New insights into meat by-products utilization

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Abstract

Meat industry generates large volumes of by-products like blood, bones, meat trimmings, skin, fatty tissues, horns, hoofs, feet, skull and viscera among others that are costly to be treated and disposed ecologically. These costs can be balanced through innovation to generate added value products that increase its profitability. Rendering results in feed ingredients for livestock, poultry and aquaculture as well as for pet foods. Energy valorisation can be obtained through the thermochemical processing of meat and bone meal or the use of waste animal fats for the production of biodiesel. More recently, new applications have been reported like the production of polyhydroyalkanoates as alternative to plastics produced from petroleum. Other interesting valorisation strategies are based on the hydrolysis of by-products to obtain added value products like bioactive peptides with relevant physiological effects as antihypertensive, antioxidant, antidiabetic, antimicrobial, etc. with promising applications in the food, pharmaceutical and cosmetics industry. This paper reports and discusses the latest developments and trends in the use and valorisation of meat industry by-products.

Keywords: animal by-products, meat by-products, offal, skin, bones, trimmings, bioactive peptides, hydrolysed proteins, biodiesel

1. Introduction

Meat industry generates large volumes of by-products like blood, bones, meat trimmings, skin, fatty tissues, horns, hoofs, feet, skull and viscera among others that are costly to be treated and disposed ecologically (Ryder, Ha, El-Din Bekhit and Carne, 2015). These costs can be balanced through innovation to generate added value products that increase its profitability. On the other hand, unappropriated treatment or handling of such by-products raised relevant crisis in the past such as the spread of the spongiform encephalopathies. The European Commission published the Regulation (EC) 1069/2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) 1774/2002. Later, the European Commission published the Regulation (EC) 142/2011 that was implementing the Regulation 1069/2009. Rules were also provided by the Food and Drug Administration (FDA, 2004) to prevent the establishment and spread of bovine spongiform encephalopathy (BSE) in the United States, including a prohibition on the use of high-risk, cattle-derived materials that can carry the BSE agent which are defined as specified risk material. This means that adequate disposal of by-products may increase the cost to processors and makes necessary to produce new substances or products capable to cover the disposal costs (Toldrá, Mora, Aristoy and Reig, 2012).

It must be taken into account that certain meat by-products can be considered as foods of interest depending on the country and local traditions while in other places they can be considered as inedible foods (Ockerman & Basu, 2004a). In fact, some by-products with high nutritional value like blood, liver, lung, heart, kidney, brains, spleen and tripe constitute part of the diet and culinary recipes in many countries worldwide (Nollet & Toldrá, 2011). Of course, the nutritional composition depends on each particular type of by-product and the animal species from which they are obtained (Honikel, 2011). Other by-products like lard may be used for cooking.

Meat by-products may constitute a valuable resource if handled properly to produce added value substances or products (Zhang, Xiao, Samaraweera, Lee & Ahn, 2010, Toldrá and Reig, 2011). Efficient use of by-products may arise up to 11.4% and 7.5% of the gross income of beef and pork (Jayathilakan, Sultana and Radhakrishna, 2012).

There is a large variety of meat by-products but, in general, most of them contain good amounts of nutrients like essential amino acids, minerals and vitamins (Aristoy & Toldrá, 2011, Honikel, 2011, Kim, 2011), constituting good valorization opportunity for the meat industry (Valta, Damala, Orli, Papadaskalopoulou, Moustakas, Malamis and

Loizidou, 2015). There are numerous applications based on new or improved technologies for processing meat by-products like edible food ingredients for the food, feed and pet food industry (see Figure1). Meat by-products can be considered as raw materials for the generation of biomolecules of interest like protein hydrolysates with relevant bioactivities or enzymes (Lasekan, Abu Bakar and Hashim, 2013), extracts with functional properties (Chernukha, Fedulova and Kotenkova, 2015) or bioactive peptides (Mora, Reig and Toldrá, 2014; Martínez-Alvarez, Chamorro and Brenes, 2015).

