positioning over the IEF gel strip. Other instruments of interest are the Octopus, a continuous-flow device for isoelectric focusing in an upward flowing liquid curtain, and the Gradiflow, where different pI cuts are obtained by a multi-step passage through two compartments buffered at different pH values (Righetti et al. 2003).

Depending on the complexity of the samples, the separated fractions can be analyzed directly by MS or in some cases they may undergo a subsequent separation step in a second dimension, generally SDS-PAGE, to separate the proteins according to their molecular weight. In the first case, the possible presence of ampholytes may imply an extra step to remove them and avoid disturbance in MS.

2.2.3.4 Chromatographic Methods

Liquid chromatography (LC) techniques are the most commonly used in proteome pre-fractionation prior to in-depth analysis. The separation of the different proteins is achieved according to their charge, hydrophobicity, size, or specificity. In some cases, chromatographic methods can also be used to eliminate some interference substances (e.g., salts) coming from previous enrichment steps.

Among LC fractionation methods, ion-exchange chromatography (IEX) is probably the most used, with proteins being separated according to their pI. Acidic proteins are usually fractionated by anion-exchange chromatography whereas basic proteins are fractionated by cation-exchange chromatography. IEX has been often used to separate milk proteins as reported by Gómez-Ruiz et al. (2007a), who used cation-exchange chromatography to separate sheep milk caseins.

Reverse phase LC (RP-LC) separates proteins according to their hydrophobicity. Proteins are adsorbed on a stationary phase carrying hydrophobic groups, and are eluted with increasing concentration of an organic solvent, generally acetonitrile. RP-LC is widely used in proteomics in combination with IEX and MS analysis, usually in shotgun multidimensional strategies that are used as an alternative to 2-D-PAGE technology. A special case of chromatography based on hydrophobic interactions uses a high concentration of lyotropic salts (frequently ammonium sulphate) to expose the hydrophobic parts of proteins towards the hydrophobic patches of

solid-phase sorbents. Desorption is promoted by using a decreasing concentration of the lyotropic salts.

Pre-fractionation with chromatographic methods is also used to investigate post-translational modifications such as glycosylation or phosphorylation, to cite perhaps the two most important. These modifications are mainly studied using affinity chromatography (AC). This chromatography utilizes highly specific biological interactions (i.e., antigen–antibody, receptor–ligand, enzyme–substrate/inhibitor, etc.). AC results are quite adequate for accessing low concentrated proteins in complex samples, in some cases through the depletion of high abundance proteins that remain bound to the column. Examples of AC are heparin chromatography, broadly used for studying microbial proteins, or lectin chromatography that is specially used for glycoproteins (Lee and Lee 2004; Azarkan et al. 2007). Immobilized metal affinity chromatography (IMAC) is used to enrich phosphoproteins. This chromatography is based on formation of coordinate bonds between basic groups on protein surface and metal ions. The major drawback is that little or no binding to Fe(III) or Ga(III) charged resins is observed at neutral pH, and using low-pH buffers may provoke protein denaturalization or precipitation in the column (Schmidt et al. 2007).

Some other techniques such as size-exclusion chromatography (SEC) separate proteins according to their molecular mass, as the second dimension of 2-D-PAGE. However, unlike 2-D-PAGE this chromatography can be used under nondenaturing conditions allowing the study of protein complexes. As an example, SEC has been used for the evaluation of the bread-making quality of hard spring wheat flours (Ohm et al. 2009). Additional methods based on the use of chip-based arrays are gaining importance recently, with surface-enhanced laser desorption/ionization (SELDI) one of their maximum exponents (Righetti et al. 2005). Unlike chromatography separation, here only retained proteins are eventually studied and the other proteins are removed by one or more washing steps. Subsequently, the use of a pulse of laser light provokes the desorption of the proteins of interest which are converted into gaseous ions and analyzed by MS, typically using time of flight (TOF) analyzers.

2.3 Protein Digestion

Once the proteins have been isolated from interfering compounds (other food components such as lipids, nucleic acids, phenolic compounds, or carbohydrates) they are usually analyzed by 1D or 2-D SDS-PAGE, depending of the complexity of the sample. Gel electrophoresis analysis is typically followed by protein digestion, a key procedure prior to the identification of proteins by MS. However, in some cases digestion is carried out without electrophoretic separation. For instance, direct digestion of a mixture of proteins is adequate when a broad survey of the identifiable protein components is desired or to minimize the loss of peptides by binding to the polyacrylamide when characterizing post-translational modifications (Kinter and Sherman 2005).

Different proteolytic agents are used for protein digestion, including enzymes such as trypsin, different endoproteases (Lys-C, Arg-C, Asp-N, Glu-C), or chymotrypsin, as well as chemical reagents such as hydroxylamine or cyanogens bromide. The specificity of the amide bond or bonds cleaved by these reagents allows the obtaining of specific peptides that facilitate the interpretation of their mass spectra and database search. Trypsin is certainly the most popular reagent because it shows many advantages compared to other enzymes and chemical reagents, in addition to its relatively low cost of production and high purity. This enzyme cleaves amide bonds at the C-terminal side of Lys and Arg residues except when these bonds are to Pro. Apart from this selectivity, Arg and Lys are common amino acids distributed through most proteomes such that tryptic cleavage yields peptides with an average length suitable for MS. Finally, trypsin cleavage yields peptides containing a strongly basic residue (Lys or Arg) at the C-terminal, a fact that facilitates the interpretation of collision-induced dissociation (CID) mass spectra (Couto et al. 2011).

When talking about protein digestion in proteomic studies we mainly think of two types of digestion: “in-gel” digestion and “in-solution” digestion. Most proteomic studies perform in-gel digestion of proteins previously separated by their charge and/or their molecular weight (1D or 2-D SDS-PAGE). Identification of proteins from polyacrylamide gels offers a number of important advantages compared to gel-free approaches, such as higher dynamic range of analysis of protein mixtures (ratio of lowest to highest abundance protein detectable) or removal of low molecular weight impurities before the MS analysis. In-gel protein digestion was first established by Rosenfeld et al. (1992). The typical steps of the method have remained the same since then, although small variations have been introduced to improve its performance. Destaining, reduction, and alkylation of Cys, enzymatic cleavage of proteins into peptides, and extraction of peptides from the gel are described as essential steps in obtaining high-quality mass spectra. Analysis by electrospray (ESI)-MS, less tolerant to salts, requires an additional desalting step which is optional for MALDI-MS (Granvogl et al. 2007)

Despite its widespread use, conventional tryptic digestion is very time consuming, with a typical digestion time in the range of several hours to half a day (Park and Russell 2000). This is a clear limitation to the production of high-throughputs in proteomic analysis. Therefore, in recent times many efforts have been focused on developing efficient and fast protein digestion methods. Several alternatives to the standard protocol have been proposed, many of them based on the use of electromagnetic waves (i.e., electromagnetic radiation), such as microwaves, infrared (IR) radiation, and ultraviolet (UV) light to accelerate protein digestion (Chen et al. 2011; Dycka et al. 2012). Among these strategies, the use of IR-assisted digestion seems to be the most promising approach due to its safety compared to the other electromagnetic waves. Other alternatives are the use of modified trypsin, for instance, by reductive methylation which decreases autolysis and shifts its optimal catalytic temperature to 50–60°C. This modified trypsin allows the reduction of digestion times from 16 h to 30 min without losing efficiency. For in-solution digestion, immobilized trypsin systems as part of a microchip bioreactor offer a

very efficient alternative to conventional methods. As an example, the use of a fiber-based microchip bioreactor provides on-chip digestion in less than 5 s with similar tryptic digests to those obtained by the conventional in-solution tryptic digestion (Fan and Chen 2007).

