

# Extraction and improvement of protein functionality using steam explosion pretreatment: advances, challenges, and perspectives

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**Abstract** Protein has become an increasingly valuable food component with high global demand. Consequently, unconventional sources, such as industrial and agroindustrial wastes and by-products, emerge as interesting alternatives to meet this demand, considering the UN Sustainable Development Goals and the transition to a circular economy. In this context, this work presents a review of the use of Steam Explosion (SE), a green technique that can be employed as a pretreatment for various waste materials, including bones, hide/leather, feathers, and wool, aiming the extraction of protein compounds, such as low molecular weight biopeptides, gelatin, and keratin, as well as to enhance the protein functionality of grains and meals. The SE technique and the main factors affecting the process's efficiency were detailed. Promising experimental studies are discussed, along with the mechanisms responsible for protein extraction and functionality improvement, as well as the main reported and suggested applications. In general, steam explosion favored

yields in subsequent extraction processes, ranging from 27 to 95%, in addition to enhancing solubility and functional protein properties. Nonetheless, it is crucial to maintain the continuity of research on this topic to drive advancements in ensuring the safety of the extracted compounds for use in consumable products and oral ingestion.

**Keywords** Steam explosion · Pretreatment · Protein · Yield · Functionality

## Introduction

Food security is a socioeconomic and public health priority and one of the most challenging topics globally, with food and nutrition responsible for maintaining human health and preventing noncommunicable diseases (Afshin et al. 2019; Akbari et al. 2022). However, population growth and the need for nutritional quality are worrying factors for both food scientists and policymakers at global levels (Di Prima et al. 2022).

The population increase is estimated to reach 9.7 billion in 2050 and 10.4 billion around 2100 (UN 2022). While statistical data point to 11 million deaths attributed to dietary risk factors in 2017 and 821 million people (10.9% of the world's population) was malnourished in 2018 (Afshin et al. 2019; FAO 2019). In addition, in 2021, the UN reported that 2 billion people faced food insecurity, an increase of 350 million compared to the period before the Covid-19 pandemic, a fact that restricts the reach of UN SDG 2—Zero Hunger until 2030 (Roush 2023).

The data presented to drive the search for foods rich in nutrients, mainly protein, which is the most important food macronutrient for human growth due to its structural and functional constituents, which provide essential amino acids

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for the maintenance of the metabolic activity of the human body (Akharume et al. 2021). Furthermore, to meet consumer demands and individual dietary needs, diversification of protein sources and functions is essential for food security and product development and manufacturing (Otero et al. 2022).

Thus, unconventional sources of protein from industrial and agro-industrial by-products, such as bones (Dong et al. 2021; Qin et al. 2020, 2022), leather wastes (Rigueto et al. 2022; Scopel et al. 2019, 2020a), feather wastes (Guo et al. 2020; Zhang et al. 2015; Zhao et al. 2012), wool wastes (Tonin et al. 2006), Brewer's spent grain (Kemppainen et al. 2016; Rommi et al. 2018), soybean meal (Zhang et al. 2013, 2017) and Camellia seed cake (Zhang et al. 2019, 2022), emerge as opportunities to be explored. These opportunities align with the call for transitioning to a circular economy to meet the SDGs and set goals for achieving economic, environmental, and social sustainability (Aiking and Boer 2020; Yada 2018). For this, it is important to search for protein extraction techniques that guarantee nutritional quality applicable to marketable products, satisfactory yield, reduction of cost, and process time, allied to the lowest environmental impact.

In this context, steam explosion (SE) is a technique that involves the application of saturated steam (between 0.5 to 2 MPa and 150 to 210 °C) for short time, with subsequent decompression, which involves the release by instantaneous pressure and sample cooling (Dong et al. 2021; Guo et al. 2020), and has recently been studied as a green technique for sample pretreatment to extract protein compounds such as keratin (Zhang et al. 2015; Zhao et al. 2012), gelatin (Scopel et al. 2019, 2020a), low molecular weight bioactive peptides (Dong et al. 2021; Guo et al. 2020), and protein functionality improvement (Zhang et al. 2017, 2019).

The main advantages of SE as a pretreatment technique are the reduction of energy consumption, costs, and processing time, lower environmental impact due to the replacement or reduction in the use of chemical agents, and increased extraction yield in subsequent processes (Scopel et al. 2020a, b).

This work aimed to present a review of the use of steam explosion as a pretreatment technique for industrial and agro-industrial wastes to extract protein compounds and improve protein functionality. The main reported mechanisms are described, as well as reported and suggested applications and future perspectives, taking into account social, economic, and environmental aspects.

## Methodology

The search for articles was conducted by inserting the terms "STEAM" AND "EXPLOSION" into the Scopus database.

These terms were present in the titles, abstracts, and/or keywords of experimental articles published between 1990 and 2022. The results were interpreted by a script implemented for this purpose, in which keywords and data patterns were searched in the abstracts of the articles, resulting in a list with the extracted data in a table form.

Terms were queried to characterize mentions of raw materials (straw, rice straw, wheat straw, bagasse, sugar-cane bagasse, eucalyptus, hardwood, wood, softwood, aspen wood, bamboo, corn stover, cornstalk, wheat bran, soybean, bone, backbone, father, skin, seed cake, soybean), products (alcohol, ethanol, bioethanol, biofuel, hydrogen, methane, gas, biogas, xylose, protein, keratin, gelatin) and regular expression patterns to identify temperature and pressure data.

Plots were created in R software (R Core Team 2022) to graphically demonstrate the summarization of the extracted data, with bar charts for raw materials and products mentioned in temporal order, as well as kernel density graphs for the temperature data related to the facets of both products and raw materials.

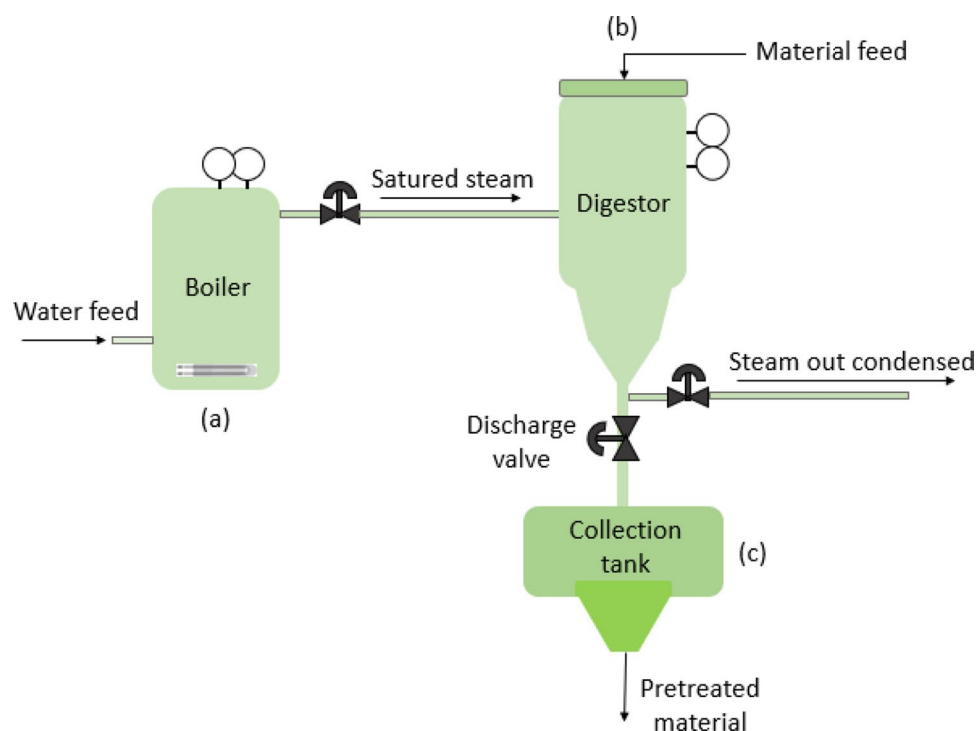
The results were interpreted by a script implemented for this purpose, in which keywords and data patterns were searched in the abstracts of the articles, resulting in a list with the extracted data in a table form.

## Steam explosion technique

The conversion of residues and biomass into compounds of greater added value often requires the pretreatment of the sample, aiming to increase the process's yield and extract the desired components without causing degradation. A pretreatment technique that has been highlighted is a steam explosion, which is generally applied for the treatment of lignocellulosic materials (Dong et al. 2021; Qin et al. 2022).