Other applications are addressed towards inedible products like fertilizers, substances of interest for the chemical or pharmaceutical industry or energy generation (see Figure 1). Energy generation is an active area mainly focused on the biodiesel production from waste animal fats (Banckovic-Illic, Stojkovic, Stamenkovic and Veljkovic, 2014; Adewale, Dumont and Ngadi, 2016) or even a second generation of bioderived diesel fuel, also known as bio gas oil (Balandincz and Hancsók, 2015).

This manuscript reports and discusses the latest developments and trends in the use and valorisation of meat industry by-products.

2. Food applications

Applications as functional ingredients

Bioactive peptides are sequences usually between 2 and 20 amino acids that exert a biological function in one or several of the physiological systems in human being. In this sense, hypocholesterolemic, antioxidant and antithrombotic peptides have been described to modulate the cardiovascular system whereas mineral binding and immunomodulatory peptides act in gastrointestinal and immune systems, respectively. Some groups of peptides are able to participate in multiple system reactions. Thus, opioid agonist and antagonists can act on nervous, gastrointestinal, and immune systems, whereas antimicrobial peptides can modulate gastrointestinal and immune systems (Lafarga and Hayes, 2014).

Bioactive peptides need to be liberated from their origin protein in order to exert the biological function as they are inactive within the parent protein (Vercruisse, Van Camp, and Smagghe, 2005). Some bioactive peptides are released during food processing either in fermentation or curing stages, whereas others are generated during gastrointestinal digestion. The main problem of naturally generated peptides is the difficulty in controlling the hydrolysis conditions because many endogenous enzymes are acting at the same time and a wide profile of peptides showing different sizes and characteristics is generated (Mora, Gallego, Escudero, Reig, Aristoy & Toldrá, 2015). For this reason, the digestion of protein extracts under controlled hydrolysis conditions using known enzymes such as alcalase, pepsin, thermolysine, trypsin, etc., allows the control of the generated bioactive peptides as well as the obtention of more homogeneous batches.

The use of by-products as a source of bioactive peptides has been extensively studied during the last years. In this sense, blood and collagen, very important by-products from slaughterhouses and meat industry, have been the most assayed (Ryder, El-Din Bekhit, McConnell and Carne, 2016).

Blood is a rich source of proteins where hemoglobin, an iron-containing protein, is the most abundant complex (Ofori and Hsieh, 2014). It is obtained all around the world and even though is used as food ingredient in Europe, Asia, and Africa, its production is more copious than needed. Its value as a source of bioactive peptides has been studied in both the cellular fraction (hemoglobin cells) and the plasma fraction, and their hydrolysates have been described to exert antimicrobial, antioxidant, ACE-inhibitory, and opioid activities (Chang, Wu and Chiang, 2007). However, antimicrobial peptides derived from hemoglobin hydrolysates have been the most studied (Nedjar-Arroume et al., 2004; Marya, Kouach, Briand and Guillochon, 2005; Briand and Guillochon, 2006, 2008). Bovine hemoglobin hydrolysate obtained with pepsin in the presence of 30% ethanol resulted in the novel identification of 67-106, 73-105, 99-105, and 100-105 fragments of the α-chain of bovine hemoglobin. These peptides exert an antibacterial activity against *Kocuria luteus A270*, *Listeria innocua*, *Escherichia coli*, and *Staphylococcus aureus* with a MIC between 187.1 and 35.2 µM as well as an ACE inhibitory activity with IC_{50} values from 42.55 to 1,095 µM (Adje et al 2011a). On the other hand, Hu et al. (2011) identified the peptide VNFKLLSHSLLVTLASHL from α-chain bovine hemoglobin showing antimicrobial activity against *E. coli*, *S. aureus*, and *Candida albicans* when assessed. The minimal peptide sequences necessary to show antimicrobial activity after a pepsin enzyme digestion of α- and β-chain hemoglobin proteins have been described to be KYR and RYH, respectively, and were studied against *E. coli*, *Salmonella enteritidis*, *L. innocua*, *Micrococcus luteus*, and *S. aureus* (Catiau et al 2011a, 2011b). The sequences obtained from blood protein hydrolysates in recent years are shown as Table 1.

