



# Strategies for Porcine Liver Valorization as a Source of Food Ingredients

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## Abstract

**Purpose of Review** The sustainable consumption and production goal and the decline of offal consumption have led to the interest in seeking alternatives for porcine livers, the largest edible gland. To that aim, we reviewed the potential of porcine livers as a source of food ingredients together with the use of eco-innovative processes and technologies for their valorization.

**Recent Findings** It is possible to extract and transform various compounds and fractions into food ingredients with tailored techno-functional properties using eco-innovative strategies involving microbial, enzymatic, physical, and chemical processes. These strategies can also contribute to improving the efficacy of different extraction and transformation processes as well as enhance sensory properties.

**Summary** Porcine liver is an interesting source of valuable compounds with multiple food applications and health benefits. Through extraction, processing, and transformation, these compounds can yield versatile food ingredients, thereby optimizing the profitability of this resource for human consumption through alternative presentations and potentially diminishing consumer reluctance compared to the whole liver.

**Keywords** Bioactive peptides · Protein hydrolysates · Techno-functional properties · Antioxidant activity

## Introduction

The growth in population increases the global demand for food and protein sources. To satisfy this demand and feed the world sustainably, it is paramount to make better and more responsible use of all available resources, as this contributes to reducing environmental impact [1]. Edible offal and other

non-edible parts (hereafter also referred to as co- and by-products, respectively) represent about 30% of a pig's live weight [2]. Therefore, achieving maximum utilization and valorization of all co- and by-products by the food, pharmaceutical, cosmetic, agricultural, and bio-energy industries can mitigate the negative impact of such production, promote a circular economy, and represent an opportunity for innovation and competitiveness in the meat industry.

Porcine offal can be considered an excellent food source and some of them are particularly rich in essential amino acids, vitamins, and minerals [3, 4]. Therefore, it is possible to take advantage of offal's nutritional properties by consuming it directly or incorporating it into a variety of food products. Liver and blood have been added to the elaboration of several traditional specialties such as pâté, haggis, and blood and liver sausages. Despite this, offal has a low commercial value nowadays, even though some and their derivatives can be considered delicacies depending on cultural heritage [5]. This low value is partly explained by the progressive decrease in the consumption of animal co-products throughout the 21st century in Europe [6]. For instance, in Spain, the ratio between the kg of consumed

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offal and that of meat decreased from 4.8 to 1.6% over the period 1990–2022 [7]. The main reasons for the negative attitude of Spanish consumers towards meat products containing liver and offal extracts seem to be mainly related with sensory aspects and health-related concerns [8]. However, this global decrease can be attributed to several factors affecting purchasing decisions, including dietary changes, increasing demand for convenient products [9], the risk of health hazards (e.g., bovine spongiform encephalopathy) [10], heavy metal accumulation such as Cd and Pb [11], and their association with low-income consumers [12].

Among offal, the liver is the largest viscera, which accounts for approximately 1.5% of a pig's live weight [4, 13]. The porcine liver is less popular among Western consumers compared to livers from other animals (lamb, calf, beef, or chicken) due to its less tender texture, fishy smell, and strong, earthy taste associated with high levels of amino acids such as glycine and glutamic acid. Metallic and bitter aftertastes have also been linked to the liver, which could be related to the presence of iron/blood and bile production, respectively, as well as a slightly sweet aftertaste. The imbalance between livers generated from pig slaughtering and those intended for direct consumption explains why part of porcine liver production ends up as pet food and animal feed. Therefore, processing porcine livers into bioactive and techno-functional food ingredients represents an interesting strategy with a greater economic impact. The use of liver and other offal ingredients in the formulation of different meat products can be easily accepted if they do not sacrifice sensory pleasure and provide clear and reliable information highlighting the positive aspects linked to their use. These issues and the approaches to address them have been summarized in Fig. 1. This review aims to summarize different strategies and technologies for the valorization of porcine livers as food ingredients.

