Synthesis, extraction and identification of meat bioactive peptides: a review

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Abstract. The consumption of meat should consider the concept of functional food. The meat had a high quality protein and contain of bioactive peptide compounds. Amino acid was component of bioactive peptides compound. It joined by covalent bonds known as amide or peptide bonds. A lot of research was currently focused on the bioactive peptide compounds isolated from myofibril and sarcoplasmic proteins with the synthesis, extraction, and identification methods. This study used a systematic review to get the structure of amino acids that the source of bioactive components and the principle of synthesis, extraction and identification of bioactive peptide in the meat. This paper highlights were finding on the structure of amino acid in the meat. The proportion of amino acids was also different in each animal body location. The result identified that more than 170 peptides were released from the main structure of the myofibril (actin, myosin) and sarcoplasmic muscle proteins, and the synthesis, extraction and bioactive peptide identification in the meat as well as their potential use as functional food.

Keywords: Bioactive, Peptides, Meat, Synthesis, Extraction, Identification

1. Introduction

The patterns of meat consumption in the world community have changed. Meat consumption is considered beneficial by most humans but dangerous for some human as well [1]. Meat is important to human and has great role for brain and intellectual development in human evolution [2]; [3]; [4]. Meat has a high quality protein [5] such as amino acids, vitamins, minerals and bioactive components that are essential for the body [6] and it contains of bioactive peptides as well as number of studies are focusing on the development of functional food from meat [7]; [8]; [9]. The provision of meat should consider to the concept of functional food that is a source component and has a positive effect on the physiology and health [6]. Bioactive components in foods are defined as a compound that has health benefits both prevention and treatment [5]. There are several benefits of bioactive components [6] such as antihypertensive ([10]), antioxidant [11] anti-cancer [12], antimicrobial [13], as opioid compounds [14], mineral binders [15], immunomodulatory [16], decreased of cholesterol [17] and anti-diabetic activity [18].

The activity of the peptides is determined by the amino acid composition and sequence that they are released from the precursor protein where they are encrypted. The natural processes within the body are modulated by the interaction of specific amino acid sequences that form part of proteins [19]. Protein
are found in the plant and animal origins that can be the potential sources of bioactive peptide encrypted in their structure [20]; [21]. Although the correlation between structure and functional properties is not well established, many bioactive peptide include a peptide residue length between 2–20 amino acids [22], and the hydrophobic amino acids in addition to proline, lysine or arginine groups. Bioactive peptide compound has been considered the new generation of biologically active regulators that can prevent such as oxidation and microbial degradation in foods. It can be used for the various medical conditions, thus increasing the quality of life [23]. Recently, functional foods [24] and nutraceuticals [25] have received much attention, particularly for the impact that they pose on human health and their use in the prevention of certain diseases.

There are many research currently focused on the generation of bioactive peptide compounds [26] that isolated from myofibril and sarcoplasmic proteins of cattle beef [27], myofibril proteins of pork [28], leather and blood of cattle [29]; [30] and pork [31]. There has been identification of bioactive peptide compound that isolated from myofibril and sarcoplasmic of various meat and method of synthetic, extraction and identification among individual experiments but some of them has not been summarizing. The main objective of this study was to compare the various scientific reports about the structure of meat amino acids, the source and type of bioactive components in the meat, and their bioactive peptide synthesis, the type, content and benefits as well as the principles of extraction and identification. The research attempted to find the structure of amino acid, the synthesis, extraction and bioactive peptide identification in the meat as well as their potential use as Functional food.

2. Materials and methods
This research applied a systematic review method. The references were relevant studies published in various scientific journals and indexed in electronic databases such as Google Scholar, Science Direct, and Scopus Journal on the method of synthesis, extraction and identification to get structure of amino acid and bioactive peptide in the meat. The keywords used in the search strategy were “Meat Bioactive Peptide”, “Meat Protein”, “Synthesis of Meat Protein”, “Extraction of Meat Protein”, and “Identification of Meat Bioactive Peptide”. We also reviewed the identified trials and review articles in reference lists to find any other potential proper reports. The literature search was conducted through a literature database within the last 20 years for journal and more 20 years for book chapter.