The generation of bioactive peptides depends to a high extent on the enzymes and substrate used in the hydrolysis. In fact, the hydrolysis degree determines the extent of hydrolysis whereas the digestion conditions (temperature, pH, and time) are very important to obtain the bioactive peptides. On the other hand, peptide size and amino acid sequences are crucial for the bioactive potential of the peptides (Yu, Hu, Miyaguchi, Bai, Du and Lin, 2006). As an example, antimicrobial peptides have been shown to be mostly hydrophobic as higher hydrophobicity is necessary in the affinity with the outer membrane of microbials. In fact, there is an interaction with negatively charged membrane phospholipids by tyrosine residues together with arginine and lysine which can act as peptide anchors in membranes (Lopes, Fedorov and Castanho, 2005). ACE-inhibitory peptides, also well-studied in hemoglobin hydrolysates, have been described to contain proline, lysine or aromatic residues. In fact, ACE binding is influenced by a proline residue at any of the three last positions of the C-terminal site. Antimicrobial and ACE-inhibitory peptides derived from bovine and porcine hemoglobin and plasma have been described in Table 1. Some opioid peptides with potential to have an effect on nervous and gastrointestinal systems have also been described from animal blood sources (Zhao et al., 1997, 1994; Kapel et al., 2003; Froidevaux et al., 2008). However, there is a lack of studies about the antioxidant capability of hemoglobin-derived peptides.

Collagen is the most abundant protein in many by-products obtained from meat industry. In fact, it is the main constituent in skin, hide, bones, and cartilages. The nutritional value of collagen is very low because it lacks essential amino acids but, on the other hand, collagen is very useful as a source of bioactive peptides (Morimatsu, 2008, Dierckx and Smagghe, 2011). Despite many recent studies have been focused on the bioactive properties of collagen hydrolysates, most of the published studies have been focused on fisheries by-products. In collagen hydrolysates, ACE-inhibitory and antioxidant activities resulted to be the most relevant when enzymes such as alcalase, trypsin, chymotrypsin, neutrase, flavorenzyme, pepsin, bromelain and papain were used (Saiga et al., 2008; Gómez-Guillén et al. 2011; Di Bernardini, Mullen, Bolton, Kerry, O'Neill & Hayes, 2012). In this sense, Herregods et al (2011) reported that thermolysin hydrolysate showed the highest *in vitro* ACE inhibitory activity as well as an important *in vivo* antihypertensive effect in spontaneously hypertensive rats. Recently, a MALDI-ToF mass spectrometry methodology has been used to determine the animal origin from collagen trypsinated peptides in food preparations and galenic formulations. The differentiation between pork and bovine gelatin was performed through the mass spectra (Flaudrops et al., 2015).

Technological applications

The cellular fraction that contains red blood cells, white blood cells and platelets, can be used as colour enhancer for sausages even though it has limited applications in foods due to the dark colour of hemoglobin, sensory adverse effects or even hygiene (Ofori & Hsieh, 2011). Better flavor can be obtained if hemoglobin is removed and used to replace fat in meat products (Viana, Silva, Delvivo, Bizzotto & Silvestre, 2005).

A heme iron polypeptide that helps for a better iron absorption can be generated through enzymatic hydrolysis of hemoglobin (Nissenson, Berns, Sakiewickz, Ghaddar, Moore & Schleicher, 2003).

Interesting technological properties for food processing can be obtained from blood proteins (Hsieh and Ofori, 2011). So, immunoglobulins, fibrinogen and serum albumin contribute to gelation and emulsification (Cofrades, Guerra, Carballo, Fernández-Martín & Jiménez-Colmenero, 2000) while other plasma proteins contribute to proteins cross-linking (Kang & Lanier, 1999), proteins enrichment (Yousif, Cranston and Deeth, 2003) or foaming (Del, Rendueles and Díaz, 2008). High antioxidant activity has been reported in red blood cell fractions from sheep, pig, cattle and red deer (Bah, Bekhit, Carne and McConnell, 2016). Also, antimicrobial activity against *E. coli*, *S. aureus* and *P. aeruginosa* was reported in sheep white blood cells (Bah et al., 2016).

