Strategies for designing novel functional meat products

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Received 7 February 2006; received in revised form 29 April 2006; accepted 29 April 2006

Abstract

In recent years, much attention has been paid to physiological functions of foods due to increasing concerns for health. Although there has been limited information of physiological functions of meat until recently, several attractive meat-based bioactive compounds, such as carnosine, anserine, l-carnitine, conjugated linoleic acid, have been studied. Emphasizing these activities is one possible approach for improving the health image of meat and developing functional meat products. This article provides potential benefits of representative meat-based bioactive compounds on human health and an overview of meat-based functional products. Strategies for designing novel functional meat products utilizing bioactive peptides and/or probiotic bacteria, is also discussed. This article focuses particularly on the possibility of meat protein-derived bioactive peptides, such as antihypertensive peptides. There are still some hurdles in developing and marketing novel functional meat products since such products are unconventional and consumers in many countries recognize meat and meat products to be bad for health. Along with accumulation of scientific data, there is an urgent need to inform consumers of the exact functional value of meat and meat products including novel functional foods.

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Keywords: Meat; Meat products; Functional foods; Bioactive peptides; Antihypertensive peptides; Probiotics; Prebiotics

1. Introduction

Most people have a concern for their diet from a health aspect. Dietary food guides, such as the USDA food guide pyramid, are tools to inform the public about diet, nutrition and health (Lachance & Fisher, 2005). In the food guide pyramid, meat is categorized as a protein food group along with poultry, fish and eggs. Undoubtedly, meat is a major source of food proteins with high biological value in many countries. Meat is also an excellent source of some valuable nutrients such as minerals and vitamins (Biesalski, 2005; Chan, 2004; Mulvihill, 2004). Some of these nutrients (e.g., iron, vitamin B12, and folic acid) are either not present or have inferior bioavailability in other foods. Regrettably, consumers often associate meat with a negative image that meat contains high fat and red meat is regarded as a cancer-promoting food (Ovesen, 2004a, 2004b; Valsta, Tapanainen, & Mannisto, 2005). Also, intake of sodium chloride, which is added in most meat products during processing, has been linked to hypertension (Ruusunen & Puolanne, 2005). For these reasons, ingestion of meat and meat products is often avoided to reduce the risk of cancer, obesity and other diseases. However, such view disregards the fact that meat plays a critical role in maintenance of human health. Although the importance of meat and meat products in consumer health has been discussed extensively in scientific articles (Arihara, 2004; Biesalski, 2005; Cassens, 1999; D’Amicis & Turrini, 2002; Desmond & Troy, 2004; Enser, 2000; Fernández-Ginés, Fernández-López, Sayas-Barberá, & Pérez-Alvarez, 2005; Garnier, Klont, & Plastow, 2003; Gregory, 2004; Higgs, 2000; Jiménez-Colmenero, Carballo, & Cofrades, 2001, 2006; Kues & Niemann, 2004; Ovesen, 2004a, 2004b; Tarrant, 1998; Valsta et al., 2005; Verbeke, Van Oeckel, Warnants, Viaene, & Boucque, 1999), efforts...
toward better consumer education and information concerning meat ingestion might be still insufficient in many countries.

In recent years, much attention has been paid to the tertiary functions of foods (Dentali, 2002; Hasler, 1988; Heasman & Mellentin, 2001; Pszczola, Katz, & Giese, 2002; Schmidl & Labuza, 2000). Tertiary functions are the roles of food components in preventing diseases by modulating physiological systems. Examples of such functions of foods are anticarcinogenicity, antimutagenicity, antioxidative activity and antiaging activity. Due to increasing concerns for health, efforts have been made by food industries in many countries to develop new foods with tertiary functions. Such foods having tertiary functions are regarded as functional foods. Many food components showing tertiary functions, such as biologically active phytochemicals of vegetables, have been studied. Also, rapid progress has been made in the development of functional foods based on the results of studies on food components providing positive health benefits. For example, many studies aimed at the development of functional dairy products, such as probiotic products, have been carried out (Mattila-Sandholm & Saarela, 2004). Such meat products would open up a new market for healthier meat and meat products. The composition of animal products could be improved through manipulation of animal feed. Several studies have shown that the feeding conditions of animals affect the contents of bioactive components, such as conjugated linoleic acid and L-carnitine, in animal products (Krajcovicova-Kudlackova, Simoniec, Bederova, Babinska, & Beder, 2000; Mir et al., 2004). Such efforts could lead to the creation of differentiated meat and meat products. As representative meat-based bioactive compounds, conjugated linoleic acid, histidyl dipeptides, and L-carnitine are described here briefly.

