

BIOLOGICALLY ACTIVE PEPTIDES OF MEAT AND MEAT PRODUCT PROTEINS: A REVIEW

PART 1. GENERAL INFORMATION ABOUT BIOLOGICALLY ACTIVE PEPTIDES OF MEAT AND MEAT PRODUCTS

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Abstract

Over many years, proteins and polypeptides have aroused scientific-practical interest due to multiple functions in the metabolic processes in the body upon vital activities. Biologically active substances of protein origin have wide application in different industries, including the food industry and medicine. At present, many studies are directed towards investigation of mechanisms of formation of such physiologically valuable food components as biologically active peptides and methods of their recovery from meat raw materials and meat products. A large part of literature data confirms that mechanisms of formation of such peptides are similar irrespective of methods of their generation. Their basis is enzymatic hydrolysis of muscle tissue proteins under the action of intracellular enzymes during autolysis, digestive enzymes of the human gastrointestinal tract or commercial enzyme preparations used in laboratories or in the industry. The method of culinary and/or technological processing also affects the process of biopeptide formation in meat products, namely, their recovery and availability.

Introduction

Animal proteins in a human diet are physiologically active compounds; they have a direct action or are a substrate for enzymatic hydrolysis in processing and consumption of food. Biologically active peptides (BAPs) can be obtained through hydrolysis by intrinsic enzymes of meat raw materials, endogenous enzymes of the human gastrointestinal tract or microbial enzymes used in a technological process.

The integrity and structure of meat proteins change during autolysis or long-term storage of meat raw materials in the frozen state. A great number of peptides with the physiological activity are released during meat processing. The biological activity of food components is widely studied *in vitro*, and nowadays the attempts are made to study their *in vivo* effect on healthy people or patients with different pathologies.

This paper presents a complex review of the methods of biologically active peptide formation in meat raw materials and products.

Main part

The mechanisms of BAP formation in meat and meat products are similar (Table 1). During autolysis of muscle tissue, the proteolytic activity conditioned by endogenous enzymes (calpains and cathepsins) is a key process, which influences protein destruction and, consequently, generation and release of a large number of peptides and free amino acids [1,2].

Bauchart et al [4] in the study of aged beef found an increase in the content of BAPs <5 kDa in meat after 14 days of

storage at a temperature of 4 °C compared to their quantity in fresh meat. Fu et al. [23] also demonstrated that bioactive peptides with a size of about 3 kDa can be generated in longissimus dorsi and semitendinosus muscles during meat aging after 20 days of proteolysis.

Generation of peptides can also be caused by the oxidation processes during meat storage [24]. The oxidative status can regulate the endogenous enzymatic activity and, consequently, a degree of degradation of myofibrillar and sarcoplasmic proteins [25]. Changes in temperature and pH can affect the content of bioactive peptides due to changes in the activity of endogenous enzymes [3,26,27].

It is known that peptides with the biological activity are naturally formed in the mammalian gastrointestinal tract during metabolism of meat diet proteins under the action of the digestive enzymes such as pepsin, trypsin, chymotrypsin, elastase, and carboxypeptidase [3,28,29,30]. Therefore, to generate such potentially biologically active peptides, researchers model a process that simulates gastrointestinal digestion. The process is based on enzymatic hydrolysis with the use of different commercial exogenous proteinases obtained from animal tissues (pepsin and trypsin), plants (papain, ficin, and bromelain) and microbial sources (alcalase®, flavourzyme®, neutrase®, collagenase or proteinase K) [30,31,32]. In addition to the meat sources, several BAPs are generated by enzymatic hydrolysis of collagen from meat or slaughter by-products (trimmings, organs, blood hemoglobin) as was highlighted in several studies [24,33].