3. Result and discussion

3.1. Meat proteins
Proteins were polymer chains of amino acid monomers having the structure and function based chain polypeptide [32]. Based on the solubility, protein meat categorized among others: sarcoplasmic proteins, myofibril proteins and stromal proteins [33]. Sarcoplasmic dissolved in water with low ionic strength (<0.15), myofibrils soluble ionic salt with a stronger, while stromal was not soluble proteins consisting of collagen, elastin and reticulin [34]; [33]. The proportion of each type of protein [35] is as presented in Table 1.

3.2. Amino acids
Amino acids are part of the structure of a protein was characterized by the central carbon atom α, which has a biological function based functional groups (R) are different [32].
Table 1. The proportion of protein components in meat.

| Component                                      | Wet Weight (%) |
|------------------------------------------------|----------------|
| Myofibril Proteins                             |                |
| Myosin                                         | 11.5           |
| Actin                                          | 5.5            |
| Conectin (Titin)                               | 2.5            |
| Nebulin                                        | 0.9            |
| Trophysynin                                    | 0.3            |
| Troponin C, I dan T                            | 0.3            |
| A, β actinin                                    | 0.6            |
| Myomesin                                       | 0.2            |
| Desmin, Filamin dll                            | 0.4            |
| Sarcoplasmic Proteins                          | 5.5            |
| Glyceraldehyde Phosphatase Dehydrogenase       | 1.2            |
| Aldolase                                        | 0.6            |
| Creatine Kinase                                | 0.5            |
| Enzymes of Glycolysis                          | 2.2            |
| Myoglobin                                      | 0.2            |
| Haemoglobin                                    | 0.6            |
| Tissue Proteins and Organella                  | 2.0            |
| Collagen                                       | 1.0            |
| Elastin                                        | 0.05           |
| Mitochondrial (Cytochrome and Non Suluble Enzyme)| 0.95          |
| Substance of Non Suluble Proteins              | 1.65           |
| Creatinine                                     | 0.55           |
| Inosine Monophosphate                          | 0.30           |
| Di, Tri-Phospopyridine Nucleotides             | 0.10           |
| Amino Acids                                    | 0.35           |
| Carnosine, Anserine                            | 0.35           |

Source: [35].

3.2.1. Amino acids structure. Some amino acids based on the structure of the side chain (R) was illustrated in Figure 1.

3.2.2. The content of amino acids in meat. The amino acid content in meat was affected by the type of livestock and feed nutrients [36]. There were differences in the ratio of amino acids arginine, histidine and lysine that is 2 : 3 : 1 on the lamb and 1.5 : 2.0-2.2 : 1 on pork [37]. The presence of essential amino acids in chicken meat original Bali such as threonine, methionine, valine, phenylalanine, isoleucine, leucine, and lysine. The proportion of amino acids is also different in each animal body location [38]. The content of histidine (g/100g) in droughmaster beef [39], 0.476 (the neck meat), 0.660 (in short rib meat), 0.952 (flank), 0.753 (rump), dan 1,000 (longisimus dorsi.) [35]. There is distribution of amino acids in proteins of myofibrils including aromatic groups, proline, basic and acidic. The recent research showed that the content of essential amino acids in different Peking duck thigh meat significantly higher than in the chest, but did not different significantly in Muscovy ducks, with cysteine higher percentage of chicken [40].

3.2.3. Free Amino Acids (FAAs). The important biochemical changes in the meat, caused more natural proteolysis activity in the flesh or by microbial enzymes that generated of free amino acid (FAAS), low molecular weight peptides, aldehydes, organic acids and amines [41]. The recent research showed the phase changes occur both essential and non-essential during storage [42], storage of meat at refrigerator temperature will occur further proteolysis particularly creatine and creatinine [43].
3.3. Bioactive compounds in meat

Bioactive compounds were the compounds in foods that had health functions and the ability to prevent the propagation of disease [45]. The health function include: hypcholesterolemic, antioxidative, antithrombotic, antimicrobial, opioid and immunomodulatory [46].

