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Microbial enzymes for bioconversion of poultry waste into added-value products

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ABSTRACT

The continuous growth of poultry industry results in an increased amount of waste provided from both production facilities and processing plants. The processing of poultry meat results in massive quantities of solid waste as feathers, viscera, bones, and dead on arrival. The use of enzymes for bioconversion of such byproducts into materials with increased value is an interesting strategy. Enzymes can be useful to convert poultry waste into feed and fertilizers. The hydrolysis of animal byproducts can also generate bioactive peptides, which are important molecules that may exert physiological effects in vivo, as antioxidant, angiotensin-I converting enzyme (ACE)-inhibitory and dipeptidyl peptidase-IV (DPP-IV)-inhibitory activities. Thus, poultry byproduct hydrolysates show potential for use as functional ingredients. This review presents an overview on useful microbial enzymes for bioconversion of poultry waste, such as proteases, lipases, combined enzyme preparations and, especially, keratinases for bioconversion of feathers. A discussion about production, purification and properties of keratinases is presented. Main areas for further studies are large scale production and purification of keratinases, and development of effective processes for production of bioactive molecules from poultry waste.

1. Introduction

Poultry industry is an important and diverse component of the food sector. Poultry products, including eggs, chicken and turkey meat represents an important protein source in the diets of most people. Broiler production greatly increased from the 1980s, due to the suggested nutritional benefits of chicken meat compared with other meats and through an extraordinary increase in consumption. This increase has been also accredited to the poultry industry, which supply processed products that are easier for the customer to prepare. This industry is constantly growing and the largest producers worldwide, namely USA, Brazil and China, account for more than 40 million tons per year production (USDA, 2014). The European Union annual production is approximately 11 million tons (AVEC, 2014).

The increased production of poultry industry results in a huge amount of waste that needs to be managed. Three primary waste products are dissolved air flotation sludge, compost from dead birds and hatchery waste, and litter and manure from production facilities. The overabundance of litter and manure resulted in excessive waste application on cropland or imprudent stockpiling and dumping of waste nearby waterways (Salminen & Rintala, 2002; Simpson, 1991). Although a controlled utilization of such waste as soil fertilizer is feasible, some concerns are due to the high levels of oxygen demand, physical clogging of soil by fat accumulation, and presence of pathogens in dead, hatchery and litter compost.

The major waste materials generated in poultry processing plants are feathers, soft meat, blood, deboning residue, and also dead on arrival. These materials are currently converted into meat and bone meal, feather meal, blood meal and fats/oils by rendering process (Lasekan, Bakar, & Hashim, 2013; Salminen & Rintala, 2002). Although these meals are a good source of protein, their utilization can be limited due to nutritional restrictions associated with losses of essential amino acids (Onifade, Al-Sane, Al-Musallam, & Al-Zarban, 1998), and with high calcium, phosphorous and lysine content of the meat and bone meal (Batal & Dale, 2011).

Feathers are an abundant waste of poultry industry since they account for approximately 8% of the adult chicken weight and are constituted by about 90% protein (Onifade et al., 1998). The major insoluble structural protein of feathers is keratin, which is recognized for its recalcitrance. Keratin-rich wastes are of difficult degradation since the protein chains are tightly packed and strongly stabilized by several hydrogen bonds and hydrophobic interactions. In addition, cross-linking of polypeptide chains by several disulfide bonds provides mechanical resistance and impairs degradation by conventional proteolytic enzymes (Brandelli, 2008; Kornillowicz-Kowalska & Bohacz, 2011). However, some microbial enzymes can hydrolyze insoluble feather keratins, allowing their conversion into feedstuffs, fertilizers, and films (Gupta & Ramnani, 2006; Onifade et al., 1998). In addition, applications of these enzymes for pharmaceutical and cosmetic purposes have been also

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described (Brandelli, 2008). Proteolytic enzymes can be also useful to increase nutritional value of poultry byproducts like mechanically deboned meat (Rossi, Flores, Venzke, & Ayub, 2009) and feather meals (Odetallah, Wang, Garlich, & Shih, 2003; Pedersen, Yu, Plumstead, & Dalsgaard, 2012). Thus, enzymatic catalysis can be an interesting alternative to convert byproducts generated during poultry processing into added-value materials.

This article presents a discussion on useful enzymes that can be useful in the management of byproducts of poultry industry. Considering the relevance of protein-rich waste, emphasis is devoted to proteolytic enzymes used in bioconversion of these waste materials, in particular on the production, purification and applications of keratinases in bioconversion of keratin waste.

2. Byproducts of poultry industry

Poultry industry generates a large amount of byproducts, mostly in the form of heads, legs, bones, viscera, skin and feathers, in addition to whole carcasses in the form of dead on arrival. These materials represents a massive amount of solid waste that should be properly managed to avoid environmental damage and loss of important raw materials for feed industry and as biological resources (Jayathilakan, Sultana, Radhakrishna, & Bawa, 2012; Lasekan et al., 2013).

Animal wastes generated in the meat industry contain considerable amounts of insoluble and hard-to-degrade structural proteins like collagen, elastin and keratin, which are major constituents of bones, organs and hard tissues. These byproducts are often rich sources of protein, which can be extracted and hydrolyzed to be used as feed or functional ingredients. The protein amount of different poultry byproducts and bioactive properties of the byproduct hydrolysates are presented in Table 1.