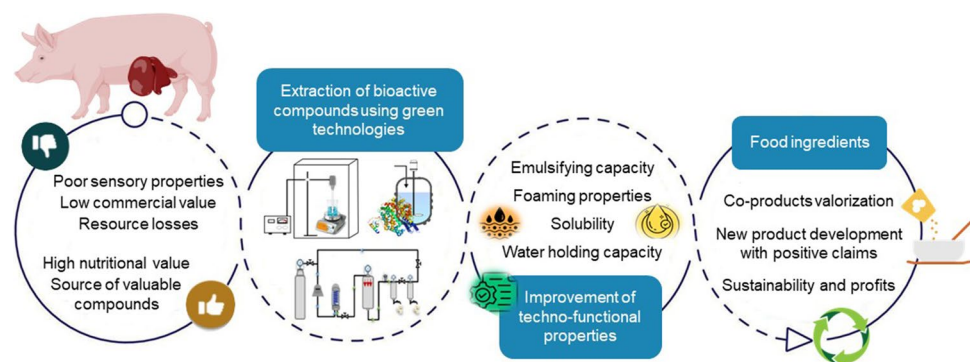
## Nutritional Value

As shown in Table 1, porcine liver can be considered a first-grade offal due to its nutritional value, protein content comparable to meat, and richness in vitamins and minerals [14, 15]. Protein contents of 180–190 g/kg are frequently found in the literature [16–18]. Relative to other edible animal by-products, the recovery of liver proteins is of particular interest since it contains all the essential amino acids and meets the requirements of the Food and

**Table 1** Chemical composition of pork liver (average value range)

Content	Range	References
Crude protein (g/kg)	180–220	[4, 16–18, 21]
Fat (g/kg)	30–40	[4, 18, 22]
Ash (g/kg)	13–14	[4, 18]
Mineral elements (mg/kg)		[4, 18, 23]
Macroelements		
P	2477	
Na	744–1269	
K	1415–3082	
Ca	70–75	
Mg	180–210	
Microelements		
Fe	148–211	
Zn	7–64	
Cu	5.5–14	
Mn	3–3.4	
Vitamins B (mg/100 g)		[4, 21]
B1	0.13–0.31	
B2	0.92–3.0	
B3	15–28	
B5	0.90–7.0	
B6	0.12–0.70	
B7	27–50	
B9	110–1000	
B12	25–40	

**Fig. 1** Strategies and advantages for porcine livers valorization as food ingredients



Agriculture Organization relative to the essential amino acids [4, 19, 20].

The lipid content of porcine liver is relatively low compared to that of beef but similar to that of the lean parts of pork meat [18, 21, 22]. Total unsaturated fatty acids account for around 53–56% of the total amount of fatty acids [4, 24] and include oleic acid (25–26%), linoleic acid (15–16%), linolenic acid (0.2–0.6%) and arachidonic acid (11%). Phospholipids are the most abundant lipid class, accounting for more than 75% of total lipids. Apart from egg yolk, porcine liver is one of the richest foods in phospholipids [25]. Phosphatidylcholine represents about 60% of total phospholipids [26]. This fraction can be recovered from porcine liver to develop stable food emulsions with properties similar to those made from other sources rich in phosphatidylcholine [27].

Regarding vitamins and minerals, porcine liver is abundant in vitamin A, with a content several times higher than that found in the meat and liver of other animals intended for human consumption [28, 29]. In addition, it contains a significant amount of vitamin B2, B3, B5, B6, B9, and B12, and in vitamin C, which is typically found in plant foods [4, 30]. The porcine liver also contains remarkable concentrations of macroelements such as phosphorus, calcium, magnesium, sodium, and potassium [4, 23]. However, porcine liver is particularly valuable as a dietary source of iron and zinc, the most abundant trace minerals in the human body. Although porcine liver is one of the richest foods in zinc [23, 31, 32], the amount of iron greatly exceeds that of zinc, whereas meat exhibits the reverse tendency [18, 21, 33]. Thus, it can be used to address iron deficiency, even though only about 20–25% of the content is present in the more easily absorbed heme form [34–36]. Despite this, it is worth noting that animal tissues and vitamin C enhance non-heme iron absorption [37, 38]. Porcine livers also contain important amounts of various microelements such as manganese, copper, chromium, and selenium [4, 23, 34, 39–41]. This nutritional richness explains the development of a powdered mixture from porcine liver, which has been used as an excellent fortifying food preparation [42].

## Compounds with Biological Activity

The porcine liver is involved in multiple metabolic reactions and can thus provide many compounds with biological activity, such as hormones and enzymes. Concerning enzymes, porcine lipases and esterases are of particular interest. Lipases are among the most used enzymes in various industrial sectors, including food, detergent/laundry, leather, textile, paper, and pulp processing industries. Their utilization to obtain free fatty acids, diacylglycerols, monoacylglycerols, and glycerol offers economic and

environmental advantages over traditional chemical hydrolysis methods. Additionally, lipases catalyze a wide range of reactions beyond hydrolysis, including esterification, inter-esterification, alcoholysis, acidolysis, and aminolysis. Therefore, many different applications within the food sector are possible [43–47]. Although microbial lipases are the most common choice for industrial applications, animal lipases can be recommended in certain circumstances. For instance, porcine pancreatic lipase has been used in synthetic applications where stereoselectivity and cost are critical factors [45, 48]. The most frequently employed esterase in synthetic procedures is porcine liver esterase, which contains multiple isoenzymes, because of its high stereoselectivity and hydrolytic activity [49, 50]. Porcine liver esterase is responsible for the activation/metabolism of drugs, including the hydrolysis of  $\beta$ -lactam antibiotics, thereby eliminating antibacterial activity, and detoxifying many other xenobiotics [50]. It is also used to catalyze the hydrolysis of pentaacetyl catechin and epicatechin for use in pharmaceutical and industrial applications [51]. The use of porcine liver aldehyde oxidase has been proposed to reduce the medium-chain aldehyde content responsible for the off-flavor associated with soy proteins [52]. Recently, it has been suggested as a source of enzymes for the hemisynthesis of phase II metabolites from anthocyanins, which are attributed to their bioactivity [53].