During each step along the protein digestion experiments extreme care is necessary to avoid contamination that can compromise MS analyses and the outcome of the study. In some cases the contamination refers to low molecular weight compounds (from either the polyacrylamide gel or the subsequent digestion of specific bands) that will not provoke erroneous protein identification but will complicate peptide detection due to higher noise levels. The use of high-purity reagents throughout the experiment, especially for gel-electrophoresis and digestion can significantly minimize this type of contamination. On other occasions, contamination with keratins, proteins derived from skin and hair, is the main problem. In this case wearing gloves and an adequate handling of the laboratory consumables (e.g., pipette tips) and reagents will limit this contamination.

2.4 Food Peptide Extraction and Fractionation

Generally, food peptide content is not as abundant as would be desirable. In addition to this, the presence of nonpeptidic constituents (i.e., lipids, sugars) may also interfere in peptide analysis. Therefore, in practice it is difficult to analyze food peptides with good accuracy without performing a sample preparation step. This sample preparation can comprise diverse procedures for isolation, purification, and pre-concentration of the analyte, more than one step being required in many cases (Poliwoda and Wiczorek 2009).

RP-LC and capillary electrophoresis (CE), are the basic analytical methods used for chemometrical analysis of food peptidome (Minkiewicz et al. 2008). In relation to CE and capillary electrochromatography (CEC), restrictions come from the small sample volume applied (nano- to picolitre) that necessitates the application of pre-concentration and pre-separation steps in samples with low peptide concentration or complex mixtures (Kasicka 2012).

In general, food samples are first subjected to a preliminary sample cleanup step to remove interfering substances and then, different fractionation steps are applied, as has been widely revised (González de Llano et al. 2004; Asensio-Ramos et al. 2009; Hernández-Ledesma et al. 2012). Several options that are summarized in Table 2.4 may be taken. Direct peptide analysis on food samples without any preparation treatment is not often reported in the literature (Cheison et al. 2010).

Peptide derivatization may be necessary in some analyses for better detection (Wang et al. 2011). Most derivatizations are developed with fluorescent labels to become detectable with fluorescence detection whose limit of detections (LODs) is about two to three orders of magnitude lower than common UV-absorption detections (Kasicka 2012). An example may be found in the determination of glutathione in must and white wine during alcoholic fermentation (Lavigne et al. 2007).

2.4.1 Extraction and Preliminary Sample Cleanup

Hydrophilic peptides are generally extracted with homogenization in water or in solutions of organic acids whereas organic solvents are used to obtain highly hydrophobic peptides. Homogenization in a mixture of organic solvents (chloroform/methanol) can be used for peptide extraction as well as for the removal of sample interferences after producing a biphasic system. By using this method, Kostyra et al. (2003) studied the opioid activity of cheese and fermented milk samples. On the other hand, homogenization in water has been widely applied on cheese, fish, meat, and cereals samples as shown in Table 2.4. Typically, the ratio of water to cheese used was 2:1 in the homogenization process, followed by an incubation step of an hour at 60°C (Gómez-Ruiz et al. 2002; Meyer et al. 2009).

Peptide extraction is usually followed by a preliminary sample cleanup for removal of other food components (i.e., proteins, lipids). Deproteinization, the most important preliminary cleanup procedure in peptide analysis, is carried out by precipitation of protein using several agents. Deproteinization could also act as a fractionation procedure for peptides because their solubility depends on the precipitant agent and its proportion (Cheng et al. 2010a,b). After precipitation, centrifugation and filtration methods are used to separate proteins from soluble peptides. In addition, the application of heat treatments or ultracentrifugation steps at high speed to eliminate the proteins has been reported (Gómez-Ruiz et al. 2007b; Ho et al. 2010).

The selectivity of precipitation directly depends on the type of precipitating agent applied. In addition to the use of organic solvents such as ethanol, methanol, or acetone, solutions containing acids such as TCA or trifluoroacetic acid (TFA) are classical protein precipitants (Juan-García et al. 2009; Escudero et al. 2010). Salting-out precipitation, based on polarity, with high concentrations of salts or precipitation by adjusting the pH to the pI of protein (Contreras et al. 2010; Pihlanto et al. 2010) are other options. A representative example is found in the isolation and identification of an angiotensin I-converting enzyme (ACE) inhibitory peptide from whole buckwheat seeds after consecutive cleanup steps of diethyl ether extraction in order to remove most of the fat content and deproteinization by adjusting the pH to the pI of buckwheat protein (Ma et al. 2006).

In some cases, the application of homogenization and/or deproteinization is enough to proceed with peptide analysis (Contreras et al. 2010). Unfortunately, most of the samples need additional steps to achieve suitable peptide isolation and concentration levels before the analysis.

2.4.2 Fractionation

2.4.2.1 Ultrafiltration

Ultrafiltration is mainly useful for fractionating peptides as well as the removal of proteins and other macromolecules based on their molecular size. Dedicated

Table 2.4 Examples of food sample preparation for peptidomic analysis

Food matrix	Extraction and clean-up	Fractionation techniques	References
<i>Dairy products</i>			
Cheese, milk, yoghurt and infant formula	Homogenization, centrifugation and deproteinization by pH adjustment	Ultrafiltration	De Noni and Cattaneo 2010
Dry-off cows milk	Centrifugation and ultracentrifugation	Ultrafiltration	Ho et al. 2010
Fermented milk	Centrifugation	SPE	Hernández-Ledesma et al. 2005
Manchego cheese	Homogenization, centrifugation, filtration and ultracentrifugation	Ultrafiltration and SEC	Taborda et al. 2007; Gómez-Ruiz et al. 2007b
Whey protein concentrate hydrolysate	Centrifugation and filtration	Ultrafiltration	Contreras et al. 2011
<i>Fish and meat</i>			
Rainbow trout muscle	Homogenization and centrifugation	Ultrafiltration and SPE	Bauchart et al. 2007
Cuttlefish protein hydrolysate	Centrifugation	Derivatization of anserine	Balti et al. 2010
Loach protein hydrolysate	Centrifugation	SEC	You et al. 2010
Dry-cured ham	Homogenization, centrifugation, filtration and deproteinization by ethanol addition	Ultrafiltration, IEX and SEC	Mora et al. 2010, 2011
Pork meat digest	Centrifugation and deproteinization by ethanol addition.	SEC	Escudero et al. 2010
<i>Eggs</i>			
Egg white protein hydrolysate	Centrifugation	SEC and IEX	Liu et al. 2010
Egg white protein hydrolysate	Centrifugation	Ultrafiltration	Miguel et al. 2004
<i>Drinks</i>			
Beer	–	SPE and SEC	Picariello et al. 2011
Must and wines	Centrifugation (not bottled wines)	Derivatization of SH functions	Lavigne et al. 2007
White and red wines	Centrifugation	Ultrafiltration and SEC	Pozo-Bayón et al. 2007