The pressure resulting from the heated steam under the sample causes a rigid rupture in the fibrous clumps of the biomass, followed by decompression. The steam penetrates the cell wall of the material, and a process of hydrolysis of the sample begins (Chen and Liu 2015), transforming the biomass into small particles due to the rupture of glycosidic bonds (Zhang et al. 2019) or protein hydrolysis (Guo et al. 2020), increasing the sample contact surface area for the next processing steps, such as acid or alkaline extraction (Rommi et al. 2018; Scopel et al. 2019) and enzymatic hydrolysis (Dong et al. 2021; Guo et al. 2020), among others.

Basically, the steam explosion system takes place inside a reactor, as exemplified in Fig. 1. The water contained in reservoir (a) is heated to the temperature at which it is transformed into saturated steam. This steam is directed to a digester (b) that contains the sample, with the heat maintained through jacketed heating. The steam entering the

**Fig. 1** Steam explosion system

digester condenses. After the steam has been in contact for a certain time with the sample contained within the digester, a discharge valve is opened, and the contents are expelled from the digester to a tank or container collection (c).

Recently, there have been technological advances in the SE technique to reduce unwanted disruptions in the molecular structure that usually occur during shearing by the traditional technique (Qin et al. 2022). Thus, some studies reported the SE technique as steam flash explosion—SFE (Zhang et al. 2014, 2015), high-density steam flash explosion—HDSFE (Zhang et al. 2013; Zhao et al. 2012), or instant catapult steam explosion—ICSE (Qin et al. 2020, 2022).

The conventional reactor model adopts the classic structure in valve-blowing mode (which contains a pneumatic ball valve) for sample release, whereas in the ICSE model, a piston is driven by pneumatic linear actuators. Additionally, in the SFE model, the sample needs to wait for the equipment's high pressure to be released. This operational parameter was optimized in HDSFE/ICSE, as it provides a much faster time for the treatment of the sample (between 0.085 s) due to the release of high energy. The density that your design can support (Yu et al. 2012; Zhao et al. 2012).

In general, the main advantage of the SE technique is the fast-processing time, which results in low energy consumption. In addition, waste with organic and/or inorganic solvents is not generated in this type of technology, which is considered a green and environmentally safe technique (Zhang et al. 2019).

Some mechanical pretreatments may precede steam explosion, such as grinding or crushing the sample, to facilitate the opening of the fibers for the penetration of steam through the surface of the material (Scopel et al. 2019; Scopel et al. 2020a, b).

The steam explosion technique has been demonstrated as an ideal pretreatment method for lignocellulosic biomasses (Yu et al. 2022), such as sugarcane bagasse (Fontes et al. 2021; Hongrattanaichit and Aht-Ong 2020; Rocha et al. 2020), wood (Besserer et al. 2022; Cebreiros et al. 2021; Walker et al. 2018) and straw from agro-industrial wastes (Álvarez et al. 2021; Díaz et al. 2022; Sun et al. 2020), mainly aimed at the production of biofuels (Gao et al. 2021; Ko et al. 2020; Rochón et al. 2022), biogas (Duran-Cruz et al. 2021; Hashemi et al. 2021; Rooni et al. 2021) and extraction of compounds of industrial interest, such as xylitol (Bonfiglio et al. 2021; Dasgupta et al. 2022; Wang et al. 2015).

The acid treatment preceding the application of SE is also commonly applied since they act as catalysts. Acidic solutions can saponify ester groups bound to hemicellulose, leading to the cleavage between hemicellulose and lignin (Ismail et al. 2021; Rojas-Pérez et al. 2022). These conditions are generally favorable in nonwoody materials (Zhang et al. 2019). The acid most commonly used as pretreatment is sulfuric acid; however, concentrations above 3% can lead to excessive degradation of hemicellulose and generate inhibitors or unwanted residues in the medium.

Calcium, sodium, and potassium hydroxide at lower temperatures ( $\sim 100$  °C) are used as an alkaline pretreatment for the breakdown of ester and glycoside side chains. However, when dealing with lignocellulosic materials, the use of alkaline agents can degrade lignin and reduce the number of total sugars obtained from the biomass (Mosier et al. 2005).

### Factors affecting the efficiency of SE treatment

The most important parameters for using steam explosion are temperature, pressure, sample composition, residence time, and particle size. These parameters are briefly described in the subtopics below.

#### Temperature

The temperature strongly influences the compounds to be treated by SE. Temperatures above 200 °C, for example, can cause exothermic degradation of sugars, contributing to the loss of volatile compounds, in addition to the disintegration of fibers due to the solubilization of hemicellulose (Lizasoain et al. 2016).

The pH of the medium can also vary under thermal stress. Scopel et al. (2019) observed a reduction in pH (from 3.20 to 3.08) in chromium (III)-tanned leather waste as the temperature increased from 130 °C to 150 °C. According to the authors, this may be related to the exposure of carboxylic groups, glutamic and aspartic acids due to the de-tanning process, where the crosslinking between chromium and the protein chains of collagen is broken, reversing the tanning process.

In addition, it is important to emphasize that the use of high temperatures during the pretreatment of protein samples by steam explosion can lead to excessive denaturation of proteins, resulting in the loss of their functional properties, as well as contributing to the degradation of amino acids, oxidation reactions, and the formation of undesirable compounds (Chakrabarti et al. 2018). Therefore, it is essential to carefully consider the process parameters, especially temperature and residence time, to prevent significant damage to proteins and preserve their functionality.

#### Pressure

The pressure is a significant parameter in the reduction of the crystallinity of samples. It happens because the fibers are elongated due to the molecular rearrangement caused by breaking hydrogen bonds during decompression. These fibers are composed of crystalline and amorphous regions, which during the SE are disarranged, allowing a reduction in the crystallinity of the material (Xu et al. 2006). Therefore, pressure is one of the most important parameters for samples

requiring a lower crystalline state for later treatments such as sheep's wool (Zhao et al. 2012).

In addition, by reducing the material crystallinity, a rearrangement of the molecules occurs and the surface area may be increased, as described by Tian et al. (2018), after exploding a chitin sample at 2.4 MPa using an ICSE, the sample surface area increased from 4.98 m<sup>2</sup>/g to 12.39 m<sup>2</sup>/g. This increase in surface area allows a reduction in processing time and reagent use in subsequent processes of chitin treatment, in this case, to obtain chitosan.

#### Sample composition

Some components present in the sample may undergo partial or total degradation during treatment with the steam explosion, especially when very high temperatures and pressures are used. For example, cysteine is an important amino acid found in the structure of keratinous materials and is usually degraded when SE is applied due to the disintegration of disulfide bonds that are linked to cysteine, which together reticulate protein chains (Zhao et al. 2012). Thus, its absence or reduced quantity may compromise the protein quality in the final product (Shavandi et al. 2017).

During thermal processing, in addition to the Maillard reaction, oxygen and carbon-based radicals can be generated, leading to oxidation processes of proteins and peptides of interest. Thus, it is essential to consider the composition of the sample, which can influence the hydrolysis reaction, where compounds such as carbohydrates, fibers, lipids, and secondary metabolites can interact with proteins and affect the type of peptides generated in hydrolysis (Chakrabarti et al. 2018).

Another critical point is related to components with strong interactions and similar solubilities. In the study by Rommi et al. (2018), Brewer's Spent Grains (BSG) was used, which has predominant fiber and protein contents, among them hordein (barley reserve proteins), glutelin (structural proteins), albumin (which can be storage proteins or enzymes) and globulin. When investigating protein and lignin fractionation using SE, the authors observed a strong co-extraction and partial co-precipitation of protein and lignin, and therefore, it was concluded that for enzymatic recovery of protein hydrolysates without promoting the co-extraction of lignin, the sample without pretreatment was more suitable.

The effect of SE in some sample constituents may also affect the sensory characteristics of the final product. Bones and keratins, for example, have aromatic amino acids such as tryptophan, tyrosine, and phenylamine, which, due to the breakage of disulfide bridges, can cause changes in the color of the final product (yellow-dark, usually) due to the release of sulfur molecules in the medium (Tonin et al. 2006).

Regarding the SE pretreatment of collagen-rich samples, aiming to extract gelatin later, it should be noted that very high pressures can lead to excessive protein breakdown (Qin et al. 2022), since the more degraded the peptide fraction, the lower bloom strength, a very important parameter and directly related to the gelatin viscosity and molecular weight, which directly impacts its applications (Rigueto et al. 2022).

#### *Residence time and particle size*

A longer residence time can lead to a greater comminution of the particles, facilitated by the easy penetration of steam into the molecular structure of the sample. In protein-based materials, Zhang et al. (2023) reported that steam has the capability of breaking and disorganizing the collagen network while penetrating through the pores of the sample, resulting in the expansion of the system and disruption of the internal structure through shear forces. This phenomenon could be beneficial in increasing the yield in protein extraction processes.

