

# Extraction of protein from food waste: An overview of current status and opportunities

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## Funding information

Future Food Beacon University of Nottingham

## Abstract

The chief intent of this review is to explain the different extraction techniques and efficiencies for the recovery of protein from food waste (FW) sources. Although FW is not a new concept, increasing concerns about chronic hunger, nutritional deficiency, food security, and sustainability have intensified attention on alternative and sustainable sources of protein for food and feed. Initiatives to extract and utilize protein from FW on a commercial scale have been undertaken, mainly in the developed countries, but they remain largely underutilized and generally suited for low-quality products. The current analysis reveals the extraction of protein from FW is a many-sided (complex) issue, and that identifies for a stronger and extensive integration of diverse extraction perspectives, focusing on nutritional quality, yield, and functionality of the isolated protein as a valued recycled ingredient.

## KEYWORDS

cavitation, enzyme-assisted extraction, food waste, liquid biphasic floatation, pulsed electric field, recycled protein, subcritical water

## 1 | INTRODUCTION

There is increasing recognition that reducing food loss and waste (FLW) represents key aspects of ensuring a sustainable and healthy diet for the global population. The United Nations (UN) Sustainable Development Goals (SDGs) Target 12.3 is to shrink the global food waste generated per capita along the food supply chain up to 50% near to 2030 (Spröte, 2019). Such a reduction would not only help to complete the SDG goal of “zero hunger,” but would also significantly improve the environmental footprint of food production. Lipinski et al. (2016) defined FLW as “The safe to eat parts of plants and animals that are either produced or harvested for human consumption but that are not ultimately consumed by people.” The recent report by FAO (2019) defines food loss (FL) associated with the food supply chain and FW as occurring at retail and consumption

level (FAO, 2019). Both FL and FW make significant contributions to overall loss of food from the human diet and vary considerably across the planet.

FLW occurs in all the segments of food life cycle (Figure 1) from harvest of crops and slaughter of livestock through to processing, retail, and consumer losses (von Massow et al., 2019). The proportion of losses post-production includes up to 42% at household level, 39% in food manufacturing, 14% in the foodservice sector, and 5% during distribution (Mirabella et al., 2014). FAO's (2019) report approximates 14% of food is lost before reaching retail. At the time of publication, equivalent data for loss at retail and consumer level were not available, but in a recent report, Berners-Lee et al. (2018) estimated that of the 3116 kcal/person/day of food energy available prior to processing and distribution, approximately 10% is lost at the wholesaler/retailer level, with a further 8% being lost at the

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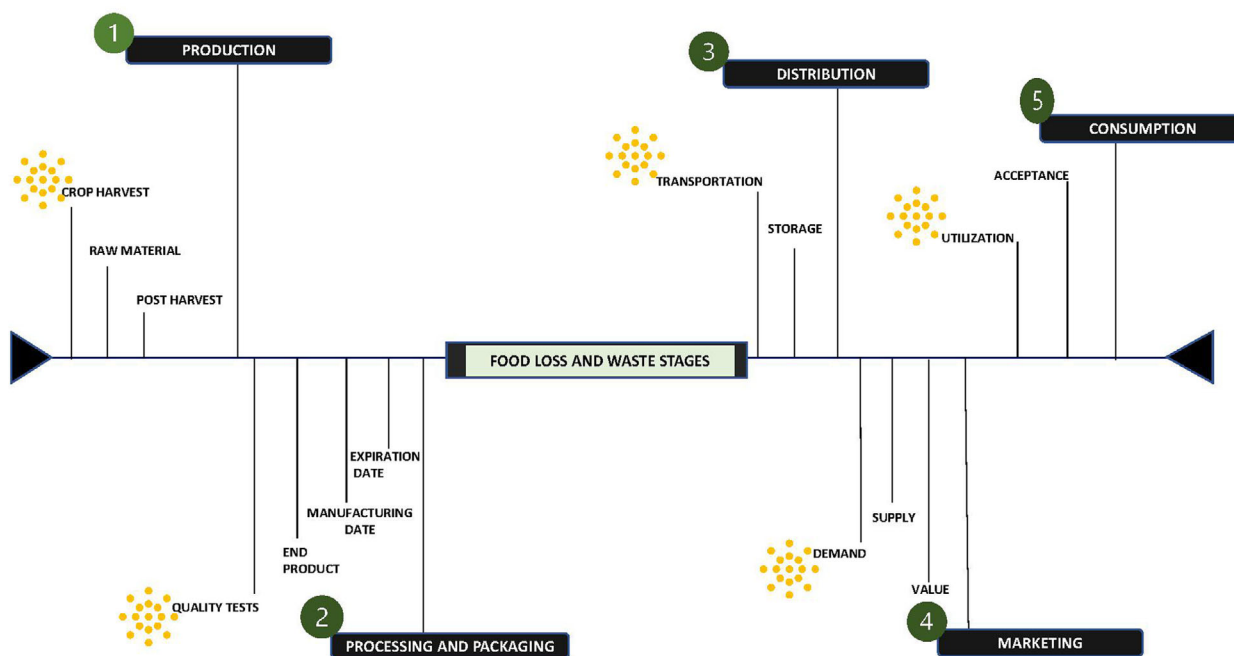


FIGURE 1 Overall food loss and waste (FLW) stages throughout the food supply chain process

level of the consumer. Several studies (Abd Razak et al., 2018; Thi et al., 2015) have demonstrated that economic growth, coupled with rising commercialization and urbanization are directly linked to increase in FW. FAO (2019) reports approximately a 13.8% FL along the food production in 2016. However, in reference to food groups, fruit and vegetables showed less loss in comparison to the tubers and roots.

FLW represents an overall loss of both macro- and micronutrients. In the face of increasing population growth and increased lifespan, and the potential impact of climate change on the production of food, there is increasing concern that our food systems should be as efficient as possible. The availability of adequate supplies of protein, which is of an appropriate quality to maintain health, has been one topic of concern. Berners-Lee et al. (2018) have recently demonstrated that, in fact, the total amount of protein produced is currently sufficient to feed the population. However, much of the protein associated with crops is actually fed to livestock that exhibit variable levels of efficiency in converting feed protein to human edible protein (Salter, 2017). Although ruminant animals have the capacity to obtain protein from nonhuman edible plants, Berners-Lee et al. estimate that approximately 50% of crop protein produced is fed to animals and only 43% of this reenters the human diet as meat, dairy, or fish. Such losses, combined with the impact of animal production on greenhouse gas emissions, have led to calls for a global transition to a largely plant-based diet (Willett et al., 2019). The impact of livestock production on global food supplies

could be further mitigated by reuse of protein extracted from FW for use in animal feed. Thus, extracted protein from such waste could either directly enter the human diet or indirectly, through use in livestock production.

In this current paper, we review the various extraction techniques used for protein from FW sources. We consider protein availability (content) and analyze the evidence relating to factors impeding the application of different extraction methods. Our aim is to highlight and identify missing links associated with to date scholarly evidence to advance development of the knowledge use for extraction of protein from FW sources for animal and human consumption. Addressing the above mentioned problems will not only help in reducing food waste but also offer potential research opportunities to explore food waste from a commercial stand-point.