Vegetable foods

Corn zein hydrolysate	Pigments extraction and centrifugation	Ultrafiltration	Tang et al. 2010
Fermented soybean extract	Filtration and dialysis	Ultrafiltration and IEX	Rho et al. 2009
Soy protein hydrolysate product	pH adjustment and centrifugation	–	Johns et al. 2011
Pea protein hydrolysate	Centrifugation	Ultrafiltration and SPE	Li and Aluko 2010
Potato protein hydrolysate	Salting-out precipitation and centrifugation	SEC	Cheng et al. 2010a, b

IEX ion exchange chromatography, *SEC* size exclusion chromatography, *SPE* solid phase extraction

membranes are mostly made of polysulfone or cellulose derivatives. Cellulose membranes have excellent hydrophilicity, which is very important in minimizing fouling, but they possess low chemical resistance and poor mechanical strength. However, polysulfone membranes provide high rigidity but foul earlier because of their hydrophobicity (Doyen et al. 2011). Commercially, membranes offer a wide range of cutoffs (500–100 kDa) and different formats including centrifugal units or cassettes for peristaltic lab systems. Fractionation of peptides has been achieved in food samples by applying ultrafiltration with more than one cutoff membrane. As an example, Samaranyaka et al. (2010) searched the presence of antioxidant and ACE inhibitory peptides in a hake protein hydrolyzate using an ultrafiltration unit with different molecular mass cutoff membranes (10, 3, and 1 kDa). In summary, ultrafiltration presents some advantages as the sample is not diluted or organic solvents are not required. Therefore, in some cases after the ultrafiltration step no additional fractionation processes are applied before analysis, such as in cheese (Bütikofer et al. 2008) or champagne wine samples (Person et al. 2004). Nevertheless, samples often need further pre-treatment procedures that mainly improve the analyte concentration.

A recent technology named electro dialysis with ultrafiltration membranes (EDUF) has been developed to fractionate peptides from complex mixtures on the basis of their electrical charge, size, or molecular weight. A conventional electro dialysis is used but some ion exchange membranes are replaced by ultrafiltration ones. This equipment has been employed for the concentration and selective separation of bioactive peptides from an alfalfa white protein hydrolyzate (Firdaous et al. 2009). A successful use of these membranes has also been reported, isolating an antihypertensive peptide from a tryptic hydrolyzate of β -lactoglobulin (Poulin et al. 2007).

2.4.2.2 Low-Pressure Liquid Chromatography

Low-pressure size exclusion chromatography (SEC) fractionates peptides on the basis of their molecular size. This technique separates analytes through a bed of porous beads where they can either enter or be excluded from the internal space of the beads based on their size. Elution occurs from the largest to the smallest analyte over time (Ly and Wasinger 2011). Several resins with different pore sizes are commercially available. Cross-linked dextran (Sephadex) resins are mostly used but polyacrylamide (BioGel P) or divinylbenzene polymers are also available as stationary phases (Poliwoda and Wieczorek 2009). Depending on the resin composition, peptides are eluted with water, organic acids, ammonia, or ammonium salts, even as alcoholic solutions that reduce potential hydrophobic interactions. For instance, Mora et al. (2011) applied SEC to fractionate peptides released during dry-cured ham processing in a Sephadex G25 column under isocratic conditions in 0.01 N HCl. Other uses have been reported, for instance, to identify ACE inhibitory peptides in white and red wines (Pozo-Bayón et al. 2007).

Low-pressure ion exchange chromatography (IEX) constitutes another technique for peptide fractionation in food analysis. In this case, peptides are fractionated according to their net surface charge/polarity. Porous or nonporous matrices with hydrophilic materials such as cellulose, cross-linked dextrans, polystyrene polymers (Dowex resins), or Bio-Rex membranes are very useful as anion or cation exchange stationary phases. These matrices are substituted with functional groups that determine the charge of the medium (e.g., quaternary ammonium, diethylaminoethyl, sulfopropyl, carboxymethyl, etc.). The ionic strength increases as the elution method can carry a large amount of salts in the elution buffer that makes samples incompatible with techniques such as MS (Ly and Wasinger 2011).

Off-line combination of IEX and SEC has been reported in some food peptide analyses. A representative example is found in the work of Liu et al. (2010), who fractionated an egg white protein hydrolyzate by SEC with Sephadex G-25 resin followed by IEX (Sephadex C-25 column) of those fractions with the highest ACE inhibitory activity. A similar fractionation strategy has also been used in the study of antioxidant peptides in a fish protein hydrolyzate (You et al. 2010), and for the evaluation of the peptide contribution to the umami taste of soy sauces (Lioe et al. 2006).

2.4.2.3 Solid-Phase Extraction

Solid-phase extraction (SPE) is based on the same principle of affinity-based separation as liquid chromatography. SPE enables retention and elution of analytes from complex mixtures, removal of interfering compounds, and sample concentration. SPE is available in normal phase, reverse-phase, and ion exchange modes, reversed-phase being one of the most used formats (Kole et al. 2011). Based on the wide range of physicochemical properties of the analytes, several commercial sorbents (e.g., C₁₈, C₈, C₂, phenyl, cyanopropyl, and ion exchange bonded materials, among others) are supplied to improve the versatility of SPE. Regarding this, in the peptidomic characterization of beer, Picariello et al., (2011) applied the samples directly onto the C₁₈ pre-packed cartridges and eluted with acetonitrile/TFA to RP-LC. In other examples, Hernández-Ledesma et al. (2005) treated the water-soluble extract of fermented milk with a Sep-Pak C₁₈ cartridge and acetonitrile elution, and a similar extraction step was used by Muguruma et al. (2009) to desalt SEC-eluted fractions from porcine myosin B.

Based on similar principles of SPE techniques, innovative size-reduced devices have recently appeared for concentration, purification, and desalting of peptides prior to analysis by MS. These devices support a membrane or microcolumn that can be of diverse nature (polar, nonpolar, and ion exchange) and feature an optimized procedure for sample preparation. For instance, in the study of trout peptidome changes during storage, Bauchart et al. (2007) used a C₁₈ membrane device prior to MALDI-TOF analysis with the aim of removing the perchloric acid used in the previous extraction. Another case is found in the study of beer peptidome in which

residual interfering sugars are removed by applying the sample on C₁₈ Zip-Tip microcolumns and peptides eluted with acetonitrile/TFA (Picariello et al. 2011).