The enzyme thrombin and fibrinogen are used for binding of meat pieces and, for instance, reconstitute meat steaks or generate meat emulsions increasing the hardness and springiness. Fibrinogen is converted by thrombin into insoluble fibrin that form fibers by aggregation. The final results is a three-dimensional network fibrin clot (Lennon, McDonald, Moon, Ward & Kenny, 2010) with more or less strength depending on the size and moisture of the pieces and the conditions of pH and temperature used (Chen & Lin, 2002). Thrombin and fibrinogen are registered under the trade mark Fibrimex® and commercialised as a binder for meat processing to manufacture restructured meat products.

Gelatin is obtained from collagen through hydrolysis and is widely used in the food industry because of its good gel-forming ability, but also as clarifying agent, stabiliser or protective coating material (Djagny, Wang & Xu, 2001; Gómez-Guillen et al., 2011).

Animal rendering yields proteins that can reduce the surface tension and produce foams (Bressler, 2009). Protein hydrolysates are also used as flavor ingredients; their sensory properties depending on the balance and content of small peptides and free amino acids (Maehashi, Matsuzaki, Yamamoto & Udaka, 1999).

3. Feed and pet food applications

Raw or rendered animal by-products have been traditionally used as ingredients in feeds and pet foods. About 25 million tonnes per year of animal by-products derived from meat industries in the US and 15 million tonnes in the European Union are processed by rendering to produce high quality fats and proteins (Hamilton, 2016). In fact, animal by-products constitute a good source of nutrients like essential amino acids, fatty acids, minerals and trace elements, B vitamins and some fat-soluble vitamins (Nollet and Toldrá, 2011; Honikel, 2011). Examples are protein or blood meals (Alexis & Robert, 2004; Pérez-Gálvez, Almécija, Espejo, Guadix and Guadix, 2011), amino acids solutions obtained from blood (Giu & Giu, 2010) or meat and bone meal ashes obtained after co-incineration (Goutand, Cyr, Deydier, Guilet and Clastres, 2008). Meat and bone meal is also a good source of essential amino acids and group B vitamins for animal feeds (Jayathilakan et al., 2012). Protein hydrolysates have been reported to be successful in aquaculture (Gilbert, Wong and Webb, 2012). Excessive bitterness in protein hydrolysates can be reduced by cleaving hydrophobic amino acids from peptides and make the palatability more appealable in pet foods (Nchienzia, Morawicki and Gadang, 2010).Rendered meat by-products are also used as ingredients for dogs pet foods (Murray, Patil, Fahey, Merchen and Hughes, 1997).

Meat by-products protein hydrolysates represent an interesting alternative to soybean meal because the absence of antinutritional factors or allergenic proteins and the presence of large amounts of all essential amino acids (Martínez-Alvarez, Chamorro and Brenes, 2015). Other by-products like hair, nail, feather and outer layer of skin containing keratin, can be profitable after hydrolysis with the enzyme keratinase (Deivasigamani & Alagappan, 2008; Lasekan, Abu Bakar and Hashim, 2015). This enzyme is predominantly a serine peptidase with a broad range of neutral-alkaline pH for activity, pH ranging 6.0-13.0, and able to hydrolyse keratin under reducing conditions (Brandelli, Sala and Kalil, 2015).

4. Energy generation applications

In recent years, biodiesel has been produced and is now replacing progressively the diesel fuel due to its advantages like being biodegradable, non-toxic and with a favorable combustion emission profile that leads to reductions in carbon dioxide, carbon monoxide, particulate matter and unburned hydrocarbons (Gerpen, 2005; Moreira, Dias, Almeida & Alvim-Ferraz, 2010). Further, the use of biodiesel does not imply significant modifications in engines.