## 2 | FOOD WASTE PROTEIN SOURCES AND USES

In the following section, we review the evidence on protein availability and food waste protein (FWP) sources. There is ample evidence (Burd et al., 2019; Eshel et al., 2019) that protein is necessary for animal and human growth, and the essential amino acids can only be obtained from foods consumed. Food proteins differ in native amino acid profiling (Table 1). At present with 57% growth, the vegetal sources dominate protein supply globally (Food & Agriculture Organization of the United Nations, 2010). From

**TABLE 1** Protein content of some common food type (© British Nutrition Foundation 2018)

Food source	Food type	Protein (g/100 g)
<b>Animal protein</b>		
Meat	Chicken breast (grilled without skin)	32.0
	Beef steak (lean grilled)	29.2
	Lamb chop (lean grilled)	31.6
	Pork chop (lean grilled)	31.6
Fish	Tuna (canned in brine)	23.5
	Mackerel (grilled)	20.8
	Salmon (grilled)	24.2
	Cod (grilled)	20.8
Seafood	Prawns	22.6
	Mussels	16.7
	Crabsticks	10.0
Eggs	Chicken eggs	12.5
Dairy	Whole milk	3.3
	Semi-skimmed milk	3.4
	Skimmed milk	3.4
	Cheddar cheese	25.4
	Half-fat cheddar	32.7
	Cottage cheese	12.6
	Whole milk yogurt	5.7
	Low fat yogurt (plain)	4.8
<b>Plant protein</b>		
Pulses	Red lentils	7.6
	Chickpeas	8.4
Beans	Kidney beans	6.9
	Baked beans	5.2
	Tofu (soya bean steamed)	8.1
Grains	Wheat flour (brown)	12.6
	Bread (brown)	7.9
	Bread (white)	7.9
	Rice (easy cook boiled)	2.6
	Oatmeal	11.2
	Pasta (fresh cooked)	6.6
Nuts	Almonds	21.1
	Walnuts	14.7
	Hazelnuts	14.1

a universal perspective, wheat, milk, and rice represent the major sources of protein (de Pee & Bloem, 2009). However, within the urbanized (cosmopolitan) cities, meat is the primary source of protein, followed by cereals (Hovhannisyan & Devadoss, 2020), whereas in the developing (less urbanized) areas of the world this order is reversed (Rampal, 2018).

The current statistics anticipate that global production of all food types is likely to continue to rise—both in terms of production and consumption (FAO & OECD, 2018). The whole world meat production augmented by 1.25% to 323 metric tons (MT) in 2017 (FAO & OECD, 2018). Similarly,

FAO's forecast for global cereal production in 2017 now stands at 2627 million tons, 16.8 million tons higher than last year's level (FAO, 2007). Likewise, global milk productivity depicted 1.4% higher production in 2017, with nearly 811 million tons production (FAO, 2018). However, there is no clear and timely data pattern between the food production and FW generated. However, some research studies focusing on FW drivers (economic, political, cultural and socio-demographic aspects) signify that base on the annual world food production (Food and Agriculture Organization of the United Nations, 2010), the amounts of FWP sources are on a rise. The FW drivers provide a rather abstract but an evident global variation in FW generated (Chalakov et al., 2016). According to Lipinski et al. (2016), on the contrary to underdeveloped countries the production of FW is estimated to be higher in developed countries with a total of 56%, of which 40% occurs in the consumption stage. This underlines an abstract but a direct relation with FW across all commodities. Moreover, FW is anticipated to amount to nearly to 126 MT by 2020 (Mirabella et al., 2014).

Generally, FW is an important resource of protein (Adhikari et al., 2018) that has the prospective to be employed as a value-added ingredient and/or product, including addition to human foods and animal feed. For a FW to be measured as a source of (valuable) protein, it has to fulfill three major (basic) requirements: (a) to encompass high protein content, (b) quality protein (well-balanced essential amino acid composition), and (c) toxic or allergic substances removed prior to its utilization as source of protein (Graf et al., 2015). FWP sources can easily be classified into animal and plant sources, based on crude protein availability and nutrition value. A number of plant by-products (Table 2) are considered important protein sources due to high nutritional value, as revealed in their essential amino acid profile; these include oat, rice, and wheat bran protein (Apprich et al., 2014; Guan et al., 2018; Tang et al., 2003). Wheat bran with 13% to 18% of proteins can be considered as a feasible good source for protein extraction, with strikingly high lysine and arginine content (Apprich et al., 2014). The bran also consists of high contents of tryptophan, tyrosine, and cysteine (Stevenson et al., 2012). Suitable due to a high protein content of 15% to 50%, oil meals (remaining after the oil extraction) mainly from seeds and plant sources have also been acknowledged as a valuable source for extracted protein (Ramachandran et al., 2007). On the contrary, pumpkin kernel meal, hop, and sea buckthorn seed meal depicted quite a poor nutritive profile, even with higher crude protein (20%) reserves (Prandi et al., 2019). Similarly, soybean curd residue, which contains viable 27% protein (dry basis), has been identified as a superior source (Li et al., 2013). Also, in primarily vegetal FWP sources, mushrooms and sugar beet flakes protein with 40% of essential amino acids

**TABLE 2** Crude protein (CP) content from food waste protein (FWP) sources

FWP source	CP (g/100 g)	Reference
Buckwheat bran	27.8	
Flaxseed	20.9	
Rapeseed press cake	35.7	Mattila et al., 2018
Wheat bran	11.05	
Hops	21.63	
Sesame cakes	37.45	
Brewer dry grain	19.96	Haile, Njonge, Asgedom, & Gicheha, 2017
Coconut cake	21.9	
Rice husk	3.9	
Soybean hull	21	
Papaya seed	25.9	Filho, Detmann, Gustavo, & Pereira, 2012
Pumpkin seed	17.6	
Almond husk	3.27	Jafari & Alizadeh, 2011
Whey permeate waste	0.25*	El-Tanboly, 2017
Cane molasses	3.2*	
Fish waste	20.58	Ho & Chu, 2019
Household kitchen waste	8.13	
Chinese restaurant	10.9	

\*Unit of measurement, %.

are considered as a possible source as a viable feed ingredient (Prandi et al., 2019). Not surprisingly, FWP sources of animal origin, including protein from fishmeal, meat and bone meal, cheese, yogurt, and whey, are also considered as good-quality sources of protein containing a high amino acid profile (Chadd et al., 2002). However, unlike plant sources, animal sources of FWP associated with mass and/or bone meal are banned in some countries due to spread of Bovine Spongiform Encephalopathy (BSE) or Transmissible Spongiform Encephalopathies (TSEs) (EFSA et al., 2016).