2.5 Future Perspectives

Current efforts are mainly focused on the improvement and development of automated systems as today sample preparation implies several labor-intensive and time-consuming handling steps. Despite the generalized use of 2-D electrophoresis, this technology has limitations mainly when dealing with proteins at varying expression levels. An alternative could be the use of automated pre-fractionation methods such as electrokinetic methodologies performed in free solution combined with one-dimensional PAGE and capillary LC-MS/MS. Recent technologies such as SELDI also imply minimal requirements for purification and separation of proteins prior to their analysis by MS. For the investigation of post-translationally modified proteins the future approach seems to be the combined use of affinity-based enrichment and extraction methods and multidimensional separation technologies prior to MS analysis. A persisting challenge is still the development of appropriate enrichment/fractionation techniques to facilitate MS analysis of membrane proteins.

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References

- Alche JD, Jiménez-López JC, Wang W, Castro-López AJ, Rodríguez-García MI (2006) Biochemical characterization and cellular localization of 11S type storage proteins in olive (*Olea europaea* L.) seeds. *J Agric Food Chem* 54:5562–5570
- Anderson AK, Guraya HS (2001) Extractability of protein in physically processed rice bran. *J Am Oil Chem Soc* 78:969–973
- Ansharullah A, Hourigan JA, Chesterman CF (1997) Application of carbohydrases in extracting protein from rice bran. *J Sci Food Agric* 74:141–146
- Asensio-Ramos M, Hernández-Borges J, Rocco A, Fanali S (2009) Food analysis: a continuous challenge for miniaturized separation techniques. *J Sep Sci* 32:3764–3800
- Azarkan M, Huet J, Baeyens-Volant D, Looze Y, Vandenbussche G (2007) Affinity chromatography: a useful tool in proteomics studies. *J Chromatogr B* 849:81–90
- Bader S, Oviedo JP, Pickardt C, Eisner P (2011) Influence of different organic solvents on the functional and sensory properties of lupin (*Lupinus angustifolius* L.) proteins. *LWT Food Sci Technol* 44:1396–1404
- Balti R, Nedjar-Arroume N, Adjé EY, Guillochon D, Nasri M (2010) Analysis of novel angiotensin I-converting enzyme inhibitory peptides from enzymatic hydrolysates of cuttlefish (*Sepia officinalis*) muscle proteins. *J Agric Food Chem* 58:3840–3846

- Barbin DF, Natsch A, Müller RK (2011) Improvement of functional properties of rapeseed protein concentrates produced via alcoholic processes by thermal and mechanical treatments. *J Food Process Preserve* 35:369–375
- Bauchart C, Chambon C, Mirand PP, Savary-Auzeloux I, Rémond D, Morzel M (2007) Peptides in rainbow trout (*Oncorhynchus mykiss*) muscle subjected to ice storage and cooking. *Food Chem* 100:1566–1572
- Berkelman T, Stenstedt T (1998) 2-D electrophoresis using immobilized pH gradients. Principles and methods. Amersham Biosciences, San Francisco, CA, USA
- Bildstein M, Lohmann M, Hennigs C, Krause A, Hilz H (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
- Bodzon-Kulakowska A, Bierzczynska-Krzysik A, Dylag T, Drabik A, Suder P, Noga M, Jarzebinska J, Silberring J (2007) Methods for samples preparation in proteomic research. *J Chromatogr B* 849:1–31
- Boye JI, Aksay S, Roufik S, Ribéreau S, Mondor M, Farnworth E, Rajamohamed SH (2010) Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques. *Food Res Int* 43:537–546
- Bütikofer U, Meyer J, Sieber R, Walther B, Wechsler D (2008) Occurrence of the angiotensin-converting enzyme inhibiting tripeptides Val-Pro-Pro and Ile-Pro-Pro in different cheese varieties of Swiss origin. *J Dairy Sci* 91:29–38
- Carpentier SC, Kris Laukens EW, Deckers P, Swennen R, Panis B (2005) Preparation of protein extracts from recalcitrant plant tissues: an evaluation of different methods for two-dimensional gel electrophoresis analysis. *Proteomics* 5:2497–2507
- Carpentier SC, Dens K, Van den houwe I, Swennen R, Panis B (2007) Lyophilization, a practical way to store and transport tissues prior to protein extraction for 2DE analysis? *Proteomics* 7:S64–S69
- Carpentier SC, Panis B, Vertommen A, Swennen R, Sergeant K, Renaut J, Laukens K, Witters E, Samyn B, Devreese B (2008) Proteome analysis of non-model plants: a challenging but powerful approach. *Mass Spectrom Rev* 27:354–377
- Chaijan M, Benjakul S, Visessanguan W, Faustman C (2006) Physicochemical properties, gel forming ability and myoglobin content of sardine (*Sardinella gibbosa*) and mackerel (*Rastrelliger kanagurta*) surimi produced by conventional method and alkaline solubilisation process. *Eur Food Res Technol* 222:58–63
- Cheison SC, Schmitt M, Leeb E, Letzel T, Kulozik U (2010) Influence of temperature and degree of hydrolysis on the peptide composition of trypsin hydrolysates of β -lactoglobulin: analysis by LC-ESI-TOF/MS. *Food Chem* 121:457–467
- Chen B, Dong JQ, Chen YJ, Wang JM, Tian J, Wang CB, Zou SQ (2007) Two-dimensional electrophoresis for comparative proteomic analysis of human bile. *Hepatob Pancreat Dis Int* 6:402–406
- Chen Q, Liu T, Chen G (2011) Highly efficient proteolysis accelerated by electromagnetic waves for peptide mapping. *Curr Genomics* 12:380–390
- Cheng Y, Chen J, Xiong YL (2010a) Chromatographic separation and tandem MS identification of active peptides in potato protein hydrolysate that inhibit autooxidation of soybean oil-in-water emulsions. *J Agric Food Chem* 58:8825–8832
- Cheng Y, Xiong Y, Chen J (2010b) Fractionation, separation, and identification of antioxidative peptides in potato protein hydrolysate that enhance oxidative stability of soybean oil emulsions. *J Food Sci* 75:760–765
- Chittapalo T, Noomhorm A (2009) Ultrasonic assisted alkali extraction of protein from defatted rice bran and properties of the protein concentrates. *Int J Food Sci Technol* 44:1843–1849
- Contreras MM, Gómez-Sala B, Martín-Alvarez PJ, Amigo L, Ramos M, Recio I (2010) Monitoring the large-scale production of the antihypertensive peptides RYLGY and AYFYPEL by HPLC-MS. *Anal Bioanal Chem* 397:2825–2832