3.3.1. Source of bioactive compounds. The grain cereals that contains some phenolic compounds such as flavonoid [47], phenolic acids and proanthocyanins are mainly found on the outer skin of the details [48]; [49], and also contains substances carotene that could reduce oxidative damage in biomolecules [50]; [51]. Phenolic compound was found in wheat that had antioxidant activity 90% dominated by ferulic acid [51]. The content of bioactive compounds in meat [52], taurine had many functions related to eye health and heart disease [53] and carnosine also plays a role in heart health [53] maintaining the balance in the muscles and joint coenzyme Q10, function as antioxidants [54], whereas creatine compounds extremely important in energy metabolism [55].

3.3.2. Type of bioactive compounds in meat. Classification of bioactive compound of meat such as: 1) derived from fat and the fatty acid derivative, for example conjugated linoleic acid (CLA), and 2) derived from protein and amino acid derivatives, especially from the class of peptides like carnosine and L-carnitine [31]. Bioactive peptides were first reported [56] when observing peptide phosphorylation of casein which lowers the baby's bones. Bioactive peptides with activity like peptide hormones or drugs that have the function of modulation through bonding interactions specific receptors on target cells to produce a physiological response. [57]. The majority of bioactive peptides known to be absorbed by the
digestive tract into the blood circulation, but the effect may mediate directly in the lumen of the stomach or through receptors in the gut [58].

3.4 Synthesis of bioactive peptides
Bioactive peptides could be derived from the precursor protein by several methods including through the process of proteolysis in the digestive tract, chemically or hydrolysis enzyme in vitro during processing and fermentation microbes [59]; [22] [60]; [61]; [62]; [63]. Most proteins contain bioactive pieces, but cannot be active without the parent protein [10]. Part of the active peptide was derived from the native protein only via proteolytic digestion, these components are able to with stand the effect of enzymes such as pepsin, trypsin, chymotrypsin, elastase and carboxypeptidases [64]. The content of the peptide in the flesh will rise during the withering post mortem. Oligopeptides level changes occur during storage of beef, pork or chicken [65]. During the shelf life of meat proteins undergo hydrolysis by proteases endogenous in meats such as calpains and cathepsins [66]. The enzymatic hydrolysis processes that contribute to improve the texture, taste and flavor of meat are not reported to affect the formation of bioactive peptides during storage, but increased I-con-verting angiotensin enzyme (ACE), which have inhibitory activity for the storage of meat. The illustration synthesis of bioactive peptides in the flesh is presented in Figure 2.

![PROTEOLYSIS](image)

**Figure 2.** Illustration of bioactive peptides synthesis from protein meat [31].

During the process of proteolytic digestion of cattle in vitro, sarcoplasmic protein degraded immediately, whereas myofibrillar chain requires prior proteolytic release and is not completely hydrolyzed by protease enzymes gastroduodenal at quite a long time [67]. The result was identified as more than 170 peptides were released from the main structure of the myofibril (actin, myosin) and sarcoplasmic muscle proteins. Some of these peptides have amino acid sequences (sequences) potential as antihypertensive compounds or antioxidants. During the identification of peptide produced during the digestion in vitro meat cook, there were 43 kinds of proteins in digestion, 20 specific proteins in the stomach, and 8 types of protein in the intestine [68].

3.5 Type of Bioactive Peptides
The bioactive peptides in the flesh consists of: group histidyl dipeptides, L-carnitine, taurine components such as peptides, ACE inhibitor, antioxidative peptides, peptide prebiotic and other promising peptide [31].
3.5.1 Histidyl dipeptides. The ratio of histidyl dipeptides differ between types of livestock, the ratio balenine: anserine on horse meat, pork, beef, lamb, chicken and kangaroo respectively 0.1.0. 0.003, 0.02, 0.01 and 0, while the ratio of carnosine: anserine at the same livestock are 93, 21, 6, 1, 0.5 and 0.1 [35]. Some of which include endogenous antioxidant in meat are: tocopherol, ubiquinone, carotenoids, ascorbic acid, glutathione, lipoic acid, ureic acid, spermine, carnosine, anserine in meat has been studied [69].