Important initiatives have been undertaken, mainly in the developed countries (Europe and the United States) as a critical strategy (policy) to battle malnutrition (protein deficiency) and to utilize protein residues and by-products (Mirabella et al., 2014). Nonetheless, still to date they are broadly either restricted and/or limited to only thickeners and foaming and gel stabilizers in high-value products (Van Dyk et al., 2013) or utilized as animal or fish feed, a fairly low-value product (Wong et al., 2016). Overall, literature search reveals that the research in the field of utilization of FWP is progressing more on a lab-

oratory rather than commercial scale. Furthermore, very few studies are found on the extraction of protein from expired sources. Recycling of expired products can be a model for an integrated ecosystem (Eissa et al., 2018; Tham et al., 2019). Expired dairy products were essentially considered as an organic fertilizer in comparison to inorganic fertilizer to grow wheat (*Triticum aestivum vulgar*) (Eissa et al., 2018). The results of the study concluded a significant increase in total chlorophyll by 22% and nitrogen (N), phosphorus (P), and potassium (K) uptake by 54%, 67%, and 14% when expired dairy powder was used in place of inorganic fertilizer. Tham et al. (2019) studied the protein recovery from expired dairy milk using alcohol-salt liquid biphasic flotation (LBF). The experiment was first conducted on a laboratory scale, where the protein recovery and separation efficiency were 94.97% and 86.289%, respectively. Interestingly, once the same optimization parameters were scaled up 40 times, the protein recovery and separation efficiency were found to be 78.92% and 85.62% high, respectively (Tham et al., 2019). Hence, more coherent and holistic studies are required for the removal of protein from FW in order to institute sustainable extraction technologies to produce high-quality protein products, fit for human consumption. These must be cost-effective and maintain the nutritional value. In parallel, economically viable techniques for extraction of protein-rich, biologically safe ingredients for animal feed may represent an efficient use of some of our FW. Animal feeding practices from FW are a complicated agenda involving various states, national, and international laws (Truong et al., 2019). Nonetheless, it is not an abandoned practice. Documented data suggest that many processing and feed production facilities pretreat the FW collected prior to animal feeding to reduce animal to animal and animal to human disease transmissions (Truong et al., 2019).

### 3 | EFFECT OF EXTRACTION TECHNOLOGIES ON FWP

In the following subsections, we review the existing extraction method employed specifically for protein. Furthermore, we explore the potential and limitations of each methodology-related parameters such as recovery yield (outcome %) and structural, nutritional, and functional alterations.

#### 3.1 | Enzyme-assisted extraction

In current years, there has been a growing interest in the use of enzymes in the extraction of protein, predominantly for food and nutraceutical purposes. Enzymes are



globular proteins sourcing from microorganisms, plants, animals, and humans, functioning as a catalyst (Robinson, 2015). Purposely in dairy (cheese, yogurt), bakery (bread making), and meat processing, enzymes are increasingly used (Raveendran et al., 2018). In fact, now an array of food-grade enzymes is also commercially available (Ramos & Malcata, 2011). Enzymes commonly used in industry at present include carbohydrase, lipase, and predominately, proteases (Ramos & Malcata, 2011). Table 3 provides an overview of each enzyme's profile that is described briefly in this section and is commonly used in the enzyme-assisted extraction (EAE) process.

EAE process is considered to be an environmentally responsive process, in which it replaces steps that include harsh chemical or physical conditions with enzymes (Pojić et al., 2018). The constituents of FWP are complex and protein frequently co-exists with pectin, starch, cellulose, and often lipids in the cells. These impurities can decrease the extraction yields of protein (Cheng et al., 2015). Consequently, during EAE, cell disruption is one of the most relevant steps to release protein from internal cell compartments in a soluble form (Cheng et al., 2015). Moreover, there is ample evidence that highlights "EAE process as a mild, non invasive, green extraction method" (De Moura et al., 2011; Demuez et al., 2015; Ramachandran et al., 2007; Robinson, 2015; Rommi, 2016; Silva et al., 2014). EAE also allows the successful extraction of amino acids such as glutamine and asparagine, usually easily destroyed as a result of acid and alkali hydrolysis (Lowenson et al., 2016). As a result, the products obtained are often more suitable for direct human consumption (Liu et al., 2016).

The application of EAE is dependent on the operational conditions, including substrate and enzyme ratio, enzyme-specific temperature and pH, and extraction time (Demuez et al., 2015). EAE of protein, along with its operational parameters, has been extensively studied in literature. A research study based on microalgae and oilseed meals (e.g., rapeseed and soybean) illustrated an independent relation between enzyme type and protein extraction (Sari et al., 2013). The results of the study did highlight an increase in extraction of protein (90% soybean meal and 50% to 80% rapeseed and microalgae) in alkaline conditions with the addition of enzymes (Protex 40xL, Protex 5L, and Protex P). Interestingly, an increase in crude protein outcome was observed irrespective of the type of enzyme being used (Sari et al., 2013). Likewise, Wang et al. (2008) reported similar results for peanut protein hydrolysate, with a higher protein yield (82.5%) at alkaline conditions (pH 8.5) using alcalase.

In an ideal reaction, enzyme and substrate react continuously till central equilibrium is attained. Ramakrishnan et al. (2013) reported a direct trend between increasing enzyme concentration and protein yield, with 76.30%

extracted protein (whole fish) using highest enzyme concentration. Similar results were reported by Benjakul and Morrissey (1997). However, it should be taken into account that continuous biochemical reactions are nonisolated assays and environmental factors (pH and temperature) have the liberty to change the flux of equilibrium either way. Research studies (Bhaskar & Mahendrakar, 2008; Gbogouri, Linder, Fanni, & Parmentier, 2004; Ramakrishnan et al., 2013) concluded a higher protein output with an increase in hydrolysis time up to 5 hr. Further increasing the time does not seem to significantly increase protein yield (Guerard et al., 2002). This could be due to the utilization of substrate molecules or unstableness and denaturing of active sites (Márquez & Vázquez, 1999). In fact, according to Chen et al. (2006) and Robinson (2015), regulatory enzymes also play a pivotal role in controlling the overall flux of the reaction.

The main concern in the selection of enzymes is the internal matrix of raw material (structure and composition) (Gildberg, 1993). In fact, protein content and overall composition of FW generated are diverse and complex, thus the role of enzyme is addressed accordingly (Table 1). Wang et al. (2008) studied the choice of enzyme for peanut seed for the extraction of protein and oil simultaneously. The study was designed, keeping in view (a) the composition of peanut seed with 24% to 28% protein and 45% to 52% oil and (b) the outer cell wall structure of cotyledon. Synergistic activities of both carbohydrase and protease were used (Wang et al., 2008). Commonly, the role of most carbohydrases (cellulases and pectinases) is to break the outer cell wall (Tu et al., 2015), whereas proteolytic enzymes hydrolyze the protein inside the cytoplasm (Ravindran & Jaiswal, 2016). In a study looking at the extraction of protein using neutrase, alcalase, pepsin, and kojizyme, Liaset et al. (2000) revealed that alcalase yielded the highest extracted protein (67.6%) and pepsin (64%). Likewise, Ramakrishnan et al. (2013) reported EAE of amino acids using alcalase and neutrase (individually and in combination) and depicted that the combination (alcalase + neutrase) produced 14 amino acids including alanine (7.59%), glycine (5.82%), histidine (3.59%), isoleucine (5.30%), leucine (9%), lysine (7.34%), methionine (2.2%), phenylalanine (4.2%), serine (4.3%), threonine (5.40%), tyrosine (3.17%), valine (7.2%), glutamic acid (9.85%), and proline (0.98%). Arginine and aspartic acid were nonresponsive to the enzymes. Studies have reported highest yield when alcalase (individually and in combination) was used (Hamada, 2000; Jarpa-Parra, 2018; Liu et al., 2016; Mudgil, Baby, et al., 2019; Pojić et al., 2018; Sari et al., 2013; Toldrá & Nollet, 2013). This is likely to be because alcalase is a food-grade endoproteinase (Ramos & Malcata, 2011) with broad specificity, enabling the hydrolysis of membranes surrounding lipid bodies, thereby