However, for materials with greater hardness or larger sizes, such as bones, the use of ICSE is more suitable because, compared to the conventional SE technique, ICSE has a higher explosion efficiency, causing a greater rupture and reduction of particles, thus increasing the extraction yield in subsequent processes (Tian et al. 2018).

Thus, the temperature, pressure, and residence time must be previously studied according to the composition and size of each sample to ensure the highest yield without the degradation of compounds of interest and the loss of quality in the final product.

### **Main applications of steam explosion as a pretreatment technique**

The main raw materials studied and products of interest made possible from the pretreatment by the steam explosion in the period from 1990 to 2022 are shown in Fig. 2a and b, respectively.

In Fig. 2a, it is noted that since 1990, wood was the most reported material in the studies; however, from 2010 onward, straw, mainly from residual sources such as wheat and rice straw, surpassed the use of wood. In 2016, a greater focus was given to bagasse, especially that generated in the production of sugarcane (sugarcane bagasse). Finally, more recently, considering the years 2020 to 2022, it appears that these materials are still on the rise in studies; however, raw materials such as bone, backbone, feather, seedcake, soybean, and skin are beginning to be used in research using steam explosion.

In parallel, Fig. 2b shows that the application of SE as a pretreatment of samples for the production of biogas and

xylose was more widespread in studies from 1990 to mid-2010, whereas from 2011 onward, bioethanol production was more widespread. Considering the last three years, bioethanol and biogas continue to be the most studied products of interest. However, it is noteworthy that in 2020, there was an increase in the frequency of the term “protein” in studies (a term that had been growing since 2010); in addition, the emergence of terms such as “gelatin” and “keratin” is evident, suggesting the use of SE to obtain protein products.

Temperature is one of the most important parameters in the SE technique, which is studied together with the sample residence time, making it possible to optimize the process and thus obtain a higher extraction yield. The main temperature ranges studied in the SE according to the raw material and the product of interest are shown in Fig. 3a and b, respectively, where it is noted that regardless of the raw material pretreated or obtained product of interest, most studies report that steam explosion experiments were conducted in the temperature range of 200–250 °C.

Under the reported conditions, the SE technique breaks the structure of the lignocellulosic matrix, composed of cellulose, hemicellulose, and lignin fractions, obtaining monomeric sugars, which can be converted into fuels and other products through fermentation (Besserer et al. 2022; Duran-Cruz et al. 2021).

Studies suggest the concept of biorefineries with multi-product production as an economic and sustainable strategy, such as obtaining biogas from the organic liquid fraction and bioethanol and xylitol from the hemicellulose fraction rich in sugar (Álvarez et al. 2021; Walker et al. 2018). Thus, including the extraction and improvement of the functionality of proteins from raw materials by SE in the context of a biorefinery is a promising alternative taking into account economic and environmental bias.

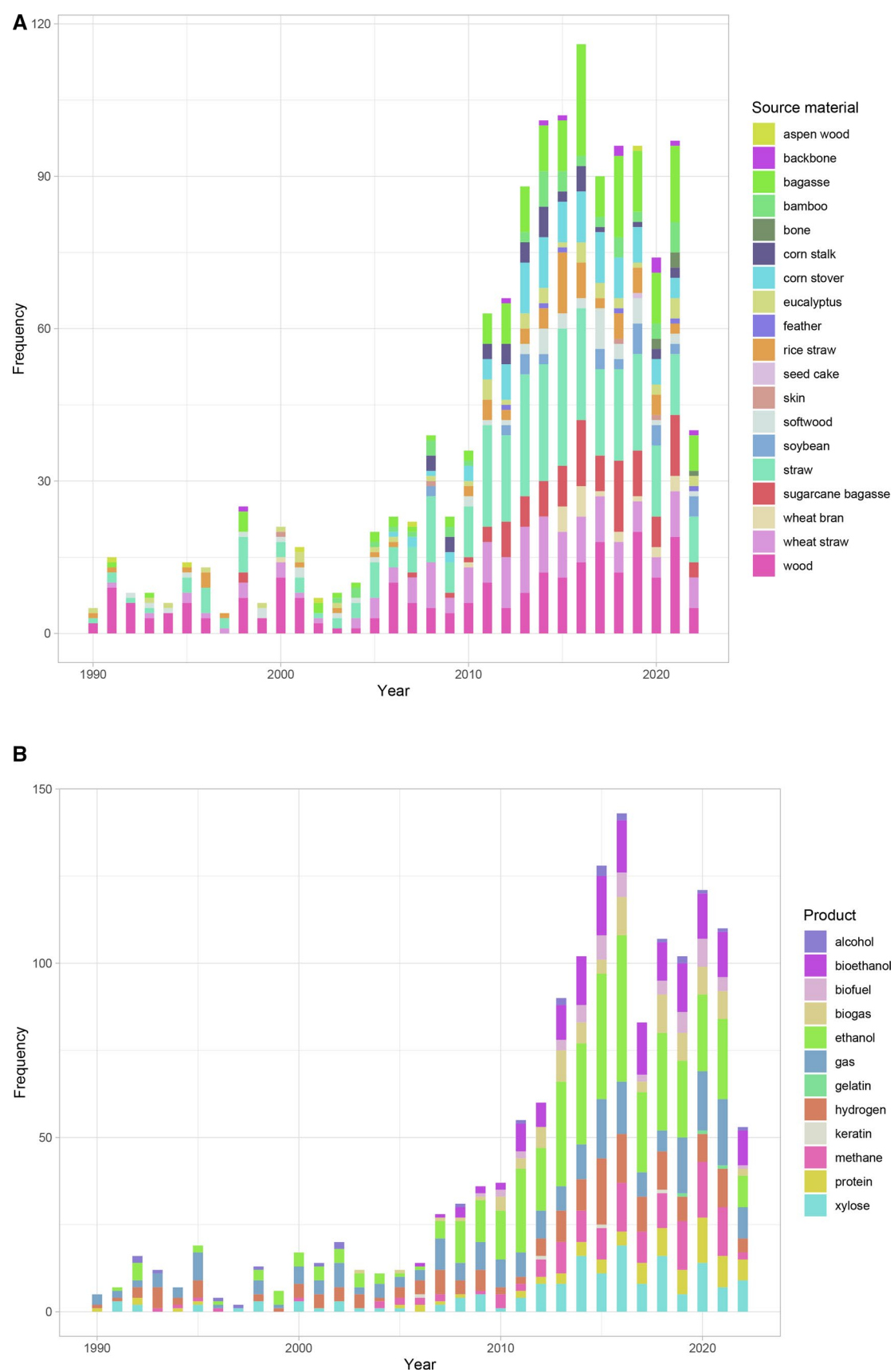
In this context, Table 1 shows the main advantages and disadvantages of using steam explosion as a pretreatment for protein extraction or to improve its functionality compared to other conventional techniques.