Regarding protein hormones, the liver secretes angiotensinogen, hepcidin, insulin-like growth factors 1 and 2, and thrombopoietin. Angiotensinogen is the precursor of angiotensin II and other angiotensin peptides, which regulate blood pressure and fluid homeostasis. Hepcidin, a liver-expressed iron-regulating hormone, also functions as a cysteine-rich antimicrobial peptide, exhibiting antifungal and antibacterial activity. Insulin-like growth factors 1 and 2 regulate growth, anabolic activities, metabolism, and aging. Thrombopoietin stimulates megakaryocyte differentiation and, thus, platelet production [54, 55].

Porcine livers are also rich in coenzyme Q10 and heparin, which could be considered for recovery. Coenzyme Q10 is the only endogenously synthesized fat-soluble antioxidant, with excellent free radical scavenging capacity, and the ability to regenerate other antioxidants such as vitamins E and C [56, 57]. It could be used as a supplement to prevent or treat heart failure, high blood pressure, and neurodegenerative diseases such as Parkinson's disease, diabetes, and cancer [58, 59]. As for heparin, it can be used as an anticoagulant to prevent blood clotting during surgery and in organ transplants and to prevent gangrene [60]. Although heparin was initially derived from dog liver cells, most of the heparin used worldwide is now derived from porcine sources.

Finally, the recovery of bile compounds should also be considered. Bile is a complex mixture secreted by the liver and stored in the gallbladder, consisting of bile acids, phospholipids, pigments, proteins, cholesterol, and some mineral

elements, among others. It is utilized for the treatment of indigestion, constipation, and bile tract disorders [60]. Bile from cattle or pigs can be purchased as a dry extract or in liquid form. Bile acids are antimicrobial compounds, with their activity depending on the degree of hydrophobicity and acidity [61]. It has been proposed to analyze its therapeutic potential to control drug-resistant bacteria [62]. Like probiotics or fecal transplantation, supplementation with specific bile acids has been suggested as a way to modify the intestinal microbiota [61]. They can also be used in animal feed to improve growth performance, antioxidant capacity, and nutrient digestibility [63]. Recently, it has been shown that porcine bile acids promote the utilization of fat and vitamin A in low-fat diets [64]. Moreover, bile acids, particularly conjugated bile acids (bile salts), are amphipathic molecules with high surface activity, able to be located at the air-water and lipid-water interfaces and self-assemble forming micelles [65]. Porcine bile phospholipids are found exclusively as phosphatidylcholine, which could be used as an emulsifier [66].

## Techno-Functional Protein Fractions and Nitrogenous Compounds

Water-soluble liver proteins represent more than 75% of total proteins [18, 67, 68]. Hemoglobin is the primary pigment in porcine liver. Consequently, this compound and the fractions containing it can be valorized similarly to those described in blood [69–71]. Moreover, the recovery of protein concentrates and fractions under different pH conditions can yield a variety of low-cost techno-functional food ingredients. Indeed, the pH-shifting technology is commonly employed in the recovery of proteins and production of surimi from fish by-products and low-value fish species [72]. The pH-shifting has been utilized to recover techno-functional proteins from goose and chicken livers [72, 73]. In all cases, extraction under appropriate conditions led to improvements in technological functionality. Ultrasound-assisted alkaline extraction has also been successfully applied to obtain protein isolates from chicken liver, resulting in increased protein yield and improved water/oil holding capacity and emulsifying properties compared to chicken liver protein isolate obtained by alkali extraction alone [74]. However, to the best of our knowledge, the use of pH-shifting on porcine liver has been primarily focused on determining its effects on nutritional quality [19]. Da Costa et al. [68] obtained freeze-dried porcine liver concentrates from the recovered fractions (soluble and insoluble) under strongly alkaline conditions but avoiding the isoelectric precipitation step. Both concentrates exhibited good emulsifying properties and demonstrated high water retention capacity when incorporated as ingredients in the formulation of a meat emulsion.