Histidyl dipeptides compounds such carnosine (β-Alanyl-L-histidine) and anserine (N-β-Alanyl-L-methyl-L-histidine) is an antioxidant that is most in the flesh. The concentration of carnosine in chicken thigh meat are 500mg per kg and 2700mg per kg on pork. Anserine is obtained in chicken meat. Both of these compounds act as antioxidants related to its ability to bind to a transition metal such as copper [31]. there is an antioxidant peptide compounds that are naturally present in the flesh, including: glutathione (c-Glu-Cys-Gly), carnosine (b-Alanyl-L-histidine), anserine (b- Alanyl-L-1-methylhistidine), and ophidine (b-Alanyl-L-3-methylhistidine) [70].

3.5.2 L-Carnitine. Many L-Carnitine (β-hydroxy-trimethyl gamma-amino butyric acid) were detected in the skeletal muscle of various animals [71]. Content of L-Carnitine in the beef thigh was1300mg per kg. This compound helps the human body to produce energy and lowers cholesterol levels and helps the body to absorb calcium and increase bone strength and chromium [31].

3.5.3 Others bioactive component. Other bioactive compounds in meat were glutathione, to have antioxidant function in toxicology and processes of pathological cell. Red meat was one of source of glutathione was 12.0-26.0 mg /100 g of beef [72]. Taurine is an amino acid essential for breastfeeding for immunity, as well as protecting the body from the oxidative stress. The Meat was a source of taurine (77.0 mg/100g beef) [5]. Coenzyme Q10 (ubiquinone) is also other bioactive compounds which demonstrate antioxidant activity, contained in beef amounted to 2mg/100 g [52]. In addition, there was also a compound of creatine and creatine phosphate which has an important role in the metabolism of muscle energy. Beef contains of creatine was 350/100 g [52]. Other components such as choline, balenine, creatinine, lipoic acid, putrescine, spermidine, and spermine were also detected in meat [31].

There were other bioactive peptide compounds in meat animals produced during the digestion process. Derived from protein digested by various digestive enzymes, such as pepsin, trypsin, chymotrypsin, elastase and carboxypeptidase [64]. The digestive enzymes produces bioactive compounds named ACE inhibitors in pork [31]. The ACE inhibitor peptides are also produced from protein of meat, such as myosin, actin, tropomyosin and troponin by pancreatic proteases [73].

3.6 Compounds and effect of bioactive peptides Identification of bioactive peptides compounds on venison from Norway based on age and gender status showed that samples taken from the Longissimus Lumborum with the results of the content of taurine were in adult male deer (72.7 mg/100g), adult females (52.1 mg/100g), male calf (107.8 mg/100g), and a female calf (137.3 mg/100g). The content of carnosine were found in adult male deer (303.1 mg/100g), adult females (275.3 mg/100g), male calf (350.3 mg/100g), and a female calf (304.6 mg/100g). The content of anserine were found in adult male deer (194 mg/100g), adult females (200.9 mg/100g), male calf (187.8 mg/100g), and a female calf (205.9 mg/100g) [74].

The content of bioactive peptides from class histidyl dipeptides on chicken and pork has been studied. The contents of carnosine (β-Alanyl-L-histidine) was 500.0 mg/kg in chicken thigh meat and 2700.0 mg/kg in meat shoulder pork [31]. The content of L-carnitine (β-hydroxy-gamma-amino butyric acid trimethyl) on beef thigh meat ranges from 1300 mg/kg of meat [71]. Other bioactive components also been studied, among others: glutathione (12-26 mg/100 g of meat [72] and taurine (77mg/100g of meat) [5]. There were significant differences between the content of bioactive peptides in beef cattle Longisimus of New Zealand (NZ) than beef cattle from the United States (US). The amount of each that was Taurine (82.8 mg/100g: 68.9 mg/100g), Carnosine (403.8 mg/100g: 308.0 mg/100g), Coenzyme Q10 (2.51 mg/100g: 1 , 23 mg/100g), Creatine (349 mg/100g: 360 mg/100g) and Creatinine
The content of bioactive peptides in the Longissimus Lumborum Romney of sheep meat was between 6 and Texel-cross from New Zealand. The comparison of each taurine content were (31.0 mg/100g: 57.3 mg/100g), Carnosine (491.1 mg/100g: 458 mg/100g), Coenzyme Q10 (1.71 mg/100g: 1.87 mg/100g), Creatine (346 mg/100g: 489 mg/100g) and Creatinine (5.9 mg/100g: 3.86 mg/100g) [5].