- Contreras MM, Hernández-Ledesma B, Amigo L, Martín-Alvarez PJ, Recio I (2011) Production of antioxidant hydrolyzates from a whey protein concentrate with thermolysin: optimization by response surface methodology. *LWT Food Sci Technol* 44:9–15
- Cookman DJ, Glatz CE (2009) Extraction of protein from distiller's grain. *Bioresour Technol* 100:2012–2017
- Couto N, Barber J, Gaskell SJ (2011) Matrix-assisted laser desorption/ionisation mass spectrometric response factors of peptides generated using different proteolytic enzymes. *J Mass Spectrom* 46:1233–1240
- Damerval C, Devienne D, Zyvy M, Thiellement H (1986) Technical improvements in two-dimensional electrophoresis increase the level of genetic-variation detected in wheat-seedling proteins. *Electrophoresis* 7:52–54
- De Mesa-Stonestreet NJ, Alavi S, Bean SR (2010) Sorghum proteins: the concentration, isolation, modification, and food applications of kafirins. *J Food Sci* 75:R90–R104
- De Moura JMLN, Campbell K, de Almeida NM, Glatz CE, Johnson LA (2011) Protein extraction and membrane recovery in enzyme-assisted aqueous extraction processing of soybeans. *J Am Oil Chem Soc* 88:877–889
- De Noni I, Cattaneo S (2010) Occurrence of β -casomorphins 5 and 7 in commercial dairy products and in their digests following in vitro simulated gastro-intestinal digestion. *Food Chem* 119:560–566
- Deak NA, Murphy PA, Johnson LA (2006) Effects of NaCl concentration on salting-in and dilution during salting-out on soy protein fractionation. *J Food Sci* 71:247–254
- Delaplace P, van der Wal F, Dierick JF, Cordewener JHG, Fauconnier ML, du Jardin P, America AHP (2006) Potato tuber proteomics: comparison of two complementary extraction methods designed for 2-DE of acidic proteins. *Proteomics* 6:6494–6497
- Domínguez H, Núñez MJ, Lema JM (1994) Enzymatic pretreatment to enhance oil extraction from fruits and oilseeds – a review. *Food Chem* 49:271–286
- Dong X, Zhao M, Shi J, Yang B, Li J, Luo D, Jiang G, Jiang Y (2011) Effects of combined high-pressure homogenization and enzymatic treatment on extraction yield, hydrolysis and function properties of peanut proteins. *Innov Food Sci Emerg Technol* 12:478–483
- Doolan IA, Wilkinson MG (2009) Comparison of the effects of various attenuation methods on cell permeability and accessibility of intracellular enzymes in *Lactococcus lactis* strains. *Int Dairy J* 19:215–221
- Doyen A, Beaulieu L, Saucier L, Pouliot Y, Bazinet L (2011) Impact of ultrafiltration membrane material on peptide separation from a snow crab byproduct hydrolysate by electro dialysis with ultrafiltration membranes. *J Agric Food Chem* 59:1784–1792
- Dycka F, Bobal P, Mazanec K, Bobalova J (2012) Rapid and efficient protein enzymatic digestion: an experimental comparison. *Electrophoresis* 33:288–295
- Escudero E, Sentandreu MA, Toldrá F (2010) Characterization of peptides released by in vitro digestion of pork meat. *J Agric Food Chem* 58:5160–5165
- Fabian C, Ju YH (2011) A review on rice bran protein: its properties and extraction methods. *Crit Rev Food Sci Nutr* 51:816–827
- Fan H, Chen G (2007) Fiber-packed channel bioreactor for microfluidic protein digestion. *Proteomics* 7:3445–3449
- Firdaus L, Dhulster P, Amiot J, Gaudreau A, Lecouturier D, Kapel R (2009) Concentration and selective separation of bioactive peptides from an alfalfa white protein hydrolysate by electro dialysis with ultrafiltration membranes. *J Membr Sci* 329:60–67
- Fukuda M, Islam N, Woo SH, Yamagishi A, Takaoka M, Hirano H (2003) Assessing matrix assisted laser desorption/ionization-time of flight-mass spectrometry as a means of rapid embryo protein identification in rice. *Electrophoresis* 24:1319–1329
- Ghaly AE, Alkoik FN (2010) Extraction of protein from common plant leaves for use as human food. *Am J Appl Sci* 7:323–334
- Gómez-Ruiz JA, Ramos M, Recio I (2002) Angiotensin-converting enzyme-inhibitory peptides in Manchego cheese manufactured with different starter cultures. *Int Dairy J* 12:697–706

- Gómez-Ruiz JA, Ramos M, Recio I (2007a) Identification of novel angiotensin-converting enzyme-inhibitory peptides from ovine milk proteins by CE-MS and chromatographic techniques. *Electrophoresis* 28:4202–4211
- Gómez-Ruiz JA, Taborada G, Amigo L, Ramos M, Molina E (2007b) Sensory and mass spectrometric analysis of the peptidic fraction lower than one thousand daltons in Manchego cheese. *J Dairy Sci* 90:4966–4973
- González de Llano D, Herráiz T, Polo MC (2004) Peptides. In: Nollet ML (ed) *Handbook of food analysis*, vol. 1. Physical characterization and nutrient analysis, vol 1. Marcel Dekker, New York, pp 125–166
- Gorg A, Weiss W, Dunn MJ (2004) Current two-dimensional electrophoresis technology for proteomics. *Proteomics* 4:3665–3685
- Granvogl B, Plösch M, Eichacker LA (2007) Sample preparation by in-gel digestion for mass spectrometry-based proteomics. *Anal Bioanal Chem* 389:991–1002
- Green BE, Martens RW, Tergesen JF, Milanova R (2010) Production of flax protein isolate. US Patent 7,820,226 B2
- Guan X, Yao HY (2008) Optimization of viscozyme L-assisted extraction of oat bran protein using response surface methodology. *Food Chem* 106:345–351
- Guttman A, Varoglu M, Khandurina J (2004) Multidimensional separations in the pharmaceutical arena. *Drug Discov Today* 9:136–144
- Hamada JS (1997) Characterization of protein fractions of rice bran to devise effective methods of protein solubilization. *Cereal Chem* 74:662–668
- Hamada JS (2000) Characterization and functional properties of rice bran proteins modified by commercial exoproteases and endoproteases. *J Food Sci* 65:305–310
- Hata S, Wiboonsirikul J, Maedab A, Kimura Y, Adachi S (2008) Extraction of defatted rice bran by subcritical water treatment. *Biochem Eng J* 40:44–53
- He C-F, Wang Y-M (2008) Protein extraction from leaves of *Aloe vera* L., a succulent and recalcitrant plant, for proteomic analysis. *Plant Mol Biol Rep* 26:292–300
- Hernández-Ledesma B, Miralles B, Amigo L, Ramos M, Recio I (2005) Identification of antioxidant and ACE-inhibitory peptides in fermented milk. *J Agric Food Chem* 85:1041–1048
- Hernández-Ledesma B, Martínez-Maqueda D, Miralles B, Amigo L, Gómez-Ruiz JA (2012) Peptides. In: Nollet ML, Toldrá F (eds) *Food analysis by HPLC*. Marcel Dekker, New York, in press
- Hey J, Posch A, Cohen A, Liu N, Harbers A (2008) Fractionation of complex protein mixtures by liquid-phase isoelectric focusing. *Methods Mol Biol* 424:225–239
- Ho CHL, Cacace JE, Mazza G (2007) Extraction of lignans, proteins and carbohydrates from flaxseed meal with pressurized low polarity water. *LWT Food Sci Technol* 40:1637–1647
- Ho CH, Chang CJ, Liu WB, Peh HC, Chen SE, Chen HY, Ho TH, Chen MT, Nagahata H (2010) In situ generation of milk protein-derived peptides in drying-off cows. *J Dairy Res* 77:487–497
- Horax R, Hettiarachchy N, Over K, Chen P, Gbur E (2010) Extraction, fractionation and characterization of bitter melon seed proteins. *J Agric Food Chem* 58:1892–1897
- Hou L, Zhu Y, Li Q (2010) Characterization and preparation of broken rice proteins modified by proteases. *Food Technol Biotechnol* 48:50–55
- Hrynets Y, Dileep AO, Xu Y, Betti M (2010) Effect of acid- and alkaline-aided extractions on functional and rheological properties of proteins recovered from mechanically separated turkey meat (MSTM). *J Food Sci* 75:E477–E486
- Hrynets Y, Dileep AO, Xu Y, Betti M (2011) Comparative study on the effect of acid- and alkaline-aided extractions on mechanically separated turkey meat (MSTM): chemical characteristics of recovered proteins. *Process Biochem* 46:335–346
- Hurkman WJ, Tanaka CK (1986) Solubilization of plant membrane-proteins for analysis by two-dimensional gel-electrophoresis. *Plant Physiol* 81:802–806
- Jiang L, Hea L, Fountoulakis M (2004) Comparison of protein precipitation methods for sample preparation prior to proteomic analysis. *J Chromatogr A* 1023:317–320