In porcine liver, salt-soluble proteins represent only a small fraction of the total protein [16, 67]. However, since NaCl is a common ingredient in many food formulations, its effects on the techno-functionality of the recovered proteins have been studied [16]. These authors examined the techno-functional properties of water-soluble and salt-soluble porcine liver proteins and found that both fractions exhibited relatively poor gelling properties. However, they demonstrated similar emulsifying properties to those of Na caseinate and served as effective foam stabilizers. Previous research reported that techno-functional properties of recovered proteins from turkey liver were enhanced by the addition of NaCl during the extraction step [75].

More recently, Feliu-Alsina and Sauer [76] focused on obtaining protein extracts with good foaming properties from ground porcine liver under mild extraction conditions. These protein extracts offer an alternative to traditional foaming agents used in food processing, such as egg whites, milk proteins, gluten, and soy proteins, which are highly allergenic [77]. According to these authors, protein extracts recovered by using buffer solutions adjusted to pH 4.0–4.75 produced voluminous and stable foams with a neutral odor thus facilitating multiple applications. Despite the relatively low yields achieved under these conditions (31.5–36.0% of total protein), the difficulty of obtaining good and affordable non-allergenic food foaming agents makes this option very interesting.

However, the non-protein fraction contains other nitrogenous compounds of technological interest. It is worth mentioning that porcine liver is also rich in nucleotides and purines, which are responsible for the umami taste and can decrease bitterness while enhancing saltiness [78]. Umami has also been shown to improve appetite and satiety [79]. The liver ranks as the second organ with the highest purine content, surpassed only by the lung, and its purine content far exceeds that of muscle tissue [25]. In contrast, the purine content of porcine liver is lower compared to that of chicken and duck livers [80]. Peptides, amino acids, amino acid derivatives, nucleotides, and purines are important taste-active and taste-enhancer compounds [81, 82]. López-Martínez et al. [83] compared porcine liver, kidney, lung, and brain as raw materials for obtaining flavor-related food ingredients. These authors reported that all four organs serve as excellent sources of sweet and umami flavor-related free amino acids. Moreover, the presence of these amino acids seems more relevant than that of potentially unpleasant ones (bitter and sulfurous).

## Tailoring Techno-Functional Properties

### Protein Hydrolysates

In addition to the high biological value of liver proteins, they can serve as a potential source of hydrolysates with



high nutritional and techno-functional value [84]. Partial or total hydrolysis methods can be used to obtain these protein hydrolysates utilizing acid, alkaline, enzymatic, and microbial processes [85]. For instance, the yeast *Monascus purpureus* has been used to obtain antioxidant hydrolysates from porcine liver with potential applications for preventing lipid oxidation in food products [86]. Presently, most research efforts concentrate on enzymatic hydrolysis due to its efficacy in breaking down protein structure and the wide range of enzymes available. Moreover, the mild processing conditions and the high specificity of the enzymes used in enzymatic hydrolysis enable the minimization of undesirable effects observed in acid and alkaline hydrolysis and microbial fermentation. These effects are primarily associated with the presence of organic solvents or toxic substances and the degradation of amino acids thus conditioning the activity of the resulting hydrolysates [87, 88].

Enzymatic hydrolysis with proteases, also called peptidases, results in a complex mixture of peptides and amino acids. Depending on the site of the peptide bond hydrolysis, enzymes can be classified as exopeptidases or endopeptidases. Exopeptidases target the terminal region of the polypeptide chain, releasing the terminal amino acid, while endopeptidases act on the peptide bonds located in the center of a protein molecule. Furthermore, enzymes used during hydrolysis can be naturally present in the substrate (endogenous or digestive enzymes) or exogenous (commercial enzymes) [89]. The utilization of exogenous enzymes (e.g., alcalase, bromelain, flavourzyme, pepsin, papain) allows a more controlled and reproducible hydrolysis process. This is why they are commonly used to produce food-grade protein hydrolysates [90].

The functionality of the obtained hydrolysates relies on their amino acid composition, molecular weight, and peptide sequence, which are directly influenced by hydrolysis conditions such as temperature, pH, and incubation time, as well as the degree of hydrolysis [91]. In addition, the enzyme/substrate ratio, enzyme specificity, and deactivation also play crucial roles [92]. Studies conducted to date have indicated that the type of enzyme primarily determines the effectiveness of hydrolysis [93]. In this connection, Verma et al. [94] observed that the percentage of hydrolyzed peptides depends on the enzyme and was higher in samples hydrolyzed with trypsin > alcalase > papain (26.82% vs. 23.56 and 19.12%, respectively). These values tend to increase linearly with hydrolysis time. Typically, the enzymatic reaction is rapid in the initial stage and then slows down over time due to a lower enzyme/substrate ratio and competitive inhibition between the non-hydrolyzed protein and the peptides that are formed during hydrolysis [94].