The carnosine contained of β-Alanyl-L-histidine and anserine was N-β-Alanyl-1-methyl-L-histidine. It had the effect of homeostasis and cell maintenance as well as endogenous (natural antioxidants) in the tissues of meat [75]. The carnosine was found in skeletal muscle and other tissues of mammals [5], plays a role in maintaining the balance [76] and also contains significant antioxidant properties [77]. The use of synthetic carnosine and ascorbic acid respectively significant influence on slowing the oxidation of the meat, but do not occur in the compound carnitine [78].

3.7 Extraction of bioactive peptides compound

The analysis of bioactive peptides constrained by the lack of a database of research on the structure of the N [79] and C-terminal [80]. The lack of research on the activity of proteolytic enzymes were specific in isolation bioactive peptides [81]. The presence of peptide compounds in food does not stand alone and always binds to a non-peptidic components (fat and sugar), it could interfere in the analysis of peptides [48]. Therefore, in practice it was difficult to analyze peptides in food with good accuracy without performing the sample preparation step. Preparation may comprise a variety of procedures for isolation, purification, and analysis of pre-concentration [82].

RP-LC and capillary electrophoresis (CE) was the method of basic analytical peptidome food chemometrical analysis [83]. In connection with the methods of CE and capillary electrochromatography (CEC), there should be restrictions on the application of sample volumes are very small (the size of the nano or unit picolitre), and should be taken pre-concentration and pre-separation in the sample with the concentration or the complex mixture of peptides low[84]. The first step can be done thorough cleaning to remove substances that interfere, then made the application of different fractionation steps as has been widely reported [85];[86]; [87]

Derivatization of peptides may be needed in some analyzes for better detection results [88]. Most derivatization was developed by the method of fluorescence (radiation of a substance with a wavelength shorter than the X-ray and UV with a limit of detection (LODs) is about two to three times lower than the absorption of UV light [84].

3.8 Identification of bioactive peptides

Some methods may be used in the separation of bioactive peptides in the process of hydrolysis [3];[61]; [62]; [89]. The methods with ultrafiltration membrane systems could be used to get a fraction of peptide hydrolysis by size, to obtain a peptide with the weight of desired molecular [61];[89]. More precise method can be done with nanofiltration. For the same purpose, it can also be done with the method of ion exchange, gel filtration, liquid chromatography (HPLC), reversed-phase liquid chromatography (RP-HPLC), and gel permeation chromatography [78];[90]; [62]. For the payload capacity of biomolecular was stronger, using electromembrane filtration techniques (EMF) [62]. The analysis with Matrix-assisted Laser Desorption / Ionization Time of Flight (MALDI-TOF) mass spectrometric analysis could be used [89].

Some of these methods could be used separately, but also required a combination of two or more methods for the production and isolation of the peptide [62]. HPLC was reported to be typically used with a UV detector or mass spectrometer [89]. Peptide fractions single can be identified using mass spectrometry and protein sequencing, while liquid chromatography can be followed by mass spectrometry (LC-MS / MS) or methods of conventional membrane filtration can also be used [61]; [62]; [91]. Schematically, the process of identification of bioactive peptides are presented in Figure 3.

3.8.1 FTIR. Fourier Transform Infrared Imaging Spectroscopy (FTIR) is a unique method to gather chemical information from biological samples with high spatial resolution (typically ~ 10 m) [92].
Further stated, FTIR can identify complex molecular D2O isotope exchange and sensitively can determine the OH groups related to the strength of hydrogen bonds. FTIR can also determine the molecular structure of the crystal hydrate and exopolysaccharide chains [93]. The FTIR used in the analysis of proteins and peptides are more common through signal absorption infrared (IR). Another spectroscopic method is more specific that can use circular dichroism (CD), ultraviolet absorption and fluorescence spectroscopy, Raman and Nuclear Magnetic Resonance (NMR). In the secondary structure analysis, NMR and Raman Spectroscopy need samples with a high protein concentration and suitable for use for the identification of small molecular weight protein with 200 amino acid residues [94].