### **Steam explosion applied to protein compounds extraction and improvement of protein functionality**

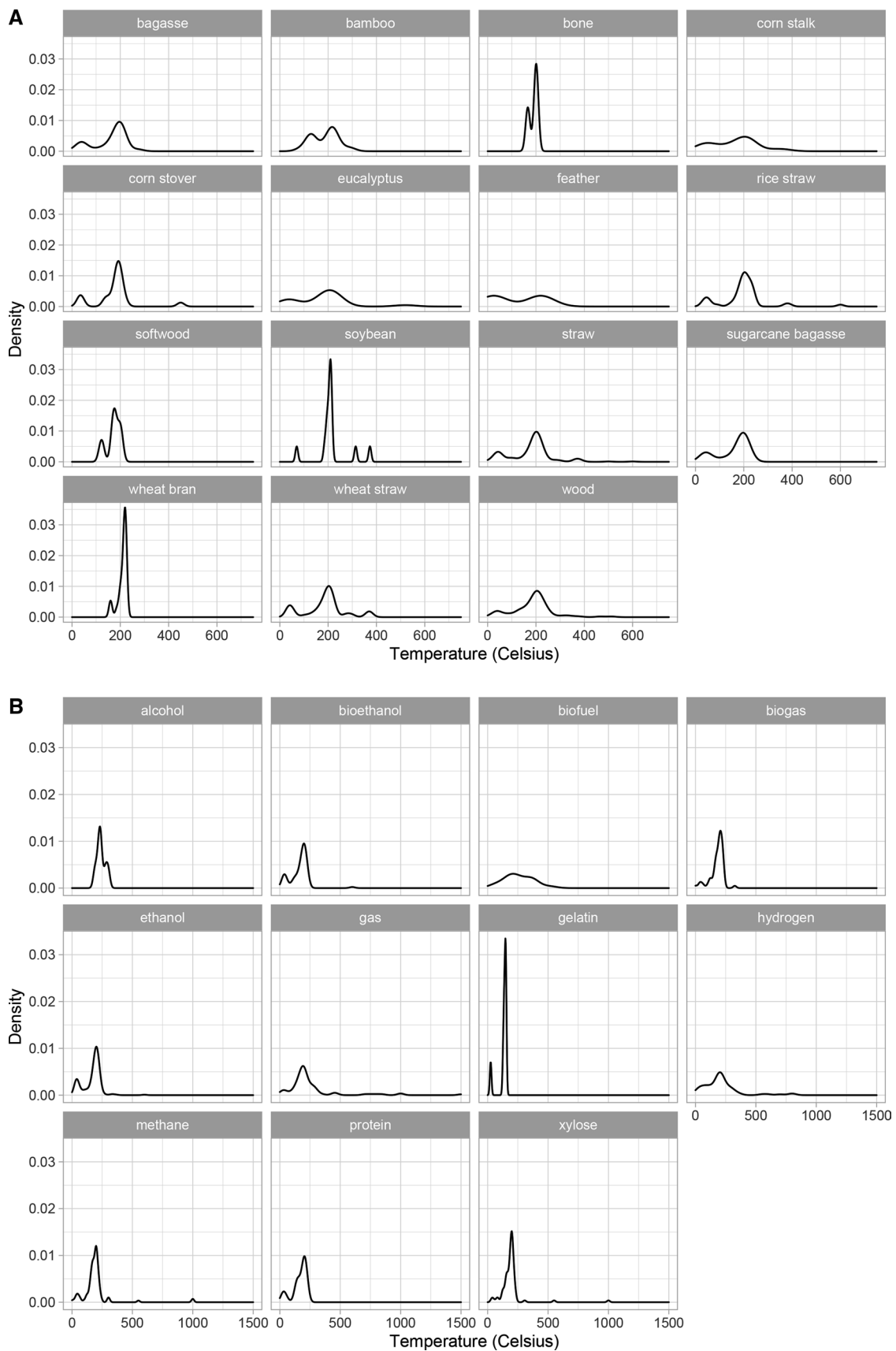
In general, the kinetic energy generated during the steam explosion can disrupt the protein microstructure and release a large number of fragmented cells, increasing the protein extraction yield. In addition, the structural rearrangement and physicochemical reaction involved altering the structure of the protein, its groups, and intermediates, impacting its functional properties (Zhang et al. 2017, 2019).

The use of SE pretreatment to extract or induce changes in protein conformations is a recent approach. Table 2





**Fig. 2** Main raw materials **a** and products of interest **b** reported in studies using steam explosion over time (1990 to 2022)



**Fig. 3** The main temperature ranges in the steam explosion, according to raw material **a** and products of interest **b**

**Table 1** Advantages and disadvantages of using steam explosion compared to other techniques, according to the compound of interest

Objective	Conventional techniques	Steam explosion	References
Gelatin extraction	<p>Advantages: Higher yield extraction; Disadvantages: Use of chemical reagents such as acids or alkalis, generating toxic residues at the end of the process</p>	<p>Advantages: 12 to 36 times reduction extraction time; Disadvantages: More aggressive conditions increase yield but reduce gelatin quality (lower molecular weight, viscosity, and bloom strength)</p>	<p>Scopel et al. (2020a) Scopel et al. (2019)</p>
Keratin extraction	<p>Advantages: The use of a strong alkali solution can extract about 94% of keratin, in addition to other reducing agents that show yields of 77 to 84%; Disadvantages: Processing aiding reagents used in chemical methods, such as sulfites, thiols, DTT, or peroxides, are harmful, often toxic, and difficult to handle</p>	<p>Advantages: Reduction of treatment time, low cost, and treatment without chemicals; Disadvantages: Destruction of cysteine and loss of essential amino acids such as tryptophan and lysine</p>	<p>Ossai et al. (2022) Guo et al. (2020) Zhao et al. (2012)</p>
Obtaining peptides from bone materials powder	<p>Advantages: No generate aggressive explosions; Disadvantages: Increased time and energy consumption, due to complex and time-consuming steps involving boiling (121 °C for 1 h), coarse grinding, drying, sieving, and grinding (ranging from 2.5 to 6 h)</p>	<p>Advantages: Less environmental pollution, low cost, convenient operation, fewer reagents, and process time and energy reduction to obtain a bone powder with a higher protein and mineral content; Disadvantages: The safety of the final product, which includes the absence of cytotoxicity, needs to be rigorously studied and proven</p>	<p>Qin et al. (2022) Cui et al. (2021)</p>
Extraction or functionality improvement of protein from grains and meals	<p>Advantages: Combined extraction using alkaline and acidic reagents can achieve a 95% yield protein extraction; Disadvantages: A large amount of acid is needed to reach the isoelectric point of the protein to recover it</p>	<p>Advantages: Higher nutritional bioaccessibility and improved functional properties (higher solubility, water, and oil-holding, emulsifier and stabilizer capacities); Disadvantages: Samples containing strong interaction and similar solubility between proteins and other compounds may result in co-extractions</p>	<p>Zhang et al. (2022) Zhang et al. (2019) Qin et al. (2018) Rommi et al. (2018)</p>



**Table 2** Steam explosion as a pretreatment/treatment technique for protein extraction/hydrolysis, or to improve protein functionality

Raw material	Product of interest	Experimental conditions		Performed <sup>1</sup> or suggested <sup>2</sup> applications	Earlier <sup>a</sup> , subsequent <sup>b</sup> or combined <sup>c</sup> treatments	Yield (%)	Main results	References
		Sample amount (g)	Saturated steam temperature (°C)	Time (min)				
Bovine bone	Bone powder	100 <sup>w</sup>	≈ 224	30	Nutrition-fortified foods <sup>2</sup>	–	The powder bone protein content was 49.67%, and more than 50% peptides < 2 kDa were obtained	Qin et al. (2022)
Fish backbones	Bioactive peptides	300 <sup>w</sup>	159	2	Supplements for food, cosmetic and pharmaceutical industries <sup>2</sup>	Enzymatic hydrolysis <sup>b</sup>	The largest molecular weight distribution of the hydrolyzate was < 3 kDa and had strong scavenging effects on DPPH (IC <sub>50</sub> = 4.24 mg/mL) and ABTS (IC <sub>50</sub> = 1.93 mg/mL)	Dong et al. (2021)
Feather wastes	Bioactive peptides	100 <sup>d</sup>	≈ 201.4	140	Food, animal feed, tissue engineering, pharmaceutical, and biomedical fields <sup>2</sup>	Enzymatic hydrolysis <sup>b</sup>	From 1 g of feather waste, 0.8 g of high-value nutrients and bioactive peptides (< 2 kDa) with antidiabetic, antihypertensive, and antioxidant activities were obtained	Guo et al. (2020)
Big Head Carp bones	Bone protein with a higher dissolution	200 <sup>w</sup>	≈ 159	20	–	Enzymatic hydrolysis <sup>b</sup>	The hydrolysis degree by alcalase (8.69%) and yield of soluble nitrogen in trichloroacetic acid (28.79%) increased with SE pretreatment, and 93% of the molecular weight distribution was less than 1 kDa	Dong et al. (2020)

Table 2 (continued)

Raw material	Product of interest	Experimental conditions		Performed <sup>1</sup> or suggested <sup>2</sup> applications	Earlier <sup>a</sup> , subsequent <sup>b</sup> or combined <sup>c</sup> treatments	Yield (%)	Main results	References
		Sample amount (g)	Saturated steam temperature (°C)	Time (min)				
Yak bone	Powder with higher solubility	100 <sup>w</sup>	≈ 198	30	–	46.16	26.14% of the peptides had a low molecular weight (< 0.5 kDa) and the solubility of the steam-blown yak bone powder protein was 97.17%, 95.29%, and 82.07% at 37, 60, and 100 °C, respectively	Qin et al. (2020)
Fish skin	Proteins with a higher hydrolysis degree	–	≈ 159	0.5	Enzymatic hydrolysis <sup>b</sup>	45.55	The fish skin protein dissolution rate was 45.55% (5 times higher than the control sample), and the relative molecular weight of the enzymatic hydrolysis products was mainly distributed below 4 kDa	Dan et al. (2020)
Grass silage fiber	Single-cell protein	–	190	15	Source of food protein: a sustainable alternative for meat products <sup>2</sup>	51	Single-cell protein can be produced from grass silage hydrolysates, and the protein yields ranged from 37 to 59 kg per ton of silage dry matter	Pihlajaniemi et al. (2020)

Table 2 (continued)