### 2. Attractive meat-based bioactive compounds

Chemicals found as natural components of foods or other ingestible forms that have been determined to be beneficial to the human body in preventing or treating one or more diseases or improving physiological performance are known as nutraceuticals (Wildman, 2000a, 2000b). In addition to various nutraceutical compounds found in plants (e.g., vegetables), several attractive meat-based bioactive substances, such as conjugated linoleic acid, carnosine, anserine, L-carnitine, glutathione, taurine and creatine, have been studied for their physiological properties (Arihara, 2004). Emphasizing these physiological activities originating from meat is one possible approach for designing healthier meat and meat products. The composition of animal products could be improved through manipulation of animal feed. Several studies have shown that the feeding conditions of animals affect the contents of bioactive components, such as conjugated linoleic acid and L-carnitine, in animal products (Krajcovicova-Kudlackova, Simoniec, Bederova, Babinska, & Beder, 2000; Mir et al., 2004). Such efforts could lead to the creation of differentiated meat and meat products. As representative meat-based bioactive compounds, conjugated linoleic acid, histidyl dipeptides, and L-carnitine are described here briefly.

#### 2.1. Conjugated linoleic acid

Conjugated linoleic acid (CLA), which was initially identified as an anticarcinogenic compound in extracts of grilled beef, is composed of a group of positional and geometric isomers of octadecadienoic acid (Gnadig, Xue, Berdeaux, Chardigny, & Sebedio, 2000; Nagao & Yanagita, 2005; Watkins & Yong, 2001). Since rumen bacteria convert linoleic acid to CLA by their isomerase, it is most abundant in fat of ruminant animals. After its absorption in a ruminant animal, CLA is transported to the mammary tissue and muscles. Beef fat contains 3–8 mg of CLA per gram of fat. The CLA content in meat is affected by several factors, such as breed, age and feed composition (Dhiman, Nam, & Ure, 2005). The CLA content of foods is also increased by heating, such as cooking and processing (Herveirah, Humeid, & Al-Ismail, 2005). As described later, probiotic bacteria promote the formation of CLA in fermented milk products (Sieber, Collomb, Aeschlimann, Eyer, & Jelen, 2004). The most common CLA isomer found in beef is octadec-9,11-dienoic acid. Since this fatty acid has anticarcinogenic activity, much interest has been shown in this compound. Recent epidemiological studies have suggested that high intakes of high-fat dairy foods and CLA may reduce the risk of colorectal cancer (Larsson, Bergkvist, & Wolk, 2005). In addition to its anticarcinogenic property, CLA has antioxidative and immunomodulative properties (Azain, 2003). CLA may also play a role in the control of obesity, reduction of the risk of diabetes and modulation of bone metabolism.

### 3. Reformulation of meat products

#### 3.1. Incorporation of functional ingredients

Although all aspects from animal production to product processing should be considered for designing healthier products, this article focuses on functional meat products mainly from the viewpoint of food processing. Potential benefits of representative meat components on human health are described, and an overview of meat-based functional products is presented. The development of novel functional meat products utilizing bioactive peptides and/or probiotic bacteria is also discussed. This article focuses particularly on the possibility of meat protein-derived bioactive peptides (e.g., antihypertensive peptides).
2.2. Histidyl dipeptides

Consumption of antioxidant-rich foods, such as fruits and vegetables, has been shown to have a preventative effect on oxidative damage in the body (Lindsay, 2000). This beneficial action of food is attributed to neutralization and reduced release of free radicals by the antioxidant potency of various compounds (Langseth, 2000). Ascorbic acid, vitamin E, β-carotene and polyphenolic compounds are representative food-derived antioxidants. Such compounds may decrease the risk of many diseases including cancer. Several endogenous antioxidants (e.g., tocopherols, ubiquinone, carotenoids, ascorbic acid, glutathione, lipoic acid, uric acid, spermine, carnosine, anserine) in skeletal muscle have been studied (Decker, Livisay, & Zhou, 2000). Both carnosine (β-alanyl-l-histidine) and anserine (N-β-alanyl-l-methyl-l-histidine) are antioxidative histidyl dipeptides and the most abundant antioxidants in meats. The concentrations of carnosine in meat range from 500 mg per kg of chicken thigh to 2700 mg per kg of pork shoulder. On the other hand, anserine is especially abundant in chicken muscle. Their antioxidant activities may result from their ability to chelate transition metals such as copper (Brown, 1981) These antioxidative peptides have been reported to play many roles, such as prevention of diseases and aging related to oxidative stress (Hipkiss & Brownson, 2000; Hipkiss et al., 1998). Recent study demonstrated the bioavailability of carnosine by determining its concentration in human plasma after ingestion of beef (Park, Volpe, & Decker, 2005).