- Jiang J, Xiong YL, Chen J (2011) Role of β -conglycinin and glycinin subunits in the pH-shifting-induced structural and physicochemical changes of soy protein isolate. *J Food Sci* 76:293–302
- Johns PW, Jacobs WA, Phillips RR, McKenna RJ, O' Kane KA, McEwen JW (2011) Characterisation of peptide molecular mass distribution in commercial hydrolysates and hydrolysate-based nutritional products. *Food Chem* 125:1041–1050
- Jorgenson JW, Evans CR (2004) Multidimensional LC-LC and LC-CE for high-resolution separations of biological molecules. *Anal Bioanal Chem* 378:1952–1961
- Juan-García A, Font G, Juan C, Picó Y (2009) Nanoelectrospray with ion-trap mass spectrometry for the determination of beta-casomorphins in derived milk products. *Talanta* 80:294–306
- Jung S (2009) Aqueous extraction of oil and protein from soybean and lupin: a comparative study. *J Food Process Preserv* 33:547–559
- Karaca AC, Low N, Nickerson M (2011) Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. *Food Res Int* 44:2742–2750
- Kašička V (2012) Recent developments in CE and CEC of peptides (2009–2011). *Electrophoresis* 33:48–73
- Keidel EM, Dosch D, Brunner A, Kellermann J, Lottspeich F (2011) Evaluation of protein loading techniques and improved separation in OFFGEL isoelectric focusing. *Electrophoresis* 32:1659–1666
- Khan SH, Butt MS, Sharif MK, Sameen A, Mumtaz S, Sultan MT (2011) Functional properties of protein isolates extracted from stabilized rice bran by microwave, dry heat, and parboiling. *J Agric Food Chem* 59:2416–2420
- Kim E-Y, Kim D-G, Kim Y-R, Hwang H-J, Nam T-J, Kong I-S (2011) An improved method of protein isolation and proteome analysis with *Saccharina japonica* (Laminariales) incubated under different pH conditions. *J Appl Phycol* 23:123–130
- Kinter M, Sherman NE (2005) The preparation of protein digests for mass spectrometric sequencing experiments, in protein sequencing and identification using tandem Mass Spectrometry. Wiley, Hoboken
- Klimek-Ochab M, Brzezinska-Rodak M, Zymaczyk-Duda E, Lejczak B, Kafarski P (2011) Comparative study of fungal cell disruption-scope and limitations of the methods. *Folia Microbiol* 56:469–475
- Kole PL, Venkatesh G, Kotecha J, Sheshala R (2011) Recent advances in sample preparation techniques for effective bioanalytical methods. *Biomed Chromatogr* 25:199–217
- Kostyra E, Kostyra H, Krawczuk S, Jarmotowska B, Sienkiewicz-Szatapka E, Grzegorzewski W (2003) Procedure for the evaluation of the opioid activity of milk products. *Pol J Food Nutr Sci* 12:39–42
- Krause JP, Schultz M, Dudek S (2002) Effect of extraction conditions on composition, surface activity and rheological properties of protein isolates from flaxseed (*Linum usitatissimum* L). *J Sci Food Agric* 82:970–976
- Latif S, Anwar F (2009) Effect of aqueous enzymatic processes on sunflower oil quality. *J Am Oil Chem Soc* 86:393–400
- Latif S, Anwar F (2011) Aqueous enzymatic sesame oil and protein extraction. *Food Chem* 125:679–684
- Lavigne V, Pons A, Dubourdiou D (2007) Assay of glutathione in must and wines using capillary electrophoresis and laser-induced fluorescence detection. Changes in concentration in dry white wines during alcoholic fermentation and aging. *J Chromatogr A* 1139:130–135
- Lee WC, Lee KH (2004) Applications of affinity chromatography in proteomics. *Anal Biochem* 324:1–10
- Lestari D, Mulderb W, Sandersa J (2010) Improving *Jatropha curcas* seed protein recovery by using counter current multistage extraction. *Biochem Eng J* 50:16–23
- Li H, Aluko RE (2010) Identification and inhibitory properties of multifunctional peptides from pea protein hydrolysate. *J Agric Food Chem* 58:493–501
- Liang Y, Hultin HO (2003) Functional protein isolates from mechanically deboned turkey by alkaline solubilization with isoelectric precipitation. *J Muscle Food* 14:195–205