Raw material	Product of interest	Experimental conditions		Performed <sup>1</sup> or suggested <sup>2</sup> applications	Earlier <sup>a</sup> , subsequent <sup>b</sup> or combined <sup>c</sup> treatments	Yield (%)	Main results	References
		Sample amount (g)	Saturated steam temperature (°C)	Time (min)				
Chromium(III)-tanned leather wastes	Gelatin	48.1 <sup>d</sup>	140	10	Polymeric films <sup>1</sup>	30	There was a reduction in extraction time (12 to 36 times) compared to conventional methods, obtaining a gelatin with a viscosity of 2.4 cP at 25 °C, a protein content of 24.6 g/L, and a molecular weight of 39 kDa, with a reduction in chromium (a factor of 16 to 96 times) and ash (11.8 to 1.2%) content by diafiltration	Scopel et al. (2020a, b)
		100 <sup>w</sup>	130	15	Alkaline hydrolysis <sup>b</sup>	27.8	There was a 1.8-fold increase in gelatin extraction with a viscosity of 6.0 cP, 5.2 Lowry protein/TKN ratio, and helix-to-coil transition temperature of 56 °C	Scopel et al. (2019)
Bovine limed hide waste	Gelatin	200 <sup>w</sup>	130	10	Membrane ultrafiltration <sup>b</sup> and Enzymatic crosslinking <sup>b</sup>	–	Gelatin's molecular weight distribution increased with higher time and temperature conditions (30–69 kDa)	Rigueto et al. (2023)

Table 2 (continued)

Raw material	Product of interest	Experimental conditions		Performed <sup>1</sup> or suggested <sup>2</sup> applications	Earlier <sup>a</sup> , subsequent <sup>b</sup> or combined <sup>c</sup> treatments	Yield (%)	Main results	References
		Sample amount (g)	Saturated steam temperature (°C)	Time (min)				
<i>Camellia oleifera</i> Abel. seed cake	Protein with improved functional properties	–	≈ 220	2	Emulsion stabilizer <sup>1</sup>	–	Camellia seed protein solubility (3.6 mg/mL) and its holding capacities of water (6.24%) and oil (4.32%) were higher after SE	Zhang et al. (2019) and Zhang et al. (2022)
Okara	Protein with improved functional properties	–	≈ 198.3	0.5	Flour products and beverages <sup>2</sup>	–	Okara water solubility content increased (50–300%) while swelling (34.6–51.9%), oil holding (27–60.9%) and water holding (22–62.5%) capacities decreased after SE	Li et al. (2019)
Brewer's spent grain	Fractionate protein	739.2 <sup>d</sup>	200	10	Multi-use food ingredients <sup>2</sup>	46–65	There was a reduction in enzymatic protein solubilization (100 to 85%), but enhanced (> 10%) the extract recovery in the centrifugation step	Rommi et al. (2018)
	Proteins with higher solubility and stability	739.2 <sup>d</sup>	200	10	Functional food and beverage <sup>2</sup>	75	Between 1.8 and 12.0% of the protein present in the grains was solubilized, whereas losses accounted for 13.4–29.1%	Kemppainen et al. (2016)

**Table 2** (continued)

Raw material	Product of interest	Experimental conditions		Performed <sup>1</sup> or suggested <sup>2</sup> applications	Earlier <sup>a</sup> , subsequent <sup>b</sup> or combined <sup>c</sup> treatments	Yield (%)	Main results	References
		Sample amount (g)	Saturated steam temperature (°C)	Time (min)				
Soybean meal	Soy protein isolate with improved physicochemical properties	600 <sup>d</sup>	207 – 217	8	–	–	After the SE pretreatment, the magnitude of the negative charge increased from -44.8 to -50.6, indicating an improvement in protein solubility and emulsion/foam stability. In addition, a greater molecular weight distribution of around 39.8 kDa was observed	Zhang et al. (2017)
Duck feathers	Keratin	100 <sup>w</sup>	≈ 201	1	Development of new biomaterials <sup>2</sup>	Alkaline extraction <sup>b</sup> 42.78	The molecular weight distribution of the extracted keratin showed some peptides of ≈ 10 kDa, indicating, in addition to keratin dissolution, protein degradation	Zhang et al. (2015)
Feather	Meal with greater digestibility	94 <sup>d</sup>	≈ 207	1	Biopolymers production <sup>2</sup>	Enzymatic hydrolysis <sup>b</sup> 91	Digestibility by pepsin of steam-exploded feather meal reached about 91% (9 times higher than feathers without pre-treatment)	Zhang et al. (2014)

Table 2 (continued)

Raw material	Product of interest	Experimental conditions		Performed <sup>1</sup> or suggested <sup>2</sup> applications	Earlier <sup>a</sup> , subsequent <sup>b</sup> or combined <sup>c</sup> treatments	Yield (%)	Main results	References
		Sample amount (g)	Saturated steam temperature (°C)	Time (min)				
Soybean meal	Protein with improved functional properties	600 <sup>w</sup>	≅207	3	Value-added food ingredient production to replace the white soy flakes <sup>2</sup>	65.66	There was a formation of protein aggregates with a molecular weight of around 669 kDa in the soybean meal, as well as increased solubility (99.33%), emulsifying activity index (36.4 m <sup>2</sup> /g), emulsifying stability index (21.53 min) and fat-binding capacity (876%)	Zhang et al. (2013)
Poultry feathers	Keratin	–	≅212	0.08	Nutrient animal feed <sup>2</sup>	72.8	Keratin solubility in KOH solution (from 3 to 72.8%) and its digestibility using pepsin (from 10.5 to 93.2%) increased	Zhao et al. (2012)
Wool	Keratin	100 <sup>w</sup>	220	10	Biomaterials for packaging, textiles, sanitary, filtration, or medical fields <sup>2</sup>	62.36	The process produced 18.66% of proteins; The temperature used in SE caused a loss of high molecular weight keratin, with proteins ranging from 3–18 kDa	Tonin et al. (2006)

<sup>d</sup>Dry basis; <sup>w</sup>Wet basis



presents a compilation of studies that aimed to apply steam explosion as a technique for extracting proteins, obtaining protein hydrolysates, and/or improving protein functionality.

It is observed in Table 2 that the protein compounds extracted from animal sources most reported by SE were keratin, gelatin, and low molecular weight peptides from feather, leather, bovine bones, and fish residues. For vegetable sources, biomass from the industrial extraction of vegetable oils, such as soybean meal and camellia seed cake, has been studied to improve the functionality of proteins, mainly related to solubility.

Regarding the treatments reported before SE application, there were acidic (10%) and alkaline treatments (5%). As a subsequent treatment, enzymatic hydrolysis is most often reported (45%), followed by alkaline (20%) and acidic extraction (10%), concentration, and purification by membranes (5%). Only one study (5%) evaluated gelatin extraction using SE with alkaline agents added to the reactor as the main technique and not just as a pretreatment.

The main mechanisms involved in steam explosion pretreatment for protein extraction or to improve its functional properties have been elucidated in some works reported in the literature and are described in the following subtopics.

### Collagen/gelatin

Collagen is a protein compound found in the skin, leather, bones, and cartilage of mammals, poultry, and fish. In the hydrolysis of collagen to obtain gelatin, the hydrogen bonds which stabilize the triple helix are interrupted. The hydrolysis of the main chain's intra and intermolecular peptide bonds occurs, originating smaller molecules (Noor et al. 2021).

Few studies related to gelatin extraction, using the SE technique are available in the literature. Scopel et al. (2019) studied for the first time the extraction of gelatin from chromium (III)-tanned leather wastes under the conditions of 130 and 150 °C for 5 and 15 min, with subsequent alkaline hydrolysis, and verified that the steam penetrated the fibrous structure of the material, breaking cross-links between Cr (III) and collagen and thus facilitating the entry of water between collagen fibers, reversing the tanning process and promotes the solubilization of proteins in the aqueous medium. Thus, with more severe conditions it was possible to obtain a higher extraction yield of gelatin because increasing the time and temperature of the process favored the greater opening of the tanned leather fibers, allowing greater water absorption and, consequently, greater protein extraction. Despite achieving an increase in extraction yield (15.5% to approximately 47% compared to gelatin extracted without pretreatment), gelatin quality was affected.

The energy generated due to the higher temperature and residence time resulted not only in the breaking of the

collagen-chromium bonds, but also in the breaking of the peptide bonds, reducing the molecular weight and, consequently, the viscosity and bloom strength of the gelatin (Scopel et al. 2019).

In a later study, Scopel et al. (2020a) extracted gelatin from the same leather waste but using SE as the main technique combined with alkaline hydrolysis, under the conditions of 130, 140, and 150 °C for 5, 10, and 15 min. The treatment at 140 °C for 10 min was considered the optimized condition, taking into account the combination between yield (30%) and gelatin quality (viscosity of 2.4 cP, protein concentration of 24.6 g/L and molecular mass of 39 kDa). Compared to traditional methods of gelatin extraction, the authors indicate a reduction of 12 to 36 times in process time.