2.3. l-Carnitine

l-Carnitine, β-hydroxy-γ-trimethyl amino butyric acid, is biosynthesized in human body, chiefly in the liver and kidneys (Held, 2005). It transports long-chain fatty acids across the inner mitochondrial membranes, where they are processed by β-oxidation to produce biological energy. Also, l-carnitine can help energy production in muscle, when we are doing hard exercise. It is detected in skeletal muscle of various animals (Shimada et al., 2005), especially abundant in beef (e.g., 1300 mg per kg of the thigh). In addition to the assistance in producing energy, l-carnitine has some biological activities (e.g., lowering levels of cholesterol) in our body (Seccombe, James, Hahn, & Jones, 1987; Shimura & Hasegawa, 1993). l-carnitine helps the body to absorb calcium to improve skeletal strength and chromium picolinate to help build lean muscle mass. Recent study demonstrated that l-carnitine blocked apoptosis and prevented skeletal muscle myopathy in heart failure (Vescovo et al., 2002). A drink product containing l-carnitine, which is marketed in the United States, is advertised as having several beneficial effects, such as maintenance of stamina and fast recovery from fatigue. Also, a product containing much l-carnitine and carnosine, which is used as a functional food ingredient, has been marketed in Japan. This product is made from a by-product of corned beef.

3. Overview of functional meat products

3.1. Functional foods and FOSHU products

The term ‘functional food’ was coined in Japan in the early 1980s (Gibson & Williams, 2000). There is no exact definition of functional food and functional food has as many definitions as the number of authors referring to it (Roberfroid, 2000). An example of typical and simple one is ‘processed foods having disease-preventing and/or health-promoting benefits in addition to their nutritive value’. This term often overlaps with nutraceuticals, medicinal foods and designer foods. Japan is also the first country to have formulated a specific regulatory approval process for functional foods (Arihara, 2004; Eve, 2000). In 1991, the concept of foods for specified health use (FOSHU) was established. According to the Japanese Ministry of Health and Welfare, FOSHU are foods that, based on knowledge of the relationship between foods or food components and health, are expected to have certain health benefits and have been licensed to bear labeling claiming that a person using them may expect to obtain that health use through the consumption of these foods. Most FOSHU products utilize functional ingredients to help in the maintenance of a healthy human body. Representative functional ingredients used for FOSHU products are oligosaccharides, dietary fibers, lactic acid bacteria, soy proteins, sugar alcohols, peptides, calcium, iron, polyphenols, glycosides, sterol esters and diacylglycerols. As of April 2006, nearly 600 FOSHU products have been approved in Japan. Although regulations for functional foods have not yet been well established in many countries (Eve, 2000; Hutt, 2000), this situation has not been a significant barrier to the development of novel functional products in the food industry. In the United States and European countries, markets for functional foods have been expanding rapidly. Health-conscious consumers have made functional foods the leading trends in the food industry.

3.2. Functional meat products

Although numerous low-fat meat products have been developed in many countries, the meat industry in most countries has been hesitant to adopt the functional trend and to introduce additional physiologically functional properties into meat products. Utilization of functional ingredients is one approach to the development of functional meat products. Such ingredients include vegetable proteins, fibers, antioxidants, probiotics and prebiotics. In fact, dietary fiber from oats, sugar beet, soy beans, apples, peas, and probiotic lactic acid bacteria have been used in the formulation of meat products (Fernández-Ginéès et al., 2005; Jiménez-Colmenero, Reig, & Toldrá, 2006). The following nine FOSHU meat products, four sausage products, one sliced ham product, two hamburger steak products and two meat ball products, have
been approved in Japan (Arihara, 2004). Dietary fibers or soy proteins have been utilized as functional ingredients in these FOSHU products. Pork sausage products containing indigestible dextrin, a water-soluble dietary fiber made from potato starch, are claimed to have beneficial effects on intestinal disorders. Another example is a low-fat sausage product containing soy proteins. It is claimed that an acceptable blood cholesterol level can be maintained by consumption of these products. Other than the approved FOSHU products, several meat products with additional fibers, proteins and calcium have been marketed in Japan. Vegetable proteins have been used in meat products for their nutritional value and functional value. Soy proteins are typical of such proteins with health-enhancing activity. They are thought to be effective for preventing cardiovascular diseases, cancer and osteoporosis. A reduced-fat sausage formulated with a modified potato starch has been marketed in the United States (Pszczola, 2002). Such dietary fiber contributes to the improvement of intestinal microflora and the reduction of fat intake.