- Liang XQ, Luo M, Holbrook CC, Guo BZ (2006) Storage protein profiles in Spanish and runner market type peanuts and potential markers. *BMC Plant Biol* 6:24–32
- Lioe HN, Takara K, Yasuda M (2006) Evaluation of peptide contribution to the intense umami taste of Japanese soy sauces. *J Food Sci* 71:277–283
- Liu J, Yu Z, Zhao W, Lin S, Wang E, Zhang Y, Hao H, Wang Z, Chen F (2010) Isolation and identification of angiotensin-converting enzyme inhibitory peptides from egg white protein hydrolysates. *Food Chem* 122:1159–1163
- Ly L, Wasinger VC (2011) Protein and peptide fractionation, enrichment and depletion: tools for the complex proteome. *Proteomics* 11:513–534
- Ma M, In YB, Hyeon GL, Yang C (2006) Purification and identification of angiotensin I-converting enzyme inhibitory peptide from buckwheat (*Fagopyrum esculentum moench*). *Food Chem* 96:36–42
- Maserti BE, Della Croce CM, Luro F, Morillon R, Cini M, Caltavuturo L (2007) A general method for the extraction of citrus leaf proteins and separation by 2-D electrophoresis: a follow up. *J Chromatogr B* 849:351–356
- Massoura E, Vereijken JM, Kolster P, Derksen JTP (1998) Proteins from *Crambe abyssinica* oil seed. I. Isolation procedure. *J Am Oil Chem Soc* 75:323–327
- Méchin V, Thévenot C, Le Guilloux M, Prioul JL, Damerval C (2007) Developmental analysis of maize endosperm proteome suggests a pivotal role for pyruvate orthophosphate dikinase. *Plant Physiol* 143:1213–1219
- Meyer J, Bütikofer U, Walther B, Wechsler D, Sieber R (2009) Hot topic: changes in angiotensin-converting enzyme inhibition and concentrations of the tripeptides Val-Pro-Pro and Ile-Pro-Pro during ripening of different Swiss cheese varieties. *J Dairy Sci* 92:826–836
- Miguel M, Recio I, Gómez-Ruiz JA, Ramos M, López-Fandiño R (2004) Angiotensin I-converting enzyme inhibitory activity of peptides derived from egg white proteins by enzymatic hydrolysis. *J Food Prot* 67:1914–1920
- Minkiewicz P, Dziuba J, Darewicz M, Iwaniak A, Dziuba M, Nalecz D (2008) Food peptidomics. *Food Technol Biotechnol* 46:1–10
- Moayed V, Omana DA, Chan J, Xu Y, Betti M (2010) Alkali aided protein extraction from chicken dark meat: composition and stability to lipid oxidation of recovered proteins. *Poult Sci* 89:766–775
- Mora L, Sentandreu MA, Toldrá F (2010) Identification of small troponin T peptides generated in dry-cured ham. *Food Chem* 123:691–697
- Mora L, Valero ML, Sánchez del Pino MM, Sentandreu MA, Toldrá F (2011) Small peptides released from muscle glycolytic enzymes during dry-cured ham processing. *J Proteomics* 74:442–450
- Moure A, Franco D, Sineiro J, Domínguez H, Núñez MJ, Lema JM (2000) Evaluation of extracts from *Gevuina avellana* hulls as antioxidants. *J Agric Food Chem* 48:3890–3897
- Moure A, Domínguez H, Zuñiga ME, Soto C, Chamy R (2002) Characterisation of protein concentrates from pressed cakes of *Guevina avellana* (Chilean hazelnut). *Food Chem* 78:179–186
- Moure A, Sineiro J, Domínguez H, Parajó JC (2006) Functionality of oilseed protein products: a review. *Food Res Int* 39:945–963
- Muguruma M, Ahmmed AM, Katayama K, Kawahara S, Maruyama M, Nakamura T (2009) Identification of pro-drug type ACE inhibitory peptide sourced from porcine myosin B: evaluation of its antihypertensive effects in vivo. *Food Chem* 114:516–522
- Murray ED (2003) Canola protein isolate functionality I. US Patent 2003/0021884 A1
- Nadal P, Canela N, Katakis I, O’Sullivan CK (2011) Extraction, isolation, and characterization of globulin proteins from *Lupinus albus*. *J Agric Food Chem* 59:2752–2758
- Nandakumar MP, Shen J, Raman B, Marten MR (2003) Solubilization of trichloroacetic acid (TCA) precipitated microbial proteins via NaOH for two-dimensional electrophoresis. *J Proteome Res* 2:89–93
- Natarajan SS, Xu C, Caperna TJ, Garrett WM (2005) Comparison of protein solubilization methods suitable for proteomic analysis of soybean seed proteins. *Anal Biochem* 342:214–220

- Natarajan SS, Xu C, Bae H, Caperna TJ, Garrett WM (2006) Characterization of storage proteins in wild (*Glycine soja*) and cultivated (*Glycine max*) soybean seeds using proteomics analysis. *J Agric Food Chem* 54:3114–3120
- Natarajan SS, Krishnan HB, Lakshman S, Garrett GM (2009) An efficient extraction method to enhance analysis of low abundant proteins from soybean seed. *Anal Biochem* 394:259–268
- Ohm J-B, Hareland G, Simsek S, Seabourn B (2009) Size-exclusion HPLC of protein using a narrow-bore column for evaluation of breadmaking quality of hard spring wheat flours. *Cereal Chem* 86:463–469
- Omana DA, Moayed V, Xu Y, Betti M (2010) Alkali-aided protein extraction from chicken dark meat: textural properties and color characteristics of recovered proteins. *Poult Sci* 89:1056–1064
- Osborne TB (1924) *The vegetable protein*. Longmans Green, New York
- Park ZY, Russell DH (2000) Thermal denaturation: a useful technique in peptide mass mapping. *Anal Chem* 72:2667–2670
- Pastorello EA, Trambaioli C (2001) Isolation of food allergens. *J Chromatogr B* 756:71–84
- Pedreschi R, Vanstreels E, Carpentier S, Hertog M, Lammertyn J, Robben J, Noben JP, Swennen R, Vanderleyden J, Nicola B (2007) Proteomic analysis of core breakdown disorder in conference pears (*Pyrus communis* L.). *Proteomics* 7:2083–2099
- Pedreschi R, Hertogi M, Lilley KS, Nicolai B (2010) Proteomics for the food industry: opportunities and challenges. *Crit Rev Food Sci Nutr* 50:680–692
- Person M, Sevestre A, Chaimbault P, Perrot L, Duchiron F, Elfakir C (2004) Characterization of low-molecular weight peptides in champagne wine by liquid chromatography/tandem mass spectrometry. *Anal Chim Acta* 520:149–158
- Picariello G, Bonomi F, Iametti S, Rasmussen P, Pepe C, Lilla S, Ferranti P (2011) Proteomic and peptidomic characterisation of beer: immunological and technological implications. *Food Chem* 124:1718–1726
- Pihlanto A, Virtanen T, Korhonen H (2010) Angiotensin I converting enzyme (ACE) inhibitory activity and antihypertensive effect of fermented milk. *Int Dairy J* 20:3–10
- Poliwoda A, Wiczorek PP (2009) Sample pretreatment techniques for oligopeptide analysis from natural sources. *Anal Bioanal Chem* 393:885–897
- Poulin J, Amiot J, Bazinet L (2007) Improved peptide fractionation by electro dialysis with ultrafiltration membrane: influence of ultrafiltration membrane stacking and electrical field strength. *J Membr Sci* 299:83–90
- Pozo-Bayón M, Alcaide J, Polo MC, Pueyo E (2007) Angiotensin I-converting enzyme inhibitory compounds in white and red wines. *Food Chem* 100:43–47
- Rho SJ, Lee J, Chung YI, Kim Y, Lee HG (2009) Purification and identification of an angiotensin I-converting enzyme inhibitory peptide from fermented soybean extract. *Process Biochem* 44:490–493
- Righetti PG, Castagna A, Herbert B, Reymond F, Rossier JS (2003) Prefractionation techniques in proteome analysis. *Proteomics* 3:1397–1407
- Righetti PG, Castagna A, Antonioli P, Boschetti E (2005) Prefractionation techniques in proteome analysis: the mining tools of the third millennium. *Electrophoresis* 26:297–319
- Rosenfeld J, Capdevielle J, Guillemot JC, Ferrara P (1992) In-gel digestion of proteins for internal sequence analysis after one- or two-dimensional gel electrophoresis. *Anal Biochem* 203:173–179
- Samaranayaka AGP, Kitts DD, Li-Chan ECY (2010) Antioxidative and angiotensin-I-converting enzyme inhibitory potential of a pacific hake (*Merluccius productus*) fish protein hydrolysate subjected to simulated gastrointestinal digestion and Caco-2 cell permeation. *J Agric Food Chem* 58:1535–1542
- Sant'Anna BPM, Freitas SP, Coelho MAZ (2003) Enzymatic aqueous technology for simultaneous coconut protein and oil extraction. *Grasas y Aceites* 54:77–80
- Saravanan RS, Rose JKC (2004) A critical evaluation of sample extraction techniques for enhanced proteomic analysis of recalcitrant plant tissues. *Proteomics* 4:2522–2532