TABLE 3 Application of enzyme assisted extraction (EAE) from protein sources

Enzyme	Enzyme type	Incubation conditions	Source (EAE)	Reference
Pepsin Alcalase	Endopeptidase	pH 2, 37 °C, 5 hr pH 6.5 to 8.5, 45 °C, 150 min	Poultry (Liver)	Chou, Wang, Lin, & Chen, 2014
Thermolysin	Metallopeptidases	pH 9.5, 37 °C, 2 hr	Bovine (Liver)	Di Bernardini et al., 2011
Papain Alcalase	Endopeptidases, Aminopeptidases, Dipeptidyl peptidases	pH 6.5, 50 °C, 6 hr pH 8, 50 °C, 6 hr pH 8, 37 °C, 6 hr	Porcine (Liver)	Verma, Chatli, Kumar, & Mehta, 2017
Alcalase		55 °C, 2 hr	Porcine (Liver)	Damgaard, Otte, Meinert, Jensen, & Lametsch, 2014
Papain Pepsin Alcalase		pH 6.5, 37 °C, 12 hr pH 3, 37 °C, 12 hr pH 8, 50 °C, 12 hr		Yu, Hsu, Chang, & Tan, 2017
Protex 40XL, Protex P, and Protex 5L	Endopeptidase	pH 2.5 to 11, 50 to 60 °C, 3 hr	Rapeseed meal	Sari et al., 2013
Pectinase B-glucanase	Carbohydrases	pH 3.5, 40 °C, 1.25 hr	Rapeseed meal	Pustjens et al., 2012
Alcalase	Endopeptidase	pH 7.5, 55 °C, 1 to 4 hr, (0.5%, 1%, and 2% EC)	Mackerel Fish Waste	Ramakrishnan et al., 2013
Pepsin Alcalase	Endopeptidase	pH 2 pH 6.5 to 8.5 40, 45, 50, 55 °C 60, 120, 150, 180 min	Quinoa	Mahdavi-Yekta, Nouri, & Azizi, 2019
Viscozyme	Carbohydrases	pH 5.5 to 6.5, 37 to 53 °C, (1.5% to 4% EC)	Okara (Soy milk)	de Figueiredo, Yamashita, Vanzela, Ida, & Kurozawa, 2018
Neutrase Alcalase Protamex Flavorzyme	Bacterial protease Endopeptidase Bacillus proteinase complex Aspergillus oryzae	pH 7, 50 °C, (2% EC), 2 hr pH 7.5, 55 °C, (2% EC), 2 hr pH 6.5, 55 °C, (2% EC), 2 hr pH 6, 50 °C, (2% EC), 2 hr	Tea leave	Shen, Wang, Wang, Wu, & Chen, 2008
Cellulase Hemicellulase Pectinex Ultra Viscozyme Alcalase	Carbohydrases Endopeptidase	pH 9, 40 to 60 °C, 1 to 3 hr	Rice	Hanmoungjai, Pyle, & Nirarjan, 2001

releasing cytoplasmic protein into smaller peptides (higher solubility) effectively.

An alternative approach is to combine aqueous enzyme-assisted extraction (AEAE) to assist multiple bioproduct extraction (Jung et al., 2006; Rommi, 2016; Wang et al., 2008). In a two-stage countercurrent soybean AEAE, De Moura et al. (2011) showed superior protein yield outcomes in comparison to basic single stage. Moreover, AEAE depicted improved functional and nutritional properties (Moure et al., 2001).

Moreover, protein isolates are commonly produced via precipitation at the isoelectric point from (a) animal sources (dairy and seafood) and (b) defatted pressed legume cakes (including soybeans, pulses, and peanuts) (Garba & Kaur, 2014). These protein isolates due to their prime functional parameters have been used as emulsifiers, stabilizers, and foaming agents and also as fortifiers to enhance the nutritional value of the end product (Garba & Kaur, 2014; Mudgil, Omar, et al., 2019). Apart from whole protein extraction, production of hydrolysate from protein isolates has been increasingly used (Kamal et al., 2018; Mudgil, Jobe, et al., 2019; Mudgil, Omar, et al., 2019). Enzymatic hydrolysis of protein has several advantages, such as higher solubility and smaller peptides (Jafar et al., 2018; Mudgil, Omar, et al., 2019).

The function of enzymes for FWP extraction on a commercial scale is a relatively new area. Generally, EAE is carried out on a laboratory scale and has potential commercial and technical limitations, including (a) uneconomical enzyme costs; (b) the current specificity of enzymes is limited for instance partial hydrolyzation of plant cell walls; and (c) enzymes are dependent on certain environmental factors (incubation temperature, substrate availability, and pH). As discussed earlier, the major issue with the application of enzymes in an industrial setup is the operational cost, with 28% to 30% associated with the raw materials (Liaset et al., 2000). Synthesis of new enzymes along with purification of enzymatic mixtures could help in reducing the cost. One such example is production of enzymes using microorganisms such as bacteria, yeast, and fungi (Raveendran et al., 2018). In comparison to animal and plant sources, microbial enzymes are commercially favored due to being cost-effective, cultural acceptance (halal sources), vegan sources, and lastly, consistent production (Terefe et al., 2014). Moreover, production of enzymes from FW sources is not an unknown arena. In fact, various different enzymes including proteases, cellulases, amylases, lipases, and pectinases particularly have been produced (Uçkun Kiran et al., 2014). Research studies focusing on customized enzymes, applied principally via genetic engineering or alongside a hybrid technique inclusive of available biodiversity, require further in-depth improved techniques. A quantitative and qualitative exploitation of

enzymatic processing on an industrial scale from FWP sources is a promising and currently relative field.

## 3.2 | Cavitation-assisted extraction

There is increasing interest in novel techniques, such as cavitation-assisted extraction (CAE) as an alternative to nongreen conventional methods (reflux, percolation, maceration using organic solvents). As narrated, the CAE process adds to the “(a) increase in temperature and pressure resulting into high mass transfer rate; (b) improved diffusion and implosion of agitating bubbles; (c) enlargement of pores; and (d) production of exceedingly reactive free radicals aiding cell disruption” (Panda & Manickam, 2019). Currently, CAE is one of the most investigated fields mainly due to its profitable advantages and future for large-scale execution. Two widely used CAE techniques are discussed here: (a) ultrasound-assisted extraction (UAE) and (b) hydrodynamic cavitation extraction (HCE) or more commonly known as high-pressure processing. However, UAE is used more commonly as a hybrid than HCE including a combination of extraction techniques. Table 4 exemplifies the application of various CAE techniques for the extraction of protein from food sources.

### 3.2.1 | Ultrasound-assisted extraction

Application of UAE is considered as a simple and more effective technique in comparison to conventional methods. Within the last decade, UAE has attracted great attention particularly for the extraction of protein (Abugabr Elhag et al., 2018; Grosso et al., 2015; Ly et al., 2018; Preece, Hooshyar, & Zuidam, 2017). The efficiency of UAE is related and dependent on processing features, including the common factors such as temperature and solvent characteristics, along with the type of ultrasonic reactor (bath or probe), operating sonication frequency, and power (Panda & Manickam, 2019).