2.1. Collagen-rich waste

The extracellular matrix that occupies the major part of the animal tissue volume is composed by a diversity of proteins and polysaccharides (mostly linked to proteins as proteoglycans) that maintain and protect the cells and tissues (Fig. 1). Among these proteins, collagens are found in all animals and are the most abundant proteins, reaching 25% of total protein mass of skin and bones (Mayne & Brewton, 1993; Van der Rest & Garrone, 1991). Byproducts that are rich in collagen can be heat-denatured and extracted leading to the formation of gelatin. Also, hydrolysates from collagen-rich waste have been investigated because of its antihypertensive characteristics, which appears to be associated with the elevated proline content (Gómez-Guillen, Giménez, López-Caballero, & Montero, 2011). Chicken leg bone is derived from deboning chicken meat, and is basically constituted by collagen, thus containing hydroxyproline as a peculiar feature. Both chicken bone and blood have been used for producing hydrolysates with potential bioactive properties (Cheng et al., 2009; Huang & Liu, 2010), as shown in Table 1. Bioactive peptides are examples of important molecules found in animal byproduct hydrolysates that can exert a physiological effect in vivo, showing potential for use as functional ingredients in animal feeds.

Table 1
Protein-rich byproducts of poultry industry as source of bioactive and functional hydrolysates.[a]

Poultry byproduct	Protein (%)	Functional and bioactive properties
Feathers and feather meal	85–99	Antioxidant, ACE inhibitor and DPP-IV inhibitor [b]
Blood and blood meal	60–80	ACE inhibitor [c]
Bone	23–24	ACE inhibitor [a]
Viscera	11–12	Antioxidant, ACE inhibitor, emulsifier [a]
Chicken skin	17–20	ACE inhibitor[d]
Offal (heads, feet, viscera)	12–15	Antioxidant [e]

[a] Essentially compiled from Lasekan et al. (2013).
[b] Fontoura et al. (2014).
[c] Huang & Liu (2010).
[d] Saiga et al. (2008).
[e] Manhiani, Northcutt, Han, Bridges, & Dawson (2013).

2.2. Feathers

Feathers, accounting to about 8% of live weight, are a major waste of poultry processing plants. The large amount of waste feathers generated each year by commercial poultry plants creates a serious environmental problem. Disposal strategies like burning in incineration plants and landfilling are restricted or banned in many countries. Feathers are mostly composed by keratin protein and this byproduct has been frequently converted into feather meal, a low-grade feed ingredient. Feather waste has been also converted into fertilizers for agricultural purpose, as bedding material, and for decorative purpose (Jayathilakan et al., 2012). The development of suitable processes to convert feather waste into digestible proteins and amino acids has been the main goal, and biotechnological approaches based on microbial enzymes have been extensively investigated in the last decade (Gupta, Sharma, & Beg, 2013; Kornillowicz-Kowalska & Bohacz, 2011). Feather hydrolysates could be converted into methane gas and fuel pellets for heating (Dudynski, Kwiatkowski, & Bajer, 2012; Ichida et al., 2001), and also utilized in the production of biohydrogen (Bálint et al., 2005). The development of an effective conversion of feather waste into fuels may address the increasing interest for energy conservation and recycling.

2.3. Miscellaneous byproducts

Heads, gizzards and blood have been typically used for meal production, whereas feet, skin, intestines and glands can be also a source of poultry fat (Sams, 2001). Keratin-rich materials like nails and beaks can be degraded by keratinolytic microorganisms (Riffel & Brandelli, 2006), and are often used in combination with viscera or blood for production of animal feed (Sams, 2001). Blood is generally treated with chemicals to prevent coagulation and dried to become a concentrated protein source known as blood meal, which shows a high content of basic (Lys and Arg) and sulfur (Cys and Met) amino acids (Márquez, Bracho, Archile, Rangel, & Benítez, 2005).

Byproducts of hatchery include egg shells, unhatched eggs, infertile eggs, and discarded chicken, which are utilized in animal feed. These meals present high calcium content, limiting its use up to 5% into feed (Jayathilakan et al., 2012).

2.4. Litter and manure

In addition to plant processing byproducts, waste from the production phase is mainly found as poultry litter and manure. These byproducts have been typically used as recycled feed and surface covering of agricultural lands (Shih, 1993; Simpson, 1991). However, poultry manure and slaughterhouse waste can be converted in methane as energy source by some anaerobic organisms (Salminen & Rintala, 2002). Thermophilic anaerobic digestion has been used to degrade chicken manure and produce biogas at high rate. The major byproduct of anaerobic digestion is biogas, which is a combustible fuel gas and can be utilized for generating electricity, heating and drying. The digester effluent is a liquid, and the aquacultural use of this byproduct has been demonstrated (Shih, 1993). Pathogenic microorganisms such as fecal coliforms are destroyed in the thermophilic digester. Also, fungi are reduced by nearly 100% and oocysts of the pathogenic protozoan *Eimeria tenella* lost both their infectivity in vivo and sporulability in vitro (Shih, 1993).

3. Useful enzymes for bioconversion of poultry waste

Advances in microbial enzyme technology offer considerable opportunity for development of low-energy consuming technologies for