Different techno-functional properties can be improved with hydrolysis. For instance, it may be used to increase proteins' solubility facilitating their utilization in food

processes. In this connection, proteins' solubility may also affect other techno-functional properties, such as emulsifying and foaming capacities. Porcine liver hydrolysates also represent an alternative to synthetic preservatives for extending the shelf-life of foods [84, 95]. Amino acids released during hydrolysis, particularly aromatic (tyrosine and phenylalanine) and hydrophobic (leucine, valine, and isoleucine) amino acids, as well as those associated with antimicrobial properties such as arginine, cysteine, glycine, histidine, and proline, are of particular interest [96]. Prolonged enzymatic reaction times result in greater protein degradation, leading to the release of more peptides and amino acids [97]. For instance, the use of flavourzyme increased the content of free amino acids during the hydrolysis of porcine liver from 4562 mg/100 g of pork liver at 4 h to 7752 mg/100 g of pork liver at 10 h. Similar trends were observed with phenylalanine, leucine, valine, and isoleucine. These findings agree with those observed by López-Pedrouso, Borrajo, et al., [98]. However, when the hydrolysates obtained with flavourzyme were compared to those obtained with alcalase, a greater amount of free amino acids was observed [96]. This enhanced efficacy of flavourzyme could be related to its mixture of endo- and exopeptidases, which confer a greater capacity to hydrolyze peptide bonds compared to enzymes containing only one type of protease and thus producing a higher number of small peptides. In contrast, the proteolysis index indicates that alcalase exhibits the highest activity (82.56%, 70.37%, 63.55%, and 59.20% for alcalase, bromelain, flavourzyme, and papain, respectively) [99]. Alcalase is one of the most used enzymes due to its broad specificity and its ability to produce hydrolysates in a short period [100]. Verma et al. [94] evaluated the antioxidant activity of protein hydrolysates extracted from porcine liver using alcalase, papain, and trypsin (Table 2). Samples hydrolyzed with trypsin showed the highest antioxidant activity, which could be related to the higher degree of hydrolysis obtained in these hydrolysates (26.82% vs. 23.56% and 19.12% for trypsin, alcalase, and papain, respectively). Regarding ABTS, trypsin hydrolysates demonstrated higher activity, with values of 86.8% compared to those obtained with alcalase and papain, which showed values below 75%. Similarly, in the case of DPPH, trypsin hydrolysates displayed values of 57.5%, whereas hydrolysates obtained with alcalase and papain yielded values below 50%.

The molecular weight of the peptides within the hydrolysates could also determine their activity. In this regard, the filtration significantly impacts the antioxidant activity of the hydrolysates, although the behavior depends on the enzymatic hydrolysis that has been carried out. Several studies indicate that whole hydrolysate samples exhibit notably higher antioxidant activity compared to their fractions, potentially due to a higher concentration of peptides and/or their synergistic effects [102]. However,

**Table 2** Antioxidant activity of bioactive peptides isolated from porcine liver after enzymatic hydrolysis

Enzyme	E/S	Incubation conditions	Deactivation	Antioxidant assays					Reference
				ABTS	DPPH radical scavenging activity	FRAP	Fe <sup>2+</sup> chelating ability	ORAC (Trolox equivalents)	
Alcalase	1:100 (w/w)	pH=8.0, 50 °C	95°C, 3 min	1068 mg ascorbic acid/100 g	562 µg Trolox/g	82.9 µmol Fe <sup>2+</sup> /100 g	n/a	53.2 mg Trolox/g	[97]
Bromelain		pH=6.0, 40 °C		392 mg ascorbic acid/100 g	427 µg Trolox/g	69.8 µmol Fe <sup>2+</sup> /100 g	n/a	45.2 mg Trolox/g	
Papain		pH=6.0, 37 °C		416 mg ascorbic acid/100 g	325 µg Trolox/g	57.0 µmol Fe <sup>2+</sup> /100 g	n/a	44.3 mg Trolox/g	
Flavourzyme	1:100 (w/w)	pH=5.5, 50 °C	95°C, 3 min	497 mg ascorbic acid/100 g	970 µg Trolox/g	48.4 µmol Fe <sup>2+</sup> /100 g	n/a	33.7 mg Trolox/g	[101]
Alcalase	1:1000 (w/w)	55°C, 2 h	95°C, 10 min	79.2%	9.9%	n/a	92%	n/a	[131]
Papain	1:100 (w/w)	pH 6.5, 50°C, 6 h	85°C, 15 min	70.6%	40.3%	12.7 mM equivalent FeSO <sub>4</sub> ·7H <sub>2</sub> O	n/a	n/a	[94]
Alcalase		pH 8.0, 50°C, 6 h		74.6%	42.3%	13.7 mM equivalent FeSO <sub>4</sub> ·7H <sub>2</sub> O	n/a	n/a	
Trypsin		pH 8.0, 37°C, 6 h		86.8%	57.5%	14.9 mM equivalent FeSO <sub>4</sub> ·7H <sub>2</sub> O	n/a	n/a	
Papain	1:100 (w/w)	pH 6.5, 50°C, 6 h	85°C, 15 min	71.2%	39.2%	11.9 mM equivalent FeSO <sub>4</sub> ·7H <sub>2</sub> O	n/a	n/a	[102]
Alcalase		pH 8.0, 50°C, 6 h		75.9%	41.8%	12.3 mM equivalent FeSO <sub>4</sub> ·7H <sub>2</sub> O	n/a	n/a	
Trypsin		pH 8.0, 37°C, 4 h		86.8%	56.7%	14.4 mM equivalent FeSO <sub>4</sub> ·7H <sub>2</sub> O	n/a	n/a	
Papain	1% (v/w)	pH 6.5, 37°C, 12 h	95–100°C, 10 min	n/a	37%	n/a	n/a	n/a	[86]
Pepsin		pH 3.0, 37°C, 12 h		n/a	55%	n/a	n/a	n/a	
Alcalase		pH 8.0, 50°C, 12 h		n/a	42%	n/a	n/a	n/a	