A group of meat products named Aplilight (e.g., pork sausages, hamburger steak, meat balls) have notable beneficial effects for consumers. These products are made with a formulation that eliminates ingredients causing allergic symptoms. Meat is less allergenic than common allergy-inducing foods, such as so-called ‘big eight’ namely milk, eggs, soya, wheat, peanuts, shellfish, fruits and tree nuts (Tarrant, 1998; Tanabe & Nishimura, 2005). However, since many meat products contain vegetable, egg and/or milk proteins, people with allergies are often affected by allergens in such ingredients. These products have been approved as allergen-free products by the Japanese Ministry of Health and Welfare.

As described above, the functional properties of meat products can be improved by adding ingredients considered beneficial for health or by eliminating components that are considered harmful. The items of functional modification in meat and meat products listed below have recently been reviewed by Fernández-Ginés et al. (2005):

- Modification of fatty acid and cholesterol levels in meat.
- Addition of vegetal oils to meat products.
- Addition of soy.
- Addition of natural extracts with antioxidant properties.
- Sodium chloride control.
- Addition of fish oils.
- Addition of vegetal products.
- Addition of fiber.

A more recent review by Jiménez-Colmenero et al. (2006) is very informative. They discussed the issue of development of functional meat products from the viewpoints of both ‘animal production strategies for improving the nutritional profiles of raw meat’ and ‘processing strategies for developing functional meat products’.

4. Utilization of meat protein-derived bioactive peptides

As described above, several attractive meat-based bioactive substances have been studied extensively. In addition to these compounds, meat protein-derived peptides are another group of promising functional components of meat (Arihara, 2006). Although the activities of these peptides in the sequences of proteins are latent, they are released by proteolytic enzymes (i.e., muscle, microbial, and digestive proteinases). Therefore, meat proteins have possible bioactivities beyond a nutritional source of amino acids alone.

Enzymatic hydrolyzates of food proteins (e.g., milk caseins) contain various physiologically functional peptides (Korhonen & Pihlanto, 2003; Meisel, 1998; Mine & Shahidi, 2005). Representative bioactivities of such peptides are:

- Antihypertensive (ACE inhibitory);
- Antioxidative;
- Opioid agonistic;
- Immunomodulatory;
- Antimicrobial;
- Prebiotic;
- Mineral-binding;
- Antithrombic;
- Hypocholesterolemic.

Although information on bioactive peptides generated from meat proteins is still limited, there is a possibility of utilizing such components for developing novel functional meat products and food ingredients.

4.1. Generation of peptides from meat proteins

Most proteins contain bioactive sequences, but those sequences are inactive within the parent proteins. Active peptide fragments are released from native proteins only via proteolytic digestion. Once such peptides are liberated, they can act as regulatory compounds. During gastrointestinal proteolysis, bioactive peptides would be generated from food proteins. Ingested proteins are attacked by various digestive enzymes, such as pepsin, trypsin, chymotrypsin, elastase and carboxypeptidase (Pihlanto & Korhonen, 2003).

The content of peptides in meat increases during postmortem aging. Changes in oligopeptide levels occur during the storage of beef, pork and chicken (Nishimura, Rhue, Okitani, & Kato, 1988). Oligopeptide levels increase in all meats during storage. During aging or storage, meat proteins are hydrolyzed by muscle endogenous proteases, such as calpains and cathepsins (Koohmaraie, 1994). Although such enzymatic hydrolysis contributes to improvement of sensory properties of meat, such as texture, taste and flavor, there has been no report on the generation of bioactive peptides in meat during postmortem aging. However, our preliminary study showed an increase in angiotensin I-converting enzyme (ACE) inhibitory activity of beef during storage (unpublished data).
Proteolytic reactions that occur during fermentation of raw sausages and dry-cured ham have been studied extensively (Toldrá, 2004; Toldrá & Flores, 1998). Primarily, meat proteins are degraded into peptides by endogenous enzymes during fermentation of meat products. Since most bacteria (e.g., lactobacilli) grown in fermented meat products have only weak proteolytic activity, the degradation of proteins is not greatly affected by the bacteria. However, lactic acid bacteria influence protein degradation by causing a decrease in pH, which results in increased activity of muscle proteases (Kato et al., 1994). During fermentation of sausages, the contents of peptides and amino acids reach about 1% dry matter of products (Dainty & Blom, 1995). Although there has been no report on the generation of bioactive peptides in fermented meat products, we measured the ACE inhibitory activities of extracts of several European fermented sausages and found that activity levels of all extracts were higher than those of extracts obtained from nonfermented pork products (unpublished data). Also, ACE inhibitory and antihypertensive activities were experimentally generated from porcine skeletal muscle proteins by lactic acid bacteria (Arihara, Nakashima, Ishikawa, & Itoh, 2004).