For example, the release of potential BAPs — angiotensin-I-converting enzyme (ACE-I, EC3.4.15.1), renin (EC3.4.23.15)

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Table 1. Brief characteristics of the processes of generation of meat biologically active peptides [3]

Product	Process	Carrier/Regulation	Functionality	Peptide sequence	Reference
Muscle tissue	Proteolysis, oxidation	Endogenous enzymes	ACE*-I activity	APPPPAEVPEVHEEVH, PPPAEVPEVHEEVH, IPITAAKASRNIA, LPLGG, FAGGRGG, APPPPAEVVP	[4,5,6]
	Enzymatic hydrolysis	Exogenous enzymes	ACE-I, antioxidant, antithrombotic, antimicrobial and antitumor activities	KRQKYD, EKERERQ, KAPVA, PTPVT, RPR, GLSDGEWQ, GFHI, DFHING, FHG	[7–15]
	Preparation	High temperature	ACE-I	SPLPPPE, EGPQPPGPVVG, PGLIGARGPPGP	[4]
Collagen	Enzymatic hydrolysis	Bacterial collagenase, exogenous enzymes, proteases from <i>Aspergillus oryzae</i>	ACE-I and antioxidant activities	AKGANGAPGIAGAPGFPGARGPSGPQGPSGPP, PAGNPGADGQPGAKGANGAP, GAXGLXGP, GPRGE, VGPV, QGAR, LQGM, LQGMH, LC	[16–19]
Dry-cured products	Proteolysis	Endogenous enzymes	antioxidant activity	DSGVT, IEAEGE, EELDNALN, VPSIDDQEELM, DAQEKLE, ALTA, SLTA, VT, SAGNPN, GLAGA, DLEE	[20,21]
Fermented products	Proteolysis	Presence of starter cultures	antioxidant activity	FGG, DM	[22]

* ACE — Angiotensin converting enzyme

and dipeptidyl peptidase-IV (DPP-IV, EC3.4.14.5) — from bovine and porcine proteins including hemoglobin, collagen and serum albumin, was assessed in the study of 2014 [34] using the *in silico* methods, peptide databases and software as well as chemical synthesis and *in vitro* analysis to confirm bioactivity of peptides. These proteins are usually found in meat by-products such as bones, blood and meat trimmings, and play a key role in the control of hypertension, development of type-2 diabetes and other diseases associated with the metabolic syndrome. New peptides included ACE-I inhibitory tripeptide Ile-Ile-Tyr and DPP-IV inhibitory tripeptide Pro-Pro-Leu corresponding to the sequences f (182–184) and f (326–328) of porcine and bovine serum albumin, which can be released after hydrolysis by the enzymes papain and pepsin, respectively.

In another work [35], the inhibitory and antioxidant activities of ACE-I sarcoplasmic proteins extracted from the pectoral muscle (*Pectoralis profundus*) of cattle (*Bos taurus*) and hydrolyzed by papain at 37 °C for 24 hours were studied. Sarcoplasmic protein hydrolysates were subjected to membrane ultrafiltration and filtrates 10 kDa and 3 kDa were obtained. As a result, 11 peptides were characterized from the total hydrolysate fraction: 15 from the fractions of 10 kDa filtrate, 9 peptides from the fractions of 3 kDa filtrate. The similarity between amino acid sequences of peptides identified in this investigation and known antioxidant and ACE-I inhibitory peptides described in the database of biologically active peptide sequences BIOPEP was found [36].

A promising source of BAPs are porcine myofibrillar proteins [37]. The enzymes pepsin, trypsin and chymotrypsin were used for *in silico* proteolysis. In intact proteins and after simulation of gastrointestinal digestion, the inhibitory peptide sequences of dipeptidyl peptidase-IV were observed most frequently. In total, the authors found 399 peptides with the antioxidant, hypotensive, anti-amnesic and stimulating or regulating different body functions activities, as well as enzyme inhibitors [38].

Other mechanisms, such as the processes of freezing and cooking can affect release and availability of meat BAPs. Freezing can denature proteins due to different chemical and physical stress mechanisms including ice formation, pH changes and low temperature [39], which leads to an increase in BAPs. Cooking can influence peptide generation and their biological activity [23,27] due to changes in the native conformation (denaturation) and disruption of intramolecular bonds caused by heating [40].