E/S enzyme/substrate ratio, n/a data not available

controversial results have been found on this issue. For instance, Verma et al. [102] observed that the fraction with the highest activity corresponded to 1–5 kDa, whereas Borrajo, Pateiro, et al., [97] found that larger molecular weights resulted in a higher level of inhibition. Hydrolysates obtained using alcalase demonstrated increased activity with larger molecular weights (562, 542, and 443 µg Trolox/g for DPPH when molecular weight cut-off of 30, 10, and 5 kDa were used, respectively). This trend was also observed for ABTS, FRAP, and ORAC assays. These results were also in line with those found with

flavourzyme [101]. In contrast, the intermediate molecular weights yielded the best results for bromelain and papain.

In addition to antioxidant activity, hydrolysates may exhibit other activities such as antimicrobial and angiotensin I-converting enzyme (ACE) inhibitory activities, making them suitable for use in the formulation of meat products [101–103]. Moreover, protein hydrolysis releases amino acids, which, as mentioned earlier, can contribute to flavor. Thus, the liver, in addition to other organs, can serve as a substrate for enzymatic protein hydrolysis to produce taste-related peptides [83]. Furthermore, certain amino acids

enhance the solubility of myosin under low ionic strengths [81]. This strategy is particularly relevant when seeking substitutes for NaCl in meat products, as improving myosin functionality is crucial for ensuring desirable gel and emulsion properties, and overall meat product quality.

### Color Compounds: Zn-Protoporphyrin

The color of meat products stands as one of the most important attributes as it influences consumers' purchasing decisions [104]. The stabilization of the red color in a wide variety of meat products is typically achieved through the addition of nitrites. However, their usage remains controversial as, under certain conditions such as low pH and high temperatures, they can react with secondary amines, forming N-nitrosamines with potential carcinogenic effects [105]. Therefore, there is considerable interest in exploring alternative strategies to produce nitrite-free and clean-label meat products while maintaining their characteristic red color. In this context, an interesting strategy is based on the formation of the red pigment Zn-protoporphyrin, which is typically found in dry-cured Parma hams and shows great stability to light and heat [106, 107]. The formation of this pigment is mainly attributed to the enzyme ferrochelatase, which progressively forms Zn-protoporphyrin from heme proteins during the long elaboration time of dry-cured hams. However, in other meat products, the formation of Zn-protoporphyrin entails some difficulties, primarily due to the shorter elaboration process, which usually lasts hours instead of several months. Thanks to the high endogenous enzyme activity of ferrochelatase, porcine livers have been proposed as raw material to obtain a coloring ingredient based on the formation of Zn-protoporphyrin [108]. The coloring capacity of this Zn-protoporphyrin-based ingredient has been examined in the production of nitrite-free sterilized pâtés, resulting in similar color and thermal stability to conventional pâtés elaborated with nitrifying salts [107].