Utilizing commercial proteases is an efficient way of releasing bioactive peptides from food proteins. Many bioactive peptides have been experimentally generated in this way (Korhonen & Pihlanto, 2003; Pihlanto & Korhonen, 2003). Proteinases from animal, plant, and microbial sources have been used for the digestion of food proteins. Several proteases have been utilized for the generation of bioactive peptides from meat proteins. In the meat industry, proteolytic enzymes have been used for meat tenderization (Dransfield & Etherington, 1981). The most commonly used enzymes for meat tenderization are the plant enzymes papain, bromelain, and ficin. In meat treated with enzymatic tenderization, peptides having bioactivities could be generated. On the other hand, effects of commercial proteases on protein breakdown and sensory characteristics of dry fermented sausages have been investigated (Bruna, Fernandez, Hierro, Ordonez, & de la Hoz, 2000). Such treatment would also generate bioactive peptides in meat products.

### 4.2. Antihypertensive peptides

The most extensively studied bioactive peptides generated from food proteins are angiotensin I-converting enzyme (ACE) inhibitory peptides (Meisel, Walsh, Murry, & FitzGerald, 2005; Vermeirssen, Camp, & Verstraete, 2004). ACE inhibitory peptides have attracted much attention because of their ability to prevent hypertension. These peptides could be used as potent functional food additives and would constitute a natural and healthier alternative to ACE inhibitory drugs.

ACE is a dipeptidylcarboxypeptidase that converts an inactive form of decapptide, angiotensin I, to a potent vasoconstrictor, octapeptide angiotensin II, and inactivates bradykinin, which has a depressor action (Li, Le, Shi, & Shrestha, 2004). Therefore, it is capable of suppressing the elevation of blood pressure by inhibiting the catalytic action of ACE. Many food protein-derived peptides are known to have an inhibitory effect on ACE. Some of these peptides have been reported to show antihypertensive effects in spontaneously hypertensive rats (SHR) by oral administration.

Among the bioactive peptides derived from meat proteins, ACE inhibitory peptides have been studied most extensively (Arihara, 2006; Vercruysse, Van Camp, & Smagghe, 2005). Fujita, Yokoyama, and Yoshikawa (2000) isolated ACE inhibitory peptides (Leu-Lys-Ala, Leu-Lys-Pro, Leu-Ala-Pro, Phe-Gln-Lys-Pro-Lys-Arg, Ile-Val-Gly-Arg-Arg-Arg-His-Gln-Gly, Phe-Lys-Gly-Arg-Tyr-Tyr-Pro, Ile-Lys-Trp) generated from chicken muscle proteins by thermolysin treatment. Arihara, Nakashima, Mukai, Ishikawa, and Itoh (2001) reported ACE inhibitory peptides in enzymatic hydrolyzates of porcine skeletal muscle proteins. Porcine muscle proteins were hydrolyzed by eight kinds of proteases. Among the digests of muscle proteins, thermolysin digest showed the most potent inhibitory activity. Two ACE inhibitory peptides (Met-Asn-Pro-Pro-Lys and Ile-Thr-Thr-Asn-Pro), which are found in the sequence of myosin heavy chain, have been identified. These peptides showed antihypertensive activity when administered orally to SHR (Nakashima, Arihara, Sasaki, Ishikawa, & Itoh, 2002). Katayama et al. (2003a, 2003b, 2004) utilized porcine skeletal muscle and respective muscle proteins for proteolytic digestion in a series of studies. They isolated a corresponding peptide (Arg-Met-Leu-Gly-Gln-Thr-Pro-Thr-Lys) from porcine tropomin C hydrolyzed with pepsin. Saiga et al. (2003a) utilized porcine skeletal muscle and respective muscle proteins for proteolytic digestion in a series of studies. They isolated a corresponding peptide (Arg-Met-Leu-Gly-Gln-Thr-Pro-Thr-Lys) from porcine tropomin C hydrolyzed with pepsin. Saiga et al. (2003a) reported antihypertensive activity of Aspergillus protease-treated chicken muscle extract in SHR. Furthermore, they isolated four ACE inhibitory peptides from the hydrolyzate. Three of those four peptides possessed a common sequence, Gly-X-Gly-X-Gly-X-Gly-X, which is homologous with that of collagen. More recently, Jang and Lee (2005) assayed ACE inhibitory activities of several enzymatic hydrolyzates of sarcoplasmic protein extracts from beef rump. An ACE inhibitory peptide (Val-Leu-Ala-Gln-Tyr-Lys) was purified from the hydrolyzate with the highest ACE inhibitory activity obtained by using the combination of thermolysin and proteinase A.