Recently, Riguetto et al. (2023) applied SE pretreatment for gelatin extraction from limed bovine hide wastes and verified that increasing the temperature from 110 to 130 °C and the processing time from 1 to 10 min significantly influenced the obtainment of gelatins with the highest contents of dry matter (7.74%), protein (28.26 g/L), viscosity (11.06 cP), molecular weight (69 kDa) and glycine, proline, and hydroxyproline amino acid content (30 g/100 g), as well as the lowest conductivity values (7888.06 µS/cm), ash content (8.60%) and pH (10.33).

Dong et al. (2021) found a similar behavior using the steam explosion as a pretreatment for fish backbones at 159 °C for 2 min, resulting in a high degree of hydrolysis of collagen fibers, with high protein content and low molecular weight peptides ( $\approx 10$  kDa).

### Keratin

Differently from fibrous proteins such as collagen and myofibrillar protein, feather keratin is compacted into  $\beta$  sheets into a supercoiled polypeptide chain with a high degree of disulfide crosslinks, hydrophobic interactions and hydrogen bonds, which confer mechanical stability, indissolubility and consequently resistance to extraction by chemicals and proteolytic enzymes (Guo et al. 2020; Zhang et al. 2015; Zhao et al. 2012).

The mechanism involved in the pretreatment of feathers by the steam explosion for keratin extraction is related to the reduction of intermolecular disulfide bonds and the crystalline structure of keratin, due to the rapid release of sufficient energy that acts from the inside out of the material (Guo et al. 2020). Initially, the saturated water vapor gradually penetrates the feathers, decomposing the highly ordered structure of the keratin into unfolded and disordered structures, due to the modification of the secondary structure (increasing the random coil/ $\beta$ -sheet structures ratio) (Guo et al. 2020; Zhao et al. 2012).

Also, the increased pressure in the system destabilizes the weak inter-sheet van der Waals stacking forces in keratin crystallite. Afterward, there is a rapid reduction in pressure, causing swelling of the keratin, exposure of hydrophilic and ionizable protein groups, and formation of amorphous regions in the structure of the protein, increasing its interaction with water, in addition to facilitating access, adhesion and catalysis of the keratin by enzymes and to increase dis-solubility in chemical solvents (Guo et al. 2020; Zhao et al. 2012).

Studies related to the steam explosion and keratin extraction show that lower temperatures can promote the extraction of up to 80% of the material (Xu et al. 2003). However, cysteine content may be reduced after pretreatment, as high temperature and pressure damage this amino acid (Chilakamarthy et al. 2021).

In a study conducted by Xu et al. (2006), the authors investigated the use of SE to obtain wool powder, which, for application, needs to have its crystallinity decreased. The authors observed that the pressure at 0.8 MPa used in processing sheep's wool was responsible for causing damage to the surface of the material, which was used as wool powder.

However, when the HDSFE is employed, the results are more promising. Zhao et al. (2012) treated feather residues through this technique and found that, although crystal down and disulfide bonds were not damaged, there was no major damage to the keratin protein chain. The authors were able to convert up to 90% of keratin into pepsin-digestible.

### Bones and fishbones

Regarding the conversion of bone materials into powder, this process is very complicated due to their characteristics of high stiffness and tenacity, especially for bones of bovine origin, since collagen organizes itself in triple colloidal structures along the longitudinal axis, while hydroxyapatite crystals orient perpendicularly in a parallel and staggered arrangement, filling the gaps between adjacent collagen fibrils (Qin et al. 2020; 2022; ShuQing et al. 2020).

However, the intense infiltration strength of saturated steam can penetrate the bone pores and tear them in a rapid decompression process, causing effects of laxity and softening, and at the same time promoting protein dissolution (Qin et al. 2022).

Qin et al. (2022) compared obtaining bone powder through the conventional method that involves grinding and cooking with the ICSE method. The authors observed that higher reaction and pressure times (2.5 MPa for 30 min) promote the opening of irregular orifices on the surface of bones, resulting in higher protein hydrolysis into peptides smaller than 2 kDa and mineral content extracted when compared with the conventional method.

In another study, however, lower pressure and reaction time were used for tuna bone SE for bone powder production. The conditions used by Cui et al. (2021) were 0.6 MPa for 5 min. Using the conventional method, the authors found a particle size of 169.76  $\mu\text{m}$ , while SE provided a size of 13.18  $\mu\text{m}$ . This 92% reduction in particle size allows better absorption of calcium present in this bone meal when used as a nutritional supplement.

### Grains and meals

Even when applied to the pretreatment of grains and meals, it is extremely important to monitor what happens to the proteins during the steam explosion, in order to preserve the nutritional properties and improve the functional properties (Kemppainen et al. 2016), which is associated with different physicochemical changes, including thermally induced protein denaturation, Maillard reaction, complex matrix breakdown, and dissociation of protein aggregates (Zhang et al. 2013, 2019).

According to Zhang et al. (2019), some secondary structures of Camellia seed cake protein treated by the steam explosion were transformed into unfolded or disordered structures, with more  $\beta$ -sheet structures and smaller amounts of  $\alpha$ -helical structure,  $\beta$ -turn structure, and random coil in comparison with the untreated sample. In the tertiary structure, the protein was partially folded, with a lower content of aromatic amino acid residues and a more polar environment.

The authors also explain the high temperature involved in the steam explosion favors the hydrolysis of glycosidic bonds of sucrose (caramelization) present in the seed cake, producing glucose and fructose, which were consumed as precursors in the Maillard reaction, a fact proven by the increased in the yellow–brown color and reduction of monosaccharides and free amino acids. Strecker degradation can also occur, where amino groups are added to the carbonyl group of reducing sugar, resulting in products such as Strecker's aldehyde, pyrazine, and pyrroles compounds characteristic of foods subjected to heat treatment (Zhang et al. 2019).

Kemppainen et al. (2016) reported that steam explosion applied to Brewer's spent grains generated pseudolignins due to condensation reactions between lignin, sugars, and sugar degradation products, which was proven by the increased association of insoluble proteins with acid-insoluble lignin. In addition, the formation of fermentation inhibitors was observed under two more severe pretreatment conditions, in particular, furfural (about 48% of the inhibitors formed), however, the combined concentration of all inhibitors did not exceed 1 g/L. Thus, furfural in low concentrations is usually found in beverages, being a precursor of aromas (Azevêdo et al. 2007).

Improvements in the foaming and fat-binding capabilities of steam-exploded soybean meal were associated with greater surface hydrophobicity and protein molecule flexibility due to disruption of film formation at the air/water interface, as well as an increase in the apolar side chain of the protein, respectively (Zhang et al. 2013, 2019).

Even with promising trends in the use of steam explosion for protein extraction and modification, few studies describe the mechanism associated with how this technique assists in the extraction, alteration of state, and functionality of proteins in residues, grains, and meals (Kemppainen et al. 2016). As previously discussed, during a steam explosion, the generation of some products derived from the Maillard reaction and other chemical reactions may occur, depending on the composition of the raw material and the process parameters, therefore, new studies on this theme are extremely important for understanding the mechanisms involved, proven by the qualitative and quantitative data.

### Application possibilities of protein compounds obtained by explosion pretreatment

As shown in Table 2, most studies suggest applying the protein compounds extracted with steam explosion pretreatment as a supplement for obtaining biopolymers, peptides, and hydrolysates for food, animal nutrition, pharmaceuticals or cosmetics, and emulsion stabilizers. In the case of applications carried out, only gelatin extracted from chromium (III)

tanned leather wastes and Camellia seed cake with improved protein functional properties were studied as a basis for obtaining polymeric films (Scopel et al. 2020b) and emulsion stabilizers (Zhang et al. 2022), respectively.