- Schmidt SR, Schweikart F, Andersson ME (2007) Current methods for phosphoprotein isolation and enrichment. *J Chromatogr B* 849:154–162
- Ser WY, Arntfield SD, Hydamaka AW, Slominski BA (2008) Use of diabetic test kits to assess the recovery of glucosinolates during isolation of canola protein. *LWT Food Sci Technol* 41:934–941
- Sereewathanawut I, Prapintip S, Watchirarujj K, Goto M, Sasaki M, Shotipruk A (2008) Extraction of protein and amino acids from deoiled rice bran by subcritical water hydrolysis. *Bioresour Technol* 99:555–561
- Sharma GM, Mundoma C, Seavy M, Roux KH, Sathe SK (2010) Purification and biochemical characterization of Brazil nut (*Bertholletia excelsa* L.) seed storage proteins. *J Agric Food Chem* 58:5714–5723
- Shen L, Wang X, Wang Z, Wua Y, Chen J (2008) Studies on tea protein extraction using alkaline and enzyme methods. *Food Chem* 107:929–938
- Sheoran IS, Ross ARS, Olson DJH, Sawhney VK (2009) Compatibility of plant protein extraction methods with mass spectrometry for proteome analysis. *Plant Sci* 176:99–104
- Song J, Braun G, Bevis E, Doncaster K (2006) A simple protocol for protein extraction of recalcitrant fruit tissues suitable for 2-DE and MS analysis. *Electrophoresis* 27:3144–3151
- Taborda G, Gómez-Ruiz JA, Martínez-Castro I, Amigo L, Ramos M, Molina E (2007) Taste and flavor of artisan and industrial Manchego cheese as influenced by the water-soluble extract compounds. *Eur Food Res Technol* 227:323–330
- Tan SH, Mailer RJ, Blanchard CL, Agboola SO (2011) Canola proteins for human consumption: extraction, profile, and functional properties. *J Food Sci* 76:R16–R28
- Tang S, Hettiarachchy NS, Shellhammer TH (2002) Protein extraction from heat-stabilized defatted rice bran. 1. Physical processing and enzyme treatments. *J Agric Food Chem* 50:7444–7448
- Tang S, Hettiarachchy NS, Horax R, Eswaranandam S (2003) Physicochemical properties and functionality of rice bran protein hydrolyzate prepared from heat-stabilized defatted rice bran with the aid of enzymes. *J Food Sci* 68:152–157
- Tang X, He Z, Dai Y, Xiong YL, Xie M, Chen J (2010) Peptide fractionation and free radical scavenging activity of zein hydrolysate. *J Agric Food Chem* 58:587–593
- Toorchi M, Nouri MZ, Tsumura M, Komatsu S (2008) Acoustic technology for high-performance disruption and extraction of plant proteins. *J Proteome Res* 7:3035–3041
- Van Het Hof KH, De Boer BCG, Tjiburg LBM, Lucius BRHM, Zijp I, West CE, Hautvast JGAJ, Weststrate JA (2000) Carotenoid bioavailability in humans from tomatoes processed in different ways determined from the carotenoid response in the triglyceride-rich lipoprotein fraction of plasma after a single consumption and in plasma after four days of consumption. *J Nutr* 130:1189–1196
- Vincent D, Wheatley MD, Cramer GR (2006) Optimization of protein extraction and solubilization for mature grape berry clusters. *Electrophoresis* 27:1853–1865
- Wang M, Hettiarachchy NS, Qi M, Burks W, Siebenmorgen T (1999) Preparation and functional properties of rice bran protein isolate. *J Agric Food Chem* 47:411–416
- Wang W, Scali M, Vignani R, Spadafora A, Sensi E, Mazzuca S, Cresti M (2003) Protein extraction for two-dimensional electrophoresis from olive leaf, a plant tissue containing high levels of interfering compounds. *Electrophoresis* 24:2369–2375
- Wang W, Vignani R, Scali M, Cresti M (2006) A universal and rapid protocol for protein extraction from recalcitrant plant tissues for proteomic analysis. *Electrophoresis* 27:2782–2786
- Wang W, Tai F, Chen S (2008a) Optimizing protein extraction from plant tissues for enhanced proteomics analysis. *J Sep Sci* 31:2032–2039
- Wang Y, Wang Z, Cheng S, Han F (2008b) Aqueous enzymatic extraction of oil and protein hydrolysates from peanut. *Food Sci Technol Res* 14:533–540
- Wang Z, Wang W, Wang W, Xu L, Chen G, Fu F (2011) Separation and determination of β -casomorphins by using glass microfluidic chip electrophoresis together with laser-induced fluorescence detection. *J Sep Sci* 34:196–201

- Watchararujji K, Goto M, Sasaki M, Shotipruk A (2008) Value-added subcritical water hydrolysate from rice bran and soybean meal. *Bioresour Technol* 99:6207–6213
- Wu FS, Wang MY (1984) Extraction of proteins for sodium dodecyl-sulfate polyacrylamide-gel electrophoresis from protease-rich plant-tissues. *Anal Biochem* 139:100–103
- Xu C, Garrett WM, Sullivan J, Caperna TJ, Natarajan S (2006) Separation and identification of soybean leaf proteins by two-dimensional gel electrophoresis and mass spectrometry. *Phytochemistry* 67:2431–2440
- You L, Zhao M, Regenstein JM, Ren J (2010) Purification and identification of antioxidative peptides from loach (*Misgurnus anguillicaudatus*) protein hydrolysate by consecutive chromatography and electrospray ionization-mass spectrometry. *Food Res Int* 43:1167–1173
- Zhang SB, Wang Z, Xu SY (2007) Downstream processes for aqueous enzymatic extraction of rapessed oil and protein hydrolysates. *Am Oil Chem Soc* 84:693–700
- Zhang SB, Lu QY, Yang H, Li Y, Wang S (2011) Aqueous enzymatic extraction of oil and protein hydrolysates from roasted peanut seeds. *J Am Oil Chem Soc* 88:727–732
- Zhen Y, Shi J (2011) Evaluation of sample extraction methods for proteomic analysis of coniferous seeds. *Acta Physiol Plant* 33:1623–1630
- Zheng Q, Song J, Doncaster K, Rowland E, Byers DM (2007) Qualitative and quantitative evaluation of protein extraction protocols for apple and strawberry fruit suitable for two-dimensional electrophoresis and mass spectrometry analysis. *J Agric Food Chem* 55:1663–1673



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