Low cost animal fat by-products are used as raw materials that are transesterified with a low molecular weight alcohol to yield a mixture of fatty acid methyl esters and glycerol as a side product (Bhatti, Hanif, Qasim & Rheman, 2008; Moreira et al., 2010). Hydro-oxygenation and hydroisomerization in tubular reactors has been proposed to increase biodiesel profitability (Herskowitz, 2008), also supercritical transesterification (Marulanda, Anitescu & Tavlarides, 2010). Other recent studies focus on the improved production of biodiesel by using ultrasounds assisted transesterification of the animal fats (Adewale et al., 2016). Animal fats have some limitations due to its protein and phosphoacylglycerols content that makes a degumming process necessary, the presence of water that requires of vacuum drying and the high content of saturated fatty acids that need to be reduced through winterization process or additives addition (Banckovic-Illic et al., 2014).

The developments have continued and nowadays a new 2nd generation, so-called bio gas oil is facing prompt application. Triacylglycerols are converted into a mixture of iso and normal paraffin via heterogeneous catalytic hydrogenation. Raw materials like brown greases have been also assayed with positive results (Baladincz and Hancsók, 2015).

5. Medical and pharmaceutical applications

Pork skin can be used as dressing for burns or skin ulcers in humans (Jayathilakan et al., 2012). Glands and organs constitute edible meat by-products with good nutritive value that are consumed in different regions of the world (Nollet and Toldrá, 2011) and, in fact, some of them are consumed for medicinal purposes in countries like China, Japan and India, or used as a source of particular pharmaceutical substances. This is the case of bile from the gall bladder, melatonin from the pineal gland, heparin from the liver, progesterone and oestrogen from ovaries, insulin from pancreas, etc. (Jayathilakan et al., 2012). Protein hydrolysates, especially those from collagen can generate peptides to be used in treatments against osteoarthritis by accumulation in the joint cartilage (Bello and Oeser, 2006). Hydrolysed collagen exerts a positive effect on bones and joints. In fact, these hydrolysates with added hyaluronic acid are being commercialised for better performance of joints and pain relief in humans.

Low molecular weight ultrafiltrates (<30kDa) obtained from pig aorta extracts were assayed with laboratory guinea pigs and such extracts were reported to exert substantial reductions in atherogenic lipoproteins, atherogenic index and total and residual cholesterol (Chernukha, Fedulova and Kotenkova, 2015).

6. Fertilizer applications

Large amounts of meat and bone meal are generated in all countries and an interesting approach is the thermochemical processing including pyrolysis, combustion and gasification. The most analysed are co-combustion with coal and pyrolysis. The resulting ashes demonstrate a high content of phosphorus which makes them suitable as fertilisers and the gas emissions are within the international regulations and contains combustibles to be used for energy production (Coutand, Cyr, Deydier, Guilet and Clastres, 2008; Cascarosa, Gea and Arauzo, 2012). The incineration of animal by-products results in good mineral fertilisers. In addition, the use of heat recovery allows for efficient energy recovery (Nujak, 2015).

7. Chemical applications

Rendered fats have many applications in cosmetic industry for products like hand and body lotions, creams and bath products. Fatty acids are used in the chemical industry for rubber and plastic polymerization, softeners, lubricants and plasticizers (Ockerman and Basu, 2006). Collagen, gelatin and glycerin are also used in chemical industry as ingredients for surfactants, paints, varnishes, adhesives, antifreeze, cleaners and polishes (Pearl, 2004). New applications using rendered fats have been reported like the production of polyhydroyalkanoates with a recombinant strain of *Ralstonia eutropha* (Riedel, Jahns, Koenig, Bock, Brigham, Bader and Stahl, 2015). Such polymer has the advantage being biodegradable and constitutes an attractive alternative to plastics produced from petroleum.

There are many applications for hides that traditionally have been used for leather-based articles like clothes, shoes, belts, handbags and purses (Ockerman & Basu, 2004b).

8. Conclusions

There are many applications of meat by-products like feed ingredients for livestock, poultry and aquaculture as well as for pet foods, energy valorisation through biodiesel production, new substances as alternative to plastics and protein hydrolysates to be used for technological purposes or as a source of bioactive peptides with relevant physiological effects. Research efforts are going ahead to produce new substances with new applications or improving those existing processes. So, the innovation is continuously addressed towards adding value and finding new applications to meat by-products.

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Legends for the figures

Figure 1.- Flow diagram of main routes of applications for meat by-products