Water is preferred and is predominantly used for the extraction of carbohydrates, glycosides, and amino acids over organic solvents and other inorganic solvents (Preece, Hooshyar, Krijgsman, et al., 2017). However, it may not efficiently extract all the preferred constituents and thus organic and inorganic solvents are used (Grosso et al., 2015; Ivanovs & Blumberga, 2017). Interestingly, it is equally vital to understand the influence of solvent to mass ratio on the UAE process. Pinchao-Pinchao et al. (2019) explain this phenomenon in reference to principle of mass transfer, where concentration gradient of the solvent to mass ratio is responsible for exchange in mass transfer. In simpler context, a higher mass to solvent ratio increases the

TABLE 4 Application of various cavitation assisted extraction (CAE) from food protein sources

Cavitation aim	Cavitation type	Reference
Protein extraction from defatted rice bran	UAE	Ly et al., 2018
Protein extraction from soybean	UAE	Preece, Hooshyar, Krijgsman, et al., 2017
Oxidation and structure of beef protein	UAE	Kang et al., 2016
Physiochemical and antioxidant properties of corn protein hydrolysates	UAE	Liang et al., 2017
Soy protein extraction	UAE	Amponsah & Nayak, 2016
Protein extraction from sunflower meal	UAE	Dabbour, He, Ma, & Musa, 2018
Wheat germ protein extraction	UAE	Zhu et al., 2009
Protein extraction from defatted rice bran	UAE	Phipek, Nagasinha, Vallisuth, & Nongyao, 2011
Protein extraction from brewer spent grain	UAE	Tang et al., 2010
Protein extraction from perilla seed meal	UAE	Zhu & Fu, 2012
Albumin extraction from defatted pumpkin seed meal	UAE	Tu et al., 2015
Extraction and functional properties of wampee seed protein	UAE	Liu et al., 2019
Protein and carbohydrates from soybean seed	UAE	Kasai & Ikehara, 2005
Soybean protein and oil extraction	UAE	Zhang, Chen, Zhang, & Wu, 2018
Bioactive properties of rapeseed protein hydrolysates	UAE	Wali et al., 2017
Processing techniques of beef	HCE	Sikes & Tume, 2014
Hemoglobin hydrolysates from porcine meat	HCE	Toldrà, Parés, Sagner, & Carretero, 2011
Protein aggregation	HCE	Duerkop, Berger, Dürauer, & Jungbauer, 2018
Zein hydrolysates bioactive extraction	UAE	Xiaofeng Ren, Zhang, Liang, Hou, & Zhou, 2017
Soy protein isolates extraction	UAE and HCE	Xian'e Ren et al., 2020

solubilization of extracted component. A growing number of laboratory-scale research studies highlight that UAE improved protein extraction combined with conventional solvent extraction. In a study looking at the extraction of protein from defatted soy flakes, Karki et al. (2010) demonstrated a protein yield of 46% at high-amplitude sonication for 120 s when compared with nonsonicated (control) samples. This increased yield may be attributed to the structural disruption occurring due to sonication. In another study, using UAE combined with water (as the solvent) on defatted peanut meal, Nguyen and Le (2019) demonstrated that ultrasonic treatment reduced the material particle size, as well as increased the protein yield by 19%. Preece, Hooshyar, Krijgsman, et al.'s (2017) study explains an indirect (reverse) relationship between protein yield

and particle size. The study showed that as the average diffusion path within the solid decreases, it facilitates the interaction of the active sites. Moreover, at the defatted peanut meal/water ratio of 1:20 (w/v), ultrasonic power of 30 W/g, pH of 6.8, temperature of 50 °C, and sonication time of 15 min, the protein yield achieved the maximum of 87.7% ± 0.7% (Nguyen & Le, 2019). Similarly, Sumari et al. (2013) found a direct effect in particle size reduction and sonication temperature. During the extraction of protein from chicken liver, Zhou et al. (2017) showed a 55% increase in protein yielded from ultrasound-assisted alkaline extraction compared to alkali alone.

The importance of reactor type and design as a processing factor was demonstrated by Panda and Manickam (2019). UAE setup generally operates either via a



commonly adopted bath-type unit or a probe-type unit. Albeit, bath-type is more commonly used due to application ease, but on an overall evaluation the protein extraction competence via probe-type was higher in comparison (Ly et al., 2018; Preece, Hooshyar, Krijgsman, et al., 2017). Conversely, Zou et al. (2017) study using UAE-alkaline extraction from chicken liver, via probe unit, observed a slump in denaturation enthalpy by 41.7%. Preece, Hooshyar, Krijgsman, et al. (2017) demonstrated the potential problems associated with escalating from laboratory- to pilot-scale extraction using UAE. They found that pilot-scale treatment of okara increased protein extraction yield by 4.2%. Moreover, more intact cells were detected in the remaining okara protein, due to 300× greater intensity in a smaller laboratory-scale treatment.

Increase in sonication power has a controlled effect on the rise in extracted protein outcome with respect to treatment time (Ly et al., 2018). Based on the frequency, UAE can be catalogued into the following three main classes: low (20 to 100 kHz), high (100 to 100 kHz), and diagnostic ultrasonic (1 to 500 MHz) (Zheng et al., 2019). Generally, low-frequency ultrasound (20 to 100 kHz) is widely applied for the extraction of protein (Mahali & G., 2019). Appropriate temperature is another crucial factor to take into account for the extracted protein via sonication (Yaqub et al., 2016). Surface plots have point up the quadratic effect (interaction between temperature changes and protein yield) such that with the increase in the applied temperature, protein yield outcomes initially elevate until optimum saturation followed by a decrease (Preece, Hooshyar, Krijgsman, et al., 2017). Continuous higher temperatures appear to decrease the protein yields due to protein denaturation (Hojilla-Evangelista et al., 2009). Denaturation is known to offer conformational changes, both reversible and irreversible (Chandrapala et al., 2011; McDonnell et al., 2014; Pearce & Kinsella, 1978). However, it is interesting to note that based on internal matrix and structure, denaturation temperature and duration vary among different sample groups (Meletharayil et al., 2016). During UAE, reversible denaturation precedes irreversible denaturation (Zhu et al., 2009). Irreversible denaturation occurs at a temperature higher than the “denaturation temperature,” which stimulates aggregation (loss in solubility index) (Tang et al., 2003). Solubility is habitually considered as a prerequisite for resulting foaming, emulsification, and gelation properties of an ingredient and/or product (Tu et al., 2015; Van der Ven et al., 2002). Abugabr Elhag et al. (2018) emphasizes on the utilization of mild temperatures for the extraction of proteins to avoid functionality losses. Synergistic effects have been demonstrated to combine extraction technologies. UAE is usually coupled with microwave-assisted extraction (MAE) or EAE to enhance protein extraction efficiency. Similarly, the

removal of polysaccharides prior to EAE was reported to enhance the use of UAE that was also combined with the use of enzymes (Ahmad et al., 2018; Ly et al., 2018).