### 4.3. Other promising bioactive peptides

Information on meat protein-derived bioactive peptides other than ACE inhibitory peptides is still limited (Arihara, 2006). Several bioactive peptides that have been derived from meat proteins and bioactive peptides that might be obtained from meat proteins in the future are discussed here briefly.

As described above, both carnosine and anserine are endogenous antioxidative dipeptides found in skeletal muscle. Apart from endogenous nonprotein peptides, several antioxidative peptides have been reported to be generated from meat proteins by enzymatic digestion. Saiga, Tanabe,
and Nishimura (2003b) reported that hydrolyzates obtained from porcine myofibrillar proteins by protease treatment (papain or actinase E) exhibited high levels of antioxidative activity in a linolenic acid peroxidation system. Among five antioxidative peptides identified from papain hydrolyzate, Asp-Ala-Gln-Glu-Lys-Leu-Glu, corresponding to a part of the sequence of porcine actin, showed the highest level of activity. Recently, Arihara et al. (2005) investigated antioxidative activities of enzymatic hydrolyzates of porcine skeletal muscle actomyosin using a hypoxanthine-xanthine oxidase system as the source of superoxide anion. Three antioxidative peptides were isolated from a papain-treated hydrolyzate of pork actomyosin and they were sequenced as Asp-Leu-Tyr-Ala, Ser-Leu-Tyr-Ala, and Val-Trp. In addition to antioxidative activity in vitro, these peptides showed physiological activity in vivo. Each of these peptides had an antifatigue effect when orally administered to mice in an experiment using a treadmill.

Opioid peptides are defined as peptides that have an affinity for an opiate receptor as well as opiate-like effects (Pihlanto & Korhonen, 2003). Basically, opioid peptides have effects on the nervous system. They also influence gastrointestinal functions. Examples of typical opioid peptides are endorphins, enkephalins, and prodynorphin. All of these typical opioid peptides have the same N-terminal sequence, Tyr-Gly-Gly-Phe. Several opioid peptides derived from food proteins have been reported. The N-terminal sequence of most of these peptides is Tyr-X-Phe or Tyr-X1-X2-Phe. The N-terminal tyrosine residue and the presence of an aromatic amino acid at the third or fourth position form a critical structure that fits with the binding site of opioid receptors (Pihlanto-Leppäla, 2001). There has been no report on the generation of opioid peptides from meat proteins. However, since opioid sequences are thought to be present in the sequences of muscle proteins, it should be possible to find opioid peptides in meat proteins by proteolytic treatment. Bovine blood hemoglobin is regarded as a minor component of meat and meat products. However, in some meat products, such as blood sausage, hemoglobin is a major component. Investigation of hemoglobin peptic hydrolyzate has revealed the presence of biologically active peptides with affinity for opioid receptors (Nyberg, Sanderson, & Glämsta, 1997; Zhao, Garreau, Sannier, & Piot, 1997).

Many studies have shown that immunomodulatory sequences exist within food proteins (Pihlanto & Korhonen, 2003). Immunomodulating peptides have been discovered in enzymatic hydrolyzates of proteins from various foods, such as milk, eggs, soybeans, and rice. However, to date, meat protein-derived immunomodulating peptides have not been reported. In addition to bioactive peptides described here, several bioactive peptides (i.e., antimicrobial, prebiotic, mineral-binding, antithrombotic, and hypocholesterolemic peptides) have been found from enzymatic hydrolyzates of various food proteins (Mine & Shahidi, 2005). It is expected that interest will be shown in research aimed at finding such meat protein-derived peptides.