An alternative strategy could involve the extraction of ferrochelatase and the intensification of its activity. Traditional methods of enzyme extraction and enzymatic activity are generally slow and, in some cases, inefficient. In this regard, the use of innovative technologies, such as ultrasounds, could enhance performance and speed in enzyme extraction processes [109, 110] as well as in enzymatic reactions to yield better results [111]. The effectiveness of ultrasound application for intensifying ferrochelatase extraction from pork liver has been addressed by comparing the kinetics of Zn-protoporphyrin formation, with ferrochelatase fractions obtained through conventional extraction, without the application of ultrasounds, and by ultrasound-assisted extraction [112]. Ultrasound application improved the rate Zn-protoporphyrin formation of the enzyme extract by up to 33.3% compared with conventional extraction. Although ultrasound

treatments did not increase the amount of extracted ferrochelatase, they enhanced its enzymatic activity, possibly due to structural changes favoring substrate location in the active site and product diffusion. Moreover, ultrasounds can expedite the conversion of heme compounds into Zn-protoporphyrin. Low and moderate ultrasound powers notably enhanced enzymatic activity in pork liver homogenates, both with and without the addition of oxyhemoglobin [113]. These authors reported that the effect of ultrasounds depended mainly on the applied ultrasonic power. Moreover, ultrasound application at low power (7.05 W/L) proved effective in intensifying the enzymatic reaction and increasing the Zn-protoporphyrin concentration compared to the conventional method without ultrasound application, halving the time needed to reach the maximum Zn-protoporphyrin concentration.

### Odor and Flavor Compounds

The Maillard reaction induces protein changes during thermal processing, contributing to the final color and aroma of the product. Liver taste and volatile profile can also be improved via Maillard reactions [114]. In their study, these authors compared the effect of Maillard reactions with xylose on chicken liver proteins, hydrolyzed chicken liver proteins, and hydrolyzed chicken liver proteins exposed to ultrasounds. Overall, the number of flavor substances significantly increased when the different protein sources were exposed to Maillard reactions. They also reported that the ultrasound pretreatment increased the degree of brown coloring and the content of flavor precursors in the hydrolyzed chicken liver after the Maillard reaction. Moreover, in sonicated hydrolyzed chicken protein, volatile compounds were increased while bitterness and astringency characteristics decreased.

Microbial fermentation stands as one of the oldest methods of food transformation and preservation, often imparting a complex and highly appreciated taste and odor to fermented foods due to amino acid metabolism. Lactic acid bacteria, *Micrococccaceae*, and surface molds are typically used in the elaboration of different meat products [82]. Liver and other co-products may possess unpleasant odors and flavors [115, 116]. Volatile analysis of porcupine livers revealed the presence of compounds such as hexanal, heptenal, and 1-octen-3-ol that contributed to the off-flavor of this product [116]. These authors compared the effectiveness of removing these undesired compounds by adding cyclodextrins and yeasts, finding that fermentation with yeast was more effective than the addition of cyclodextrins, although off-flavor compounds were not completely removed. Luo et al., [115] identified the cattle's heart, liver, lung, rumen, and intestine volatiles and compared four methods: shallot-ginger extract masking, baker's yeast fermentation,

active dry yeast, and  $\beta$ -cyclodextrin composite, and ultrasound-treated chitosan composite. While the ultrasound-treated chitosan composite was the most effective method for deodorization, yeast fermentation significantly reduced the content of characteristic flavor compounds of each cattle by-product. Furthermore, Liu et al., [117] investigated the effect of fermentation on the physical properties and aroma of lamb liver paste. The authors compared the fermentation with starter cultures (*Staphylococcus xylosus* and *Pediococcus pentosaceus*), natural fermentation without starters, and a sterilized liver paste control. Fermentation with starter cultures led to a new product with lower pH, different aroma compounds, and decreased fat oxidation during storage. In addition, instrumental brightness, redness, and yellowness values were higher in the fermented lamb liver paste with cultures. The appropriate selection of starter cultures can also reduce the formation of bitter peptides [82]. Therefore, microbial fermentation can alter the aromatic profile of livers, enabling the production of new products and ingredients with an improved and more desirable flavor.

### Other Techno-Functional Properties

Apart from odor and flavor, the applicability of liver proteins can be improved by enhancing their techno-functional properties. While much attention has been given to enhancing the techno-functional properties of plant proteins in recent years [118, 119], less focus has been placed on improving animal proteins including liver proteins. However, physical, chemical, and biological approaches can be used to functionalize proteins regardless of their origin. Protein solubility is an important property for many food applications, significantly influencing other functional properties. Chemical modifications like succinylation can help to improve protein solubility, emulsifying properties, and foaming capability by altering protein structure. For instance, Lu, Pan, et al., [120] studied the effects of succinylation modification on the structural and emulsifying properties of isolated chicken liver proteins, obtained via the pH-shift method, under different pH conditions. The authors reported that the functional attributes of chicken liver proteins (e.g., solubility and emulsifying properties) can be modulated by the pH values during the succinylation process as well as to improve the color and smell of the product. Later, Lu, Cao, et al., [121] combined xanthan gum with succinylated chicken liver protein to form a complex used as a back-fat replacer in the formulation of emulsified sausages. The authors reported that low-fat emulsified sausages with the addition of fat mimetic exhibited similar microstructure and texture to conventional high-fat sausages, demonstrating the technological feasibility of simultaneously improving the quality and nutritional characteristics of emulsified sausages while

leveraging the valorization and techno-functional properties of liver proteins.