### 3.2.2 | Hydrodynamic cavitation extraction

Interestingly, concerns about finding a substitute for thermal processing have led to alternate pressure processing extraction. Research using HCE application has been restricted to emulsification, cell disruption, and meat tenderization (Borrajó et al., 2019). The mechanism is quite similar to UAE. However, the only difference is in reference to temperature and pressure. In pressure processing, hydrodynamic cavitation is produced by passing a liquid through a small orifice (Pojić et al., 2018). According to Escobedo-Avellaneda et al. (2011), “the constriction increases kinetic energy, resulting into nucleation, bubble growth, and implosion.” In other words, with a decline in pressure, the surrounding liquid exerts hydrostatic pressure. This adiabatic compression causes a powerful microscopic mixing effect and the temperature increases by about 38 °C per 100 MPa (Asaithambi et al., 2019). Contrary to UAE, HCE is easier to scale up and utilize in a continuous process at a commercial scale (Carpenter, 2018). Moreover, the collapse intensity of the cavitation in HCE is less compared to UAE. However, the number of cavities generated is more in HCE creating a larger total volume of the cavity collapse, which makes HCE more efficient than UAE (Sikes & Tume, 2014).

Studies describing the effects of HCE of proteins from by-products are currently inadequate. According to Shah et al. (2019), a pooled processing approach, such as EAE followed by, or in conjunction with, HCE, increased proteolytic enzyme activity and hence protein yield. Application of HCE treatment as a single pass (soy slurry and okara at 100 MPa) enhanced protein yield outcomes up to 82% (Preece, Hooshyar, Krijgsman, et al., 2017). However, once the multiple iterations of HCE was applied, a dip in protein yield was observed.

CAE processing has great prospective to not only advance the current extraction techniques but also to completely transform extraction technology. However, at hand there are concerns relating to the use of HCE in terms of the denaturation of protein and the process efficiency at a magnified industrial scale. Drawbacks, particularly for the UAE process, are attenuation of ultrasound waves, along with a lack of uniformity and higher energy consumption. Negative-pressure cavitation (NPC) is another type of hydrodynamic cavitation. NPC system requires the application of negative pressure via a vacuum pump (Tian et al., 2015). Moreover, under the influence of vacuum, the system is designed to operate at room temperature

avoiding the degradation of heat-sensitive compounds (Zhao et al., 2011). NPC studies related to protein extraction are scarce. The grouping of EAE and NPC methods was formerly designed by Zhao et al. (2011). The results of the findings indicated an increase in both the destruction of cell walls (polysaccharide) and better mass shift.

### 3.3 | MAE process

Application of MAE, which started in the late 1980s, has been recognized as a noteworthy cost-effective extraction technology in the food industry (Gohi et al., 2019; Moret et al., 2019; Zarei et al., 2017). The success of the MAE process is ascribed to the destruction of cell wall from the continuous collisions of water molecules within the matrix (Ivanovs & Blumberga, 2017), resulting in exudation (release) of components within cells into the surrounding solvent medium (Grosso et al., 2015). A number of advances in MAE instrumentation have been developed, focusing on pressurized and solvent-free MAEs (Sarker et al., 2006).

MAE has been employed for a number of reasons including solubilization of cell wall polysaccharides (Kaufmann & Christen, 2002), inactivation of enzymes (de Mesa-Stonestreet, 2011), and enhancement of nutritional quality (Mahali & G., 2019). The MAE process is dependent on internal and external factors including matrix structure (thickness of cell wall), solvent type, volume (solid/solvent) ratio, and microwave treatment pressure, time, and temperature (Moret et al., 2019). Phongthai et al. (2016) studied rice bran protein extraction using MAE process. The results illustrated an increment in protein yield by about 1.54-fold as compared to alkaline extraction. Interestingly, protein digestibility remained the same. In an overview of the optimization of MAE, Tatke and Jaiswal (2011) highlight that extraction time and yield are co-dependent. Moreover, the review explains the relevance of why organic solvents (e.g., ethanol, methanol, and 2-propanol) are more commonly preferred than water. Mainly because water is known to have a high dielectric constant or relative permittivity (ideally 80.4 at 20 °C) (De Sousa et al., 2017). Permittivity is one of the fundamental parameters in MAE process that affect the propagation of electric field. In simpler terms, relative permittivity explains how well a material, in this case water, allows electric field to travel through it. Methanol (33), ethanol (25.3), and 2-propanol (21.8) have a lower relative permittivity than water, thus better suited (De Sousa et al., 2017; Lee & Park, 2011). Phongthai et al. (2016) noted a decline in protein output and protein denaturation due to high microwave power (900 to 1000 W) and prolong extraction

time. Hence, best practice to avoid protein denaturation is to use a combination of low to moderate power.

MAE of soymilk (675 W, 80 °C at 160 RPM) has resulted in a momentous increase of 24% and 44.4% in extraction yield and protein content, respectively, as compared to solvent extraction (Varghese & Pare, 2019). The increase in extraction yield is due to (a) the cleavage degree of microwaves to disorder hydrogen bond networking and (b) degradation of the cell wall (Kaufmann & Christen, 2002). Furthermore, Varghese and Pare (2019) demonstrated an increase in protein characteristics, including solubility and digestibility, of extracted soymilk in comparison to conventional milk. Study on extraction time has shown an increase in the protein yield with an increase in microwave power (600 to 1000 W) and the extraction time (60 to 120 s) (Phongthai et al., 2016). However, a previous study (Bandyopadhyay et al., 2012) reported a reverse result, where protein yield (defatted rice) decreased by about 4.21% to 10.3% with an extended extraction time passing more than 40 s. Bandyopadhyay et al.'s (2012) study focusing on de-oiled bran via viscozyme and MAE reported a maximum of 82.5% and 82.6% protein. Moreover, microwave pretreatment, followed by EAE, showed denaturation with elevation in accidental and unsystematic coil structure, resulting in higher susceptibility to Papain (Gohi et al., 2019). Ochoa-Rivas et al. (2017) demonstrated that MAE under 725 W for a period of 8 min was able to extract 100% pure protein with an extraction yield outcome of 55%. Moreover, MEA improved functional properties in terms of emulsifying index, water absorption index, foam activity index, and foam stability index.

The efficiency of protein extraction using MAE is subjective to a number of factors, including selection of closed- or open-type vessel system (Sarker et al., 2006) and nonuniform temperature distribution. Martins et al. (2019) highlight that the reason of nonuniform temperature distribution mainly in a heterogeneous food matrix system is the obstruction of ions, resulting in reduced conductivity. In other words, hot and cold regions are formed within the food matrix system, allowing irregular and uncontrolled degradation much like thawing of frozen food (Ryynänen, 1995).

### 3.4 | Supercritical extraction process

Research on the use of supercritical extraction (SE) in the processing of bioorganic waste is gaining momentum. Supercritical water is universally known as a potent alternative against conventional protein extraction methods (Grosso et al., 2015). This extraction technique uses high-pressure hot-water (100 and 374 °C) treatments (Grosso et al., 2015). As the water temperature increases to 250 °C, it

allows dissolution of hydrophobic complex (Herrero et al., 2006). It is mainly due to decrease in relative dielectric constant from 80 to 27 (Herrero et al., 2006). Additionally, even in the absence of external catalyst, proteins and carbohydrates can be hydrolyzed in supercritical water (Taylor & King, 2002). The extraction mechanism of SE was recently explained by Zhang et al. (2019), as four successive steps. Generally, desorption at various active sites under high elevated temperature and pressure. Followed by diffusion of the extracts in the matrix. Third step is critically dependent on the solute partitioning from the sample matrix (Zhang et al. 2019). The final step is the elution (Ong, Cheong, & Goh, 2006).