4.4. Utilization of peptides for meat products

Bioactive peptides derived from meat proteins are promising candidates for ingredients of functional foods, including meat products. Although bioactive peptides, such as ACE inhibitors, have not yet been utilized in the meat industry, meat products with such activity could open up a new market. Several food products containing ACE inhibitory peptides, such as sour milk and soup products, have been successfully marketed for hypertensives (Arihara, 2006). There are two commercially available dairy products containing Ile-Pro-Pro and Val-Pro-Pro, which are generated from milk protein by fermentation with Lactobacillus helveticus (Nakamura, Yamamoto, Sakai, & Takano, 1995; Seppo, Jauhiaine, Poussa, & Korpela, 2003). Calpis Amiel-S drink has been approved as a FOSHU in Japan. The Finnish fermented milk drink Evolution, developed by Valio Ltd., contains the same tripeptides as those in Amiel-S. Both products have been tested in studies using SHR and in clinical human trials. Another beverage containing milk protein-derived ACE inhibitory peptides was developed in Japan and has been approved as a FOSHU (Sugai, 1998). The thermolysin digest of dried bonito containing antihypertensive peptides has also been used in a soup product in Japan. A FOSHU sour milk product utilizing caseinphosphopeptide (CPP) has been developed in Japan. CPP is a milk protein-derived peptide acting as mineral trappers resulting in enhancement of the absorption efficiency of calcium (Gagnaire, Pierre, Molle, & Leoni, 1996). In the future, hydrolyzates of meat proteins and their corresponding bioactive peptides might be utilized for physiologically functional foods.

Generation of bioactive peptides in meat products, such as fermented meat products, is a possible direction for introducing physiological functions. Bioactive peptides would be generated in fermented meat products since meat proteins are hydrolyzed by proteolytic enzymes during fermentation and storage. Developing functional fermented meat products could be a good strategy in the meat industry. On the other hand, rediscovery of traditional fermented meats as functional foods is also of interest. Many traditional fermented foods, such as fermented dairy products, have been rediscovered as functional foods (Farnworth, 2003). Numerous physiologically active components, including bioactive peptides, have been discovered in these traditional fermented foods. For these reasons, traditional fermented meats are attractive targets for developing new functional meat products.

5. Development of probiotic meat products

5.1. Probiotics and probiotic foods

The use of probiotic lines is another attractive approach for designing functional meat products. Probiotics are defined as ‘live microorganisms which, when administered in adequate amounts (as part of food), confer a health ben-
efit on the host’ (Stanton et al., 2003). Representative probiotic bacteria are intestinal strains of *Lactobacillus* and *Bifidobacterium*. According to the scientific basis for isolation and defining probiotic bacteria, desirable properties of such bacteria (Brass & Schiﬃn, 2000) are:

- Human origin.
- Resistance to acid and bile toxicity.
- Adherence to human intestinal cells.
- Colonisation of the human gut.
- Antagonism against pathogenic bacteria.
- Production of antimicrobial substances.
- Immune modulation properties.
- History of safe use in humans.

In the dairy industry, the main approach for improving the beneﬁcial physiological properties of products has been the development of probiotic lines of traditional fermented products (Mattila-Sandholm & Saarela, 2000). For example, *Lactobacillus rhamnosus* strain GG, a representative strain of probiotic lactic acid bacteria, has been applied to various fermented dairy products (Saxelin, 1997). Products containing the GG strain have been marketed in 30 countries under license from a Finnish company (Valio Ltd.). It has been reported that both probiotic bacteria and probiotic products have diverse functions (Agrawal, 2005; Mattila-Sandholm & Saarela, 2000; Stanton et al., 2003; Työppöne, Petäjä, & Mattila-Sandholm, 2003), such as:

- Modulation of intestinal ﬂora.
- Prevention of diarrhea.
- Improvement of constipation.
- Lowering faecal enzyme activities.
- Lowering plasma cholesterol level.
- Modulation of immune responses.
- Prevention and treatment of food allergies.
- Prevention of cancer occurrence.
- Adjuvant in *Helicobacter pylori* treatment.

### 5.2. Probiotic meat products

In recent years, the possibility of development of probiotic meat products has been discussed in the ﬁeld of meat science and industry (Arihara, 2004; Arihara et al., 1998; Erkkilä & Petäjä, 2000; Hammes, Haller, & Gänzle, 2003; Hugas & Monfort, 1997; Incze, 1998; Lücke, 2000; Pennacchia et al., 2004; Työppöne et al., 2003). By using probiotic bacteria, potential health beneﬁts can be introduced to meat products. Target products with probiotic bacteria are mainly dry sausages, which are processed by fermentation without heat treatment. Technically, it has already become possible to produce probiotic meat products. German and Japanese producers have developed meat products containing human intestinal lactic acid bacteria. In 1998, a German producer launched a salami product containing three intestinal lactic acid bacteria strains (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium* spp.). This product is claimed to have health beneﬁts and is thought to be the ﬁrst probiotic-like salami product to be marketed. Shortly after, in the same year, a Japanese producer began to market a new range of meat spread products fermented with probiotic lactic acid bacteria (*Lactobacillus rhamnosus* FERM P-15120). *L. rhamnosus* FERM P-15120 has been selected from the collection of human intestinal lactobacilli for a desirable probiotic meat starter culture (Sameshima et al., 1998). Regulations for the maintenance of product safety in Japan require the use of 200 ppm nitrite and 3.3% sodium chloride and processing at a temperature below 20°C for meat fermentation. In addition to the probiotic properties, screening was carried out to clear these hurdles.