### Stabilization and Sensory Properties Improvement

Drying serves as an economical preservation technique, enhancing product stability while reducing weight and volume, thus streamlining the processing and transportation of pork liver [122]. However, the water removal process of pork liver is energy-costly and time-consuming, which justifies its intensification using alternative technologies such as airborne ultrasonic application during hot air-drying of pork liver [123, 124]. In this regard, Sánchez-Torres et al., [124] reported that the air-borne application of ultrasounds shortened the drying time by up to 40% at 30 °C. Nevertheless, the protein solubility remained constant for liver dried from 30 to 60 °C, while drying at 70 °C led to a large reduction in the soluble protein fraction. Therefore, the application of ultrasounds during hot air-drying (< 70 °C) of pork liver could be interesting to obtain functional protein. In addition, it was found that convective drying process at moderate temperatures close to room conditions (10–20 °C) can be an effective method for preserving ferrochelatase activity since it was possible to stabilize the liver and minimize the loss of ferrochelatase activity [125].

The stability and potential application of pork liver can benefit from defatting and deodorizing processes as they may contribute to making it more palatable and acceptable to consumers while extending its shelf life. For this reason, the use of supercritical CO<sub>2</sub> and conventional vacuum steam distillation methods to minimize volatile organic compounds was investigated [126]. Vacuum steam distillation showed a higher affinity for reducing or eliminating sulfur compounds, furans, and ketones, while supercritical CO<sub>2</sub> showed greater effectiveness in reducing or eliminating acids, alcohols, aldehydes, halogenated compounds, nitrogenous compounds, esters, and hydrocarbons. As a result, compounds responsible for the off-flavor in pork liver, such as (E, E)-2,4-heptadienal (fishy), 1-octen-3-ol (mushroom), and 1-nonanol (fatty and green), can be efficiently removed by supercritical CO<sub>2</sub>. Moreover, the fat content of the samples treated with supercritical CO<sub>2</sub> was lower compared to non-CO<sub>2</sub> samples [126]. In this sense, supercritical CO<sub>2</sub> can be used for fat extraction in foods with high protein content as an alternative to other conventional techniques [127].

However, the extracted lipids can have an undesirable odor and relative instability during processing and storage. For these reasons, the encapsulation may be of interest as this technology is widely used to mask flavor, color, and odor. Indeed, the formulation of meat products with encapsulated liver oils improved the sensory characteristics of



the product and minimized oxidation throughout the storage [128, 129]. Encapsulation is also used for the controlled release and protection of bioactive and labile compounds. As described earlier, enzymatic hydrolysis is the most convenient and widely used method to obtain peptides with the desired functionality and/or biological effects. In some cases, it may be necessary to protect these bioactive peptides from thermal treatments, storage, and physiological modifications to obtain the benefits. For this reason, de Souza et al., [130] studied the encapsulation of porcine liver hydrolysate using different coating materials (Arabic gum, maltodextrin, whey protein, a combination of whey protein with maltodextrin, xanthan gum, and soy protein isolate) and two encapsulating methods (spray-drying and freeze-drying). The encapsulated peptides were exposed to gastrointestinal simulation and found that encapsulation was effective in preserving the antioxidant and antimicrobial activities of the pork liver hydrolysate.

## Conclusions

The porcine liver is recognized as an excellent source of nutrients and compounds with diverse applications in food and other industrial sectors. Through conventional and innovative methods, such as high-power ultrasounds and supercritical fluid extraction, these compounds, along with lipid and proteinaceous fractions, can be effectively extracted from the liver. These techniques not only improve processing efficiency but also offer environmentally friendly solutions. As illustrated in Fig. 1, the extracted compounds and fractions can be directly utilized or further modified to enhance specific techno-functional properties like solubility, color, and emulsifying capacity. Similar strategies can be applied to other offal and co-products, promoting their valorization. The resulting ingredients offer various benefits, including reducing the reluctance towards the consumption of whole viscera and replacing allergenic ingredients like soy protein isolates and additives such as nitrites. Consequently, the development of these ingredients and their use in reformulation processes and new product development with positive claims (e.g., clean label, allergen-free) present opportunities for innovation and competitiveness. However, it is essential to assess the profitability and consumer acceptance of final products in each case. The valorization of porcine livers and offal as food and food ingredients adheres to the principles of the circular economy and contributes to sustainable consumption and production patterns.

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## Declarations

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

**Competing Interests** The authors declare no competing interests.

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