An effective extraction strategy, applied to defatted rice bran, was described by Hata et al. (2008), in which the protein yield and antioxidant activity were observed to be in a straight line with high temperature. Another study described using subcritical aqueous acetone (Chiou et al., 2012). The outcome of the study explained a relation between solvent concentration and protein content, such that protein content augmented with increasing acetone concentration up to 40% only. Increase in solubility of rice bran protein is observed due to hydrolysis of large insoluble protein into smaller peptides (Yver et al., 2012). Thus, the solubility increases due to cell wall lyses and hydrolysis of protein at higher temperatures (Sharif et al., 2014). Protease prehydrolysis accompanied by supercritical water treatment to mine protein from soy meal was described by Lu et al. (2016). A significant increase in protein extraction yield (59.3%) due to lower dielectric constant above 100 °C was found in comparison to conventional yield (16.4%). During the SE process, moisture removal is a fairly difficult task requiring additional procedures (evaporation and/or chemical dehydration), thus affecting the protein purity.

### 3.5 | Pulsed electric field

Pulsed electric field (PEF) is an electricity-based (non-thermal), processing technique (Buchmann et al., 2019a). Even though the conception of PEF was pragmatically introduced about 50 years ago, PEF can be still considered as a promising technology mainly due to the modern developments in the industrial (food) applications (Buchmann et al., 2019b; Gad & Jayaram, 2011; Jaeschke et al., 2019). PEF technology has several advantages over heat extraction methods, as it preserves nutritional value, flavor, texture, and color (Drahansky et al., 2016). The current focus of the PEF technique is predominantly on (a) killing microorganisms nonthermally and (b) cell disruption, for improvement of metabolite extraction (Gad & Jayaram, 2011; Gulzar & Benjakul, 2020).

The fundamental mechanism of the PEF is the generation of short pulses of high electric fields (10 to 80 kV/cm) with intervals varying from microseconds to milliseconds (Sharma et al., 2014). The most recognized mechanisms for PEF-induced separation are electrical disruption of cell membrane or cell membrane electroporation (Calderón-Miranda et al., 1999; Sharma et al., 2014). Electroporation based on the intensity of the field strength can be overturned reversibly or completely (Batista Napotnik & Miklavčič, 2018). PEF augments permeabilization without any disadvantageous effect (Gudmundsson & Hafsteins-son, 2001; Kumar et al., 2017).

The application of PEF for the extraction of protein from FWP sources is a fairly new concept and has only been carried out on a few high-protein products. PEF in combination with EAE (2 hr) from Mussel exhibited 77.08% extraction outcome of protein (Zhou et al., 2017). Furthermore, it was observed that an increment of electric field strength (10 to 20 kV/cm) aids in increasing the protein yield (Altunakar, 2007), but an added increase shows adverse effects on the protein yield. Li et al. (2016) used PEF-assisted enzymatic techniques to extract protein from abalone (*Haliotis discus Hannai* Ino) viscera, a protein-rich by-product from abalone processing. At optimum conditions (20 kV/cm, 600  $\mu$ s), this technique resulted in fully hydrolyzed protein, with improved functional properties, mainly solubility index (91.54%) and emulsifying index.

The effects of PEF on dairy protein extraction were described by Xu et al. (2015), who showed the presence of cell disruption and extraction of  $\beta$ -LG band. In a recent study of waste meat with the use of high voltage followed by low voltage, Ghosh et al. (2019) generated  $78 \pm 8$  mg/ml protein content. Paritosh et al.'s (2017) results are consistent with that of Ghosh et al. (2019) with high protein extraction from meat waste. The ways in which extraction is assisted by PEF vary profoundly. Food internal matrixes (structure and composition) are the initial contributing factor to PEF efficiency, but so far received no attention. Based on the research carried out so far on inactivation of microorganisms in products, it can be postulated that an increase in temperature can occur during PEF treatment depending on sample composition and processing conditions (Wouters et al., 2001). An increase in temperature can also be understood in terms of intrinsic resistance, which can be due to a particular particle (thickness and composition of cell wall) or a special structure (emulsions). Additionally, Wouters et al. (2001) concluded phase transitions of lipids and proteins, which only highlight the possible application of PEF for protein extraction in food systems. Internal system parameters (pH and conductivity) influence the PEF process, but they have been studied with various microorganisms only (Gad & Jayaram, 2011; Torres-León et al., 2018; Wouters et al., 2001). In

addition, where on one hand electromechanical compression and electric field causes tension and increase in cell permeability. It is also observed to adversely entrap air bubbles in the treatment chamber causing less uniformity resulting into lower efficiency (Altunakar, 2007).

### 3.6 | Liquid biphasic flotation

LBF is a promising purification method that combines solvent sublation (SS) and aqueous two-phase extraction system (Lee et al., 2016). Self-descriptive LBF works on the basic principle of floatation process (Kyzas & Matis, 2019) and the system consists of a glass column equipped with a sintered disk, linked to a compressed air system (Sankaran et al., 2018). Interestingly, the dimensions of the glass column and porosity of the sintered disk are not limiting and can be upscaled if required (Tham et al., 2019). Bubbles are mainly generated using regulated compressed air into the glass column containing the sample along with an organic solvent (Chia, Chew, et al., 2019). The air bubbles are then used to capture (adsorb) the active compounds (surfactants). The adsorption level is depended on the surface hydrophobicity and hydrophilicity of the active compounds (Zhuo et al., 2018). The entrapped active compounds in the bubbles then dissolve in the organic solvent phase placed on top of the aqueous solution (Sankaran et al., 2018). In other words, the top and bottom layers include lower and higher polarity molecules, respectively (Sankaran et al., 2019). Organic solvents used in the process are based on the composition of the sample and the affinity of the extracted component (usually ethanol and methanol) (Lee et al., 2016). Conventionally, organic SS has been used over the years as part of liquid–liquid flotation. More specifically, protein liquid–liquid flotation caused a major drawback in the structure of a protein (denaturation) (Sankaran et al., 2018). However, LBF is known to have documented high separation efficiency (Chia, Chew, et al., 2019; Chia, Mak, et al., 2019; Sankaran et al., 2019; Zhuo et al., 2018) and unlike other extraction methods, LBF produces high concentration coefficient (minimal protein loss).

Recently, it is being recognized as one of the eco-friendly processes for industrial application. Since 1896, ATPS has been used for the extraction of various types of separation of specific cell receptors, extractive fermentation, drug residues in food, wastewater treatments (Iqbal et al. 2016). However, research studies in reference to protein extraction from the food industry and more specifically from FW are very limited. Pereira and Coutinho's (2019) study on the crude feedstocks at large scale has attracted the most interest. Tham et al.'s (2019) study that investigates protein extraction from expired milk products suggests high opti-

mization in protein yield outcome (94.97%) and also separation efficiency (86.289%). Moreover, the study suggests an experimental design ensuring commercialization, with equal higher protein yield outcome (78.92%).