It was shown that several BAPs are also released from meat products during drying or aging [41]. Proteolytic degradation, which occurs during aging of dry cured ham or during sausage fermentation and forms aroma and texture, results in generation of peptides with low molecular weight (3–5 kDa) and free amino acids [7,42]. In fermented meat products, protein degradation is influenced by different variables such as product composition, processing conditions and starter cultures. Proteolytic degradation by endogenous enzymes and lactic acid bacteria affects the peptide content. In particular, the presence of lactic acid bacteria causes a decrease in pH, which leads to higher activity of muscle endogenous proteases [43].

Generation of bioactive peptides in meat autolysis

Biologically active peptides can be generated under the action of endogenous proteases in the process of meat autolysis. Proteolysis by endogenous enzymes is the most important phenomenon that takes place during meat aging. Endopeptidases, such as calpains and cathepsins, first of all, are responsible for hydrolysis of proteins into large fragments and oligopeptides, which influences meat texture during aging and the initial stages of the rigor mortis processes. Later due to exopeptidases, such as aminopeptidases and carboxypeptidases, small peptides and free amino acids will be formed [44].

Meat aging influences its taste, tenderness, moisture binding capacity (MBC), color and juiciness. A detailed

study of the biochemical processes occurring during meat aging improves the understanding of their development. Monitoring of these processes allows revealing biomarkers of meat product quality [45]. Di Luca et al. [46] studied changes in proteome of muscle exudate during the normal period of meat aging (seven days) in genetically similar pigs from one population with the same meat quality characteristics. It was found that several quality meat indicators significantly changed during autolysis especially at the latest stage of aging. For example, from the 3rd to the 7th day, meat tenderness significantly increased, color parameter CIE b* of muscles also changed compared to the period of rigor mortis, and cooking losses changed as well. These data illustrate structural changes that take place in pork muscles during their aging, which was significantly reflected on their proteomic profiles. Three key protein groups (stress proteins, metabolic enzymes and structural proteins), which significantly changed during meat aging, were identified [47].

Another significant peculiarity resided in the fact that quantity of proteins associated with stress reduced. Monitoring of these changes is usually performed using myofibrillar or sarcoplasmic proteomic fractions. These observations in the more available substrate, that is, in the muscle exudate, allows complimenting previous studies, showing, for example, that vinculin correlates with the moisture binding capacity and peroxiredoxin-6 with meat tenderness. These protein biomarkers have potential for monitoring fresh meat quality and predicting a course of autolysis [47,48].

Hydrolysis by enzymes of different origin

The most common methodology of BAP generation is hydrolysis of proteins by commercial enzymes of microbial, plant or animal origin. In meat and meat products, Flavourzyme from *Aspergillus oryzae*, as well as Neutrase and Alcalase from *Bacillus subtilis* and *Bacillus licheniformis*,

respectively, are most widely used for generation of bioactive peptides. In addition, proteases of plant origin, such as bromelain and papain, were described as interesting enzymes for meat protein hydrolysis due to their role in meat tenderization. These enzymes show wider specificity compared to other enzymes, such as trypsin and pepsin, cleaving peptide bonds from a wide spectrum of areas and often acting either as endopeptidases or as exopeptidases hydrolyzing amino acids from N- and C-terminal ends [45].

Conclusion

Meat and meat products are one of the main sources of biologically active peptides.

With proteins as the main meat components, BAP generation occurs either under the action of endogenous muscle enzymes in the processes of autolysis and aging, or exogenous enzymes during digestion in the gastrointestinal tract, or with the use of commercial enzymes in laboratories or industrial processes under controlled conditions. During meat storage, peptide formation can also be caused by the oxidation processes. In addition, freezing processes and cooking can affect recovery and availability of meat BAPs. BAPs can be released from meat products during drying or aging. Mechanisms of BAP formation in meat and meat products are similar.

However, despite clear identification of BAPs, there is a growing need for studying interactions of a food matrix, especially when the aim is to use bioactive peptides as a functional ingredient. Qualitative assessment of these peptides for the better understanding of their impact on health and bioavailability is necessary for advance in this field. We will discuss this question in the second part of the review.

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