![Diagram of bioactive peptides identification]

**Figure 3.** Schema of bioactive peptides identification [61].

Analysis with limited sample CD method needs purification of the protein solution, thus as not to interfere with the absorption of light. Various methods will be able to give a description of the structure, interactions and conformational changes in peptides and proteins. Infrared spectrum can be divided into three main groups: 1) a far-reaching (<400.0 cm⁻¹), and 2) mid-infrared (4000.0 - 400 cm⁻¹) and close (13000.0 - 4000 cm⁻¹). Basically, spectrum 4000-2500 cm⁻¹ is able to identify the structure of OH, CH and NH. Spectrum 2000.0 - 1500 cm⁻¹ to C = C and C = O carbonyl cluster is one of the most easily recognizable absorption in the infrared spectrum. It usually depends on the intensity of spectrum that can catch the band, and depends also on the type of C = O bonds which may occur in the spectrum 1830-1650 cm⁻¹. Nonetheless, metal carbonyls absorbs above 2000 cm⁻¹, C = N bond also occurs in this spectrum and usually stronger [93].

3.8.2 NMR. The Nuclear Magnetic Resonance (NMR) spectroscopy is the method of branch which is based on the fact that many atomic nuclei could be oriented by magnetic strong and would absorb the radiation of radio frequency. The parameters could be measure on the resulting spectral lines (line, intensity, line width, multiplicities and transient versus time) could be interpreted in terms of molecular structure, conformation, molecular motion and other processes [95]. NMR spectra of organic
compounds identified through the position of the absorption peak, also called resonance position or precession frequency. The screening of chemical molecules certainly provides the information of how atomic nuclei in the molecule bound. The detection of clusters of molecules on bioactive peptide compounds by using NMR [96] is presented as in Table 4. NMR is used to determine the profile of meat exudate in the process of withering and food safety control [96]. Several standards of bioactive peptides can be used, among others: taurine (a molecular weight of 125.15 g/mol, a final concentration of 0.5 M), anserine nitrate salt (molecular weight 303.3 g/mol, a final concentration of 0.5 M), L-alanine (molecular weight of 89.09 g/mol, a final concentration of 0.5 M), L-serine (molecular weight of 105.09 g/mol, a final concentration of 0.5 M), L-valine (molecular weight of 117.15 g/mol, a final concentration of 0.5 M), L-leucine (molecular weight 131.18 g/mol, and a final concentration of 0.5 M [95].

| Peak | Compound Abbreviation | Group | 1H (ppm) | Mult./J(µHz) | Assignment Data |
|------|------------------------|-------|----------|-------------|----------------|
| 222  | Inosine phosphate      | CH-2  | 8.51     | S           | 1H; Bibliography |
| 223  | Carnosine              | CH-2 (His) | 8.59 | S           | COSY (7.27) HSQC (136.200) |
| 224  | Anserine               | CH-2 (His) | 8.63 | S           | COSY (7.27) TOCSY (3.870) HSQC (138.000) |
| 225  | Nicotinic acid         | CH-6  | 8.62     | D           | COSY (7.600) TOCSY (8.278.940) HSQC (152.800) |
| 226  | Nicotinic acid         | CH-2  | 8.94     | S           | TOCSY (8.620:8.270:7.60) HSQC (151.40) |

Source: [96]

4. Conclusion

The present review presence of essential amino acids in Baline chicken meat original such as threonine, methionine, valine, phenylalanine, isoleucine, leucine, and lysine. The proportion of amino acids is also different in each animal body location. The result identified more than 170 peptides were released from the main structure of the myofibril (actin, myosin) and sarcoplasmic muscle proteins. Some of these peptides have amino acid sequence potential as antihypertensive compounds or antioxidants. During the identification of peptide produced during the digestion in vitro meat cook, there were 43 kinds of proteins in digestion, 20 specific proteins in the stomach, and 8 types of protein in the intestine [68].

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