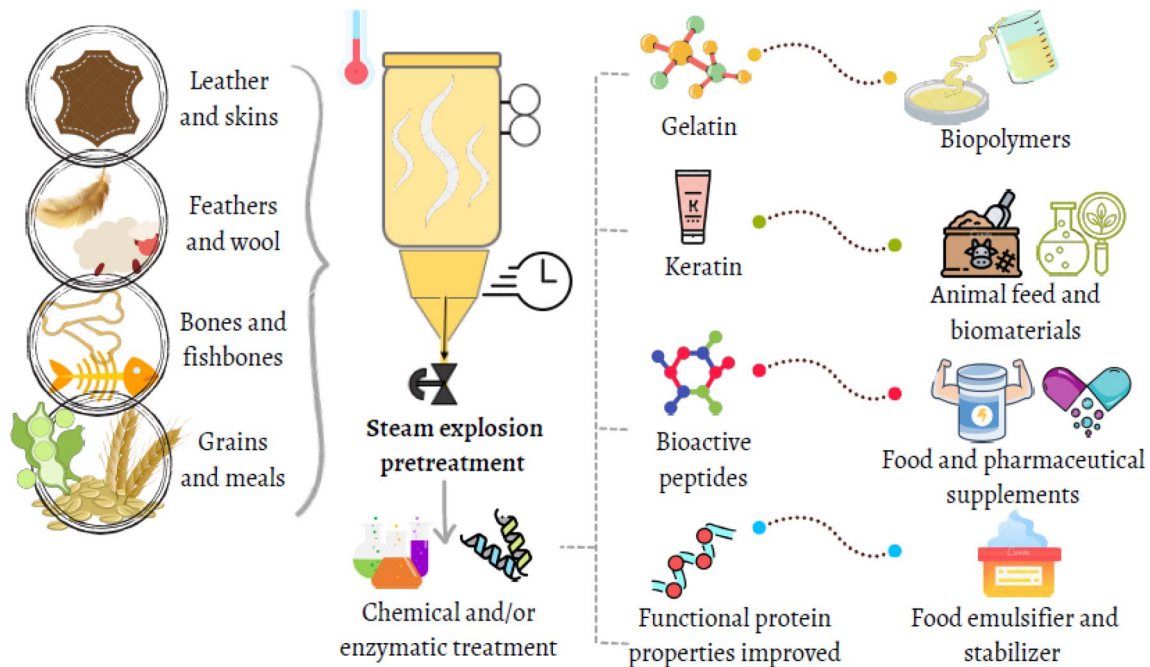
Figure 4 summarizes the main compounds addressed in this review and their suggested and performed applications, which are discussed in more detail below.

### Polymeric films

Collagen-rich skin and leather wastes are interesting alternatives for gelatin extraction to obtain polymeric films. Furthermore, when the residues come from non-tanned sources, gelatin films can be applied in food preservation, while gelatin films from tanned sources, as in the case of chromium, for example, can be used as mulching of soil for agriculture, improving crop yield and quality and contributing to food demand for the growing world population (Rigueto et al. 2022).

In this perspective, the gelatin extracted by the steam explosion in the study by Scopel et al. (2020a) and starch, cross-linked with glutaraldehyde were applied to the production of polymeric films, where it is reported that crosslinking reduced solubility by 14.5% and 42.0%, for films produced only with gelatin and with the gelatin-starch mixture. Thus, it can withstand environments with higher moisture, such as agricultural environments (Scopel et al. 2020b).

In previous works, gelatin mulching soil films promoted an 80% germination rate and increase in rapeseed height



**Fig. 4** Suggested or performed applications for protein compounds obtained from steam explosion pretreatment

(Dang et al. 2016), and maintained the mulching effect for the entire lettuce growing period, ensuring the rate of growth and dry matter accumulation (Sartore et al. 2018).

To guide studies on the application of mulching films for agriculture, the EN 17,033 standard establishes suitability parameters, which involve parameters on biodegradation (90% in up to 2 years), mechanical properties (tensile strength and elongation at break), and  $\leq 3\%$  light transmission (Rigueto et al. 2022).

### Food emulsifier and stabilizer

Protein-based emulsifiers can act as fat stabilizers and humectants in a variety of foods such as confectionery, bakery, beverage, and dairy products. To meet these purposes, molecular structure, surface hydrophobicity, and molecular weight are essential properties to promote the formation of tiny droplets (Kim et al. 2020).

Currently, due to the growing demand for clean-label food products, that is, containing natural, sustainable, and safe ingredients, the food industries have sought to replace synthetic emulsifiers, in addition to the use of alternative vegetable proteins to animal proteins origin (Kim et al. 2020).

Zhang et al. (2022) applied steam-exploded camellia seed protein (SECSP) to stabilize oil-in-water emulsions compared to soy protein isolate (SPI) and observed that SECSP-stabilized emulsions contained smaller particles (245–224 to 230–105 nm), higher  $\zeta$  potential (−39 to −43 and −44 to −64 mV), for SPI and SECSP, respectively.

The long-term stability of the emulsion is a critical point for emulsions, because for food applications, depending on the emulsifier used, the emulsion can coalesce and flocculate and result in the formation of two layers (cream and a transparent layer), impacting the visual appearance and the sensations perceived in the mouth when tasting the emulsion (Zhang et al. 2022).

Thus, the authors also evaluated creaming in emulsions stabilized with SPI and SECSP, with protein concentrations ranging from 0.5 to 2 g/100 mL, and found that creaming in SPI and SECSP emulsions at 0.5 g/100 mL occurred after 14 and 21 days of storage, respectively. For the other concentrations, after 28 days there was no cream formation for the SECSP emulsion, while for SPI only the maximum concentration tested (2 g/100 mL) managed to maintain the stability of the emulsion. These results indicate that SECSP can improve the flavor, water retention, and stability of foods such as ground meat, emulsions, and viscous and bakery products (Zhang et al. 2022).

In addition, greater stability of the SECSP emulsion was observed in *in vitro* simulations of gastric and small intestine digestion, attributed to the presence of glycosylated products resulting from pretreatment with the steam explosion,

forming protective films around the oil droplets (Zhang et al. 2022).

Other studies have also observed significant improvements in the solubility and functional properties of soybean meal (Zhang et al. 2013, 2017), however, food applications have not been studied.

### Bioactive peptides

Bioactive peptides are fragments of specific proteins, which generally have from 2 to 20 amino acids and molecular weights of less than 6 kDa. They are inactive when they are within the sequence of the parent protein, and after hydrolysis depending on their structure, amino acid sequence and composition, molecular weight, charge distribution, and other external factors such as pH, chemical treatments, and processing conditions, may exert antioxidant, antimicrobial, antihypertensive, antidiabetic, antiobesity, antithrombotic, anticancer, hypocholesterolemic, multifunctional, among others (Peighambardoust et al. 2021; Sarmadi and Ismail 2010).

Guo et al. (2020) applied steam explosion to feather residues to extract keratin, which was subsequently hydrolyzed by enzymes such as alcalase and papain, where approximately 85% of the hydrolyzate obtained had a molecular weight of less than 2 kDa. In amino acid profile determination, it was found that several peptides had N-terminal hydrophobic amino acids, including Leu and Val, with potential antioxidant activity *in vivo*, in addition to several other promising amino acids for the treatment of type 2 diabetes and blood pressure or even, act as intestinal microbiota regulators.

Dong et al. (2021) studied collagen extraction assisted by the steam explosion with subsequent enzymatic hydrolysis. It was observed that about 77% of fish backbones protein after steam explosion ranged above 10 kDa, with higher content of glycine, alanine, proline, glutamic acid/glutamine, hydroxyproline, aspartic acid, and arginine. When subjected to hydrolysis using Flavourzyme for 3 h, the molecular weight distributed mostly below 3 kDa (48% were below 1 kDa), with an increase from 211.48 to 3586.24 mg/100 mL in free amino acid content with the predominance of leucine, phenylalanine, arginine, glycine, and lysine, in addition to an increase of 6.24 times in glutamic acid and aspartic acid, which are umami amino acids, where bitter amino acids corresponded to 43.54% and sweet and umami 29.44%. The hydrolysates also showed antioxidant potential with an  $IC_{50}$  of 4.24 mg/mL for scavenging the DPPH radical and an  $IC_{50}$  of 1.93 mg/mL for the ABTS radical. In general, the authors concluded that the pretreatment by steam explosion made it possible to obtain biopeptides from collagen hydrolysates of fish backbones, with antioxidant capacity, umami flavor,



and less bitterness, promising as supplements for foodstuff, cosmetic and pharmaceutical industries.

However, regarding the perspectives of biopeptide applications in food products, toxicity and allergenicity are limiting factors due to the scarcity of research on the safety of these compounds for human health, because although there is the hydrolysis of proteins in peptides from low molecular weight, the allergenic properties derived from the original protein may remain in some peptides, as in the case of peanuts, for example (Sarmadi and Ismail 2010).

In addition to safety issues, more consistent studies are needed on the purpose of application (food or drug?), oral consumption (which involve evaluations on gastrointestinal stability and intestinal absorption), and development of techniques that make viable the large-scale production of biopeptides from proteins derived from grains and wastes, making it possible to meet market demand (Chakrabarti et al. 2018; Sarmadi and Ismail 2010).

Finally, regulations for products based on bioactive peptides on the world market may differ depending on the country or intended use. Canada and the USA, for example, do not provide any legal status for “functional food”, while Japan already has a regulated category of “foods for specific health uses”, and even markets bioactive peptides and protein hydrolysates such as Bonito peptide, Calpis, and Valtiron, from Bonito fish, sour milk and sardine muscle, respectively (Chakrabarti et al. 2018; Chalamaiah et al. 2019). A complete list of biopeptides from food proteins marketed in different countries and their regulatory policies has been reviewed by Chalamaiah et al. (2019).