Bunte, Hertel, and Hammes (2000) and Jahreis et al. (2002) have studied the utilization of probiotic lactic acid bacteria (*Lactobacillus paracasei*) as a starter culture for a moist type of fermented sausage. Human studies with healthy volunteer revealed that the consumption of such product with *L. paracasei* brought some probiotic effects. After 4 week ingestion of the product, the values of CD4 T helper cells were elevated and phagocytosis index increased. Also, the expression of CD54 (glycoprotein responding to inﬂammatory regulators) decreased and the titre of antibodies against oxidized LDL increased.

Since the effect of probiotic bacteria on the formation of CLA in media and fermented milk products has been demonstrated (Alonso, Cuesta, & Gilliland, 2003; Coakley et al., 2003; Sieber et al., 2004; Xu, Boylston, & Glatz, 2005), such effect would be also expected in fermented meat products. As described above, CLA is an attractive bioactive compound for designing functional products. Such novel functional fermented meat products with probiotics and CLA would have a market.

### 5.3. Prebiotics and synbiotics

In the food industry, much interest has also been shown in the utilization of prebiotics. Prebiotics are deﬁned as ‘non-digestible food ingredients that beneﬁcially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improve the health of the host’ (Gibson & Roberfroid, 1995). Several prebiotic substances are known to enhance the activity of probiotic bacteria (Holzapfel & Schillinger, 2002; Playne, Bennett, & Smithers, 2003). Oligosaccharides and dietary ﬁbers are representative prebiotic substances used for processed foods. Such prebiotics have been utilized for several meat products in Japan. Some desirable attributes in functionally enhanced prebiotics listed by Rastall (2000) are:

- Targeting at speciﬁc probiotics (*Lactobacillus* and/or *Bifidobacterium*).
- Active at low dosage and lack of side effects.
− Persistence through the colon.
− Protection against colon cancer.
− Enhance the barrier effect against pathogens.
− Inhibit adhesion of pathogens.

In addition to oligosaccharides and dietary fibers, the presence of prebiotic peptides has been suggested. Many studies have shown that hydrolyzates of milk proteins exhibited stimulation of the growth of lactic acid bacteria and bifidobacteria (Brody, 2000). However, the main growth factors have been identified to be the sugar moieties of glycolysated peptides. Nonglycosylated peptides derived from proteins have recently been identified by Lieple et al. (2002). They first reported nonglycosylated peptides that selectively stimulate the growth of bifidobacteria. Recently, we found that the hydrolyzate of porcine skeletal muscle actomyosin digested by papain enhanced the growth of *Bifidobacterium* strains in media (Arihara, Ishikawa, & Itoh, 2006). One of the corresponding prebiotic peptides was purified from the hydrolyzate and identified as a tripeptide (Glu-Leu-Met).

A mixture of probiotics and prebiotics, known as synbiotics which are proposed by Gibson and Roberfroid (1995), is utilized for many foods, such as fermented dairy products, in European countries and Japan. Sybiotics are foods containing both probiotic bacteria and prebiotic ingredients to provide a diet in which the intestinal growth of the probiotic bacteria is enhanced by the presence of the prebiotic, thus promoting the chance of the probiotic bacteria is enhanced by the presence of the prebiotic. The mixture of *Lactobacillus acidophilus* and *Bifidobacterium animalis* was reported by Lieple et al. (2002). They first reported nonglycosylated peptides that selectively stimulate the growth of bifidobacteria. Recently, we found that the hydrolyzate of porcine skeletal muscle actomyosin digested by papain enhanced the growth of *Bifidobacterium* strains in media (Arihara, Ishikawa, & Itoh, 2006). One of the corresponding prebiotic peptides was purified from the hydrolyzate and identified as a tripeptide (Glu-Leu-Met).

6. Concluding remarks

There are still some hurdles in developing and marketing novel functional meat products. Because such products are unconventional and consumers in many countries recognize meat and meat products to be bad for health, unlike milk and dairy products. Therefore, further studies are required to demonstrate the clear benefits of meat and meat components for human health. Along with accumulation of scientific data, there is an urgent need to inform consumers of the exact physiological value of meat and meat products including novel functional meat products. Since food safety is another critical aspect of food quality, efforts should also be directed to ensure that new functional meat products are safe. Without proof of product safety, most consumers would hesitate to adopt new foods in their diet.

References