### 3.7 | Hybrid extraction processes

Solubility is an indicator of protein extractability (Sari et al., 2013). In general, protein extraction initiates at a pH further from the isoelectric point by solubilizing the protein, which then precipitates aiding in extraction (Vincenzetti et al., 2008). According to Güzel et al. (2019), the effect of “pH on protein extraction is influenced by cell wall alterations and change in protein properties.” Table 5 illustrates research studies based on alkaline and acid-based protein extraction practices. Sari et al. (2013) stated “acid ministered extraction seems inefficient in cell wall degradation mainly as a result of the fact that the applied acid pH is closer to the protein isoelectric point than that of the alkaline experiments.” This means that the protein solubility is low due to less net charge. The other approach (De Moura et al., 2011; Moure et al., 2001) is to solubilize protein using salt solutions by ultrafiltration and diafiltration. Addition of trichloroacetic acid to acetone could increase protein concentration and improve contaminant removal (Vilhena et al., 2015; Vincent et al., 2016). Furthermore, mechanical methods can be applied for protein extraction that result in disruption of cells by shear stress and/or impact forces due to the collision of beads (Demuez et al., 2015). Alternatively, reverse micelles are exploitable for the extraction and purification of proteins. Chen et al. (2014) studied protein extraction using a forward and backward extraction system. The study noted 70.1% and 92% forward and backward protein extraction efficiency of soybean, respectively. The higher protein back-extraction was mainly dependent on pH and salt concentration. A number of studies have suggested the application of a number of the extraction methods described above in combination to maximize extraction of protein (Baker & Charlton, 2020; Ghosh et al., 2019; Maqsood et al., 2019; Sari et al., 2013).

## 4 | CONCLUSION AND FUTURE POSSIBILITIES

The present assessment of the literature sets out to review empirical, peer-reviewed studies on extraction of protein from FW. It can be seen that significant advances have been made in the field of extraction of protein from FWP sources. However, to date, the application of the extraction technology model is mostly limited to laboratory



TABLE 5 Overview of conventional and/or hybrid techniques protein yield

Extraction method	Technique model	Source	Protein yield	Reference
Acid precipitation	Conventional	Milk		de Figueiredo et al., 2018
	Hybrid	Soybean		Yver et al., 2012
Alkaline extraction	Hybrid	Rice Bran	22.07%	Phongthai et al., 2016
	Hybrid	Oat Bran (defatted)	56.2%	Guan & Yao, 2008
	Hybrid	Soybean	70%	Preece, Hooshyar, Krijgsman, et al., 2017
	Hybrid	Soybean	50%	Lu et al., 2016
	Hybrid	Soybean	90%	Sari et al., 2013
	Hybrid	Coconut milk (press cake)	43.15%	Rodsamran & Sothornvit, 2018
	Hybrid	Peanut (flour)	55%	Ochoa-Rivas et al., 2017
Mechanical extraction	Hybrid	Rapeseed (meal)	50% to 80%	Sari et al., 2013
	Hybrid	Rapeseed (defatted meal)	53%	Rommi, 2016
	Hybrid	Wheat Bran	64.1%	Hemery et al., 2011
	Hybrid	Bean (flour)	45%	Tabtabaei, Vitelli, Rajabzadeh, & Legge, 2017
	Hybrid	Lupine (Flour)	6% to 10%	Wang, Zhao, De Wit, Boom, & Schutyser, 2016
Reverse micelles	Hybrid	Wheat Germ (defatted)	45.6%	Zhu et al., 2009
Aqueous extraction	Hybrid	Rapeseed (defatted meal press)	40% to 41%	Rommi, 2016

scale. Also, it is noticeable that the extraction processes often specifically target protein yield. Overall, we see that research in the field of studying and understanding nutritive and functional changes postextraction is scarce, evident by the number of studies. This creates a loop hole in the reutilization of extracted protein due to food safety concerns, both for human and animal consumption viability. Also, it is noticeable that extraction technology models are often attributed for being environmental greener, but without keeping in view the cost factor. A holistic approach to protein extraction needs to be taken that considers not only yield but also food safety, environmental impact, and affordability. The main idea of this review paper is to strengthen the concept of recycling and reutilization of valuable extracted protein from FW as an equally valuable recycled ingredient and/or product to induce sustainability.

As highlighted by numerous authors, extraction of protein from FWP sources is a highly complex and multifaceted process. To begin with, our analysis has shown that prerequisite steps, such as extraction (removal) of fat and carbohydrates (soluble and insoluble) along with minerals, are of prime importance in order to maxi-

mize yield. In general, batch processes are carried out, which may often lead to higher protein outcome. Our understanding of the research data present suggests the implementation of a continuous process. Continuous processes (hybrid or singular processes) offer holistic benefits with lower capital costs, minimal operation and maintenance, and improved process control. Moreover, in reference to yield outcome it is equally vital to determine pretreatment parameters' effects on the extracted protein. Several studies have demonstrated that polysaccharide removal (mainly in plant FWP sources) may predict the importance of protein solubility. Solubility is patently a marker of protein extractability. Consequently, the creation of a favorable framework (optimizing pre-extraction conditions) for a more sustainable (environmentally friendly) extraction is of vital importance in the further development of techniques for isolating protein from FW. However, as yet there has been limited independent, eco-innovative research conducted on how to optimize prerequisite steps of protein extraction.

This paper also highlights diversity for future research. The use of novel (eco-innovative) technology to support the extraction of protein is more and more recognized as



a key FW recycling tool. Furthermore, future research should investigate the existing limitations of each extraction process. Another relevant area of future research concerns the potential of emergent (surfacing) extraction technologies to work in line with maintaining and/or increasing nutritional quality of protein whether to be used directly as human food (perhaps in alleviating malnutrition) or as an ingredient in animal feed. Investigations into product development, via focusing on the changes occurring in the functional properties of recycled (extracted) protein, are also required. From a commercial perspective, studies must employ strong collaboration and integration between scholarly research and industrial applications. It is critical that research must go beyond the laboratory scale and potentially shed light on the large-scale production with a nuanced account of cost and nutritional value. In parallel, it is vital that such work must continue to ensure adequate consideration of biosecurity and food safety, whether produce protein for human or animal consumption.

#### ACKNOWLEDGMENT

The authors duly acknowledge the Future Food Beacon University of Nottingham for the financial support provided (Grant No. IAHB0013).

#### AUTHOR CONTRIBUTIONS

All authors provided equal input in writing and substantiation reading of the final manuscript. The conception (design) of the work was carried out by Ms. Hina Kamal, along with drafting the manuscript. Critical revision for important intellectual content was equally completed by Dr. Cheng Foh Le, Dr. Andrew Salter and Dr Asgar Ali. Final reviewing, editing the manuscript, supervision, funding acquisition, and approval of the version to be published was provided by Dr. Asgar Ali. In addition, all authors are liable (accountable) for the work submitted in manuscript.

#### CONFLICTS OF INTEREST

The authors declare no conflicts of interests.

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**How to cite this article:** Kamal, H., Le, C. F., Salter, A. M., & Ali, A. Extraction of protein from food waste: An overview of current status and opportunities. *Compr Rev Food Sci FoodSaf*. 2021; 1–21. <https://doi.org/10.1111/1541-4337.12739>