For pharmaceutical applications, although food biopeptides claim to improve human health, regulatory requirements are more stringent, as they require the completion of clinical trials that demand high long-term costs, making pharmaceutical applications limited/inaccessible (Chakrabarti et al. 2018).

According to Mokomele et al. (2018), the steam explosion is also promising for animal nutrition, as it makes it possible to increase the *in vitro* digestibility of dry matter and the accessibility of nutrients present in the plant cell wall to the microorganisms and enzymes of the rumen, and consequently, the *in vivo* performance of beef cattle.

As shown in Table 2, the hydrolysates and biopeptides obtained in some works are also mentioned as promising for animal nutrition (Guo et al. 2020; Qin et al. 2022; Zhao et al. 2012) and cosmetic and biomedical (Dong et al. 2021; Guo et al. 2020; Tonin et al. 2006) fields, but limited to suggested applications only.

In this sense, we emphasize the importance that the advances obtained in the steps of extraction or improvement of the functionality of the proteins are not limited only to the suggested applications, thus proving the effectiveness and viability of the steam explosion as a green technique

in the context of pretreatment of samples applicable in the industrial and commercial environment, enabling advances and benefits to society and the environment.

## An overview of economic, environmental, and social aspects

In the different reported studies, it appears that the extraction or modification of the protein structure using steam explosion as a pretreatment is promising, as it is a simple operational process that includes mild extraction conditions and low environmental impact, allowing its exploitation on a large scale (Zhang et al. 2022).

Figure 5 presents the main topics related to considerations of economic bias, protein property improvement, environmental sustainability, and social responsibility to the subject of this review.

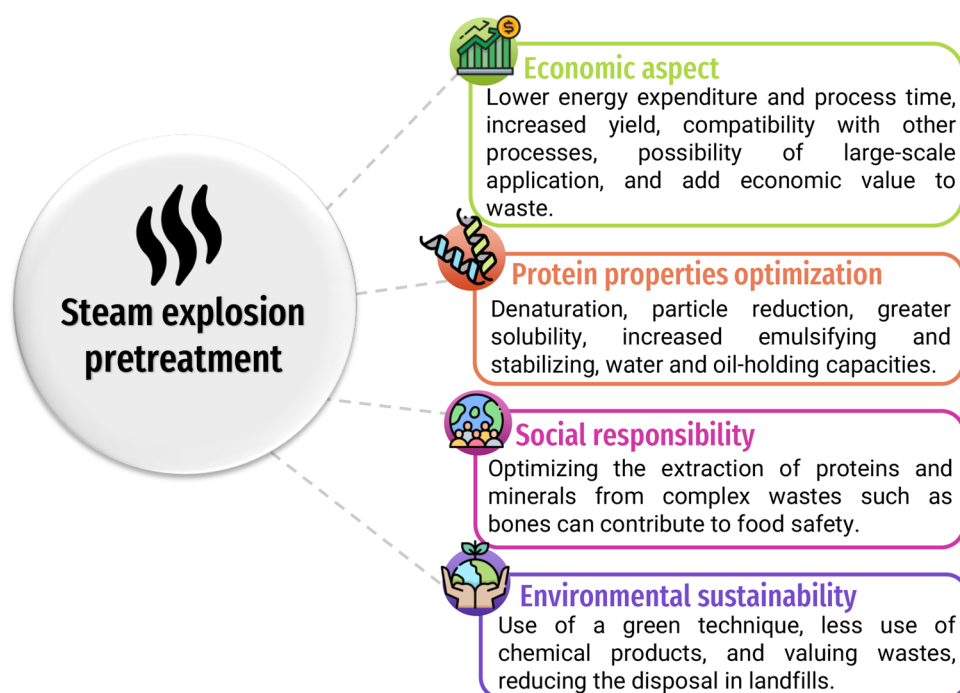
Regarding the economic aspects, Scopel et al. (2019) compared the alkaline hydrolysis process with and without SE, where an economic evaluation was performed. The authors point out that a high pH (above 12) is required for alkaline hydrolysis, resulting in the use of a greater amount of alkalizing agent and increasing the extraction cost, demonstrating that there was an increase of 1.8% in the amount of protein extraction, compared to the sample without pretreatment with SE. In addition, high temperatures (approximately 100 °C) combined with the processing time (6 to 8 h) reflect higher energy expenditure and slow processes, which are not currently desirable for industries.

Another advantage associated with this process is the low dissolution of chromium present in the gelatin samples (less than 0.9%), while in the untreated samples, it varies between 23.2% and 46.8%, contributing both to the reduction of environmental impacts as with the expansion of previously limited applications due to the high concentration of chromium in the sample (Scopel et al. 2019; Scopel et al. 2020a, b).

In the techno-economic assessment carried out by Pihlajaniemi et al. (2020) comparing the steam blast and ammonia soaking methods, it was possible to conclude that the investment cost of the ammonia soaking process (38.8 M€) compared to SE (55.8 M€) would be more suitable for applications on a smaller scale, while the SE is ideal for large-scale applications, as it allows better use of the generated steam.

The steam explosion as a pretreatment of fishbone also facilitates and reduces the costs of the collagen enzymatic hydrolysis process since concentrated acid or alkaline methods are expensive, while only the use of enzymatic hydrolysis makes it unfeasible due to low hydrolysis efficiency associated with the resistance of the bone structure, which is complex for commercial proteases (Dong et al. 2021).

**Fig. 5** Economic, environmental, and social aspects positively impacted by the steam explosion as a pretreatment technique for protein extraction/modification



As already reported, there are different technical specifications for SE equipment directly related to their performance and economic viability. Yu et al. (2012) compared two steam explosion pretreatment models, the classic valve blowing and ICSE models. The authors report that the ICSE operation is more complex, but the steam consumption is lower, 0.25 tons (duration time from 90 to 120 s) compared to the traditional explosion, which is 0.8 to 1 ton per ton of pretreated material (duration time from 15 to 20 min). As steam directly contributes to the reduction of pretreatment costs, it is suggested that process automation is essential to promote process cost reduction.

In addition to the economic and environmental advantages of the steam explosion technique, the use of steam-exploded wastes also positively impacts economic and environmental issues. According to Guo et al. (2020), the annual world generation of keratin waste destined for landfills exceeds 65 million tons (−400 \$/t biomass), resulting in a loss of more than 26,000 million dollars. In this context, the valorization of these residues for cattle feed, for example, could add a value of around 70–200 \$/t biomass.

Also, the byproducts generated during the industrial processing of grains for oil extraction may have adequate protein and amino acid levels for producing ingredients or functional protein for the food industry. In China, for example, approximately 730,000 tons of camellia seed cake are generated annually after oil extraction, with a protein content of 14 to 20%, with most of it destined for disposal in landfills (Zhang et al. 2019; Zong et al. 2015).

Valuing residual biomass from a value chain (such as grains and meals, feathers, wool, leather, skin, and bones) to obtain products with added value for another value chain has become the focus of a new bioeconomy, which takes into account the concepts of the circular economy. Therefore, the steam explosion technique can be a strong ally to achieve significant advances in this context.

Finally, when compared to other green protein extraction processes, such as ultrasound-assisted extraction, microwave-assisted extraction, subcritical water extraction, and high-pressure processing, where the systems may be delicate and the setups complex, requiring industrial-scale bulk treatment plants and posing challenges for safe and economical production (Noor et al. 2021), steam explosion stands out as a method already commonly used on an industrial scale, with simple and controllable parameters.

## Conclusions and perspectives

This review presented the steam explosion technique as a pretreatment to extract and improve protein functionality. The main raw materials studied were leather and hide wastes, feathers, wool, grains, and meals.

The main products obtained were low molecular weight peptides with antioxidant potential, gelatin suitable for the production of polymeric films, and grain and meal proteins with improved solubility and functional properties, promising for applications as food emulsion stabilizers.



Economic, social, and environmental aspects can be positively impacted by the use of the green technique of steam explosion, as well as the use of solid waste generated in the industrial and agro-industrial sectors containing protein compounds of added value.

As for perspectives, new studies are needed to more precisely elucidate the mechanisms and reactions involved during the steam explosion pretreatment, taking into account the raw material used and the variations in the process parameters. In addition, studies on the applications and safety of the obtained protein compounds are essential to promote significant advances in this context.

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**Data availability** Not applicable.

**Code availability** Not applicable.

## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethics approval** Not applicable.

**Consent to participate** The authors declare that they have contributed equally to the article.

**Consent for publication** Not applicable.

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