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PROTEOLYTIC ENZYMES FOR IMPROVING THE PROPERTIES OF MEAT PRODUCTS FROM NON-TRADITIONAL RAW MATERIALS

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In this scientific article the influence of enzymatic treatment on the quality of meat products from non-traditional raw materials (camelina, horse meat, mutton, beef) was studied. Bromelain (0.5-0.2%), papain (0.1-0.3%) and ficin (0.1-0.5%) as well as their combination were used as enzymes. To improve functional and technological characteristics, a multicomponent brine containing plant components (sea buckthorn powder, pumpkin powder and goji berry extract, rosemary extract), plant enzymes, phosphates and protein hydrolysates were used. The results showed that the use of enzymes contributed to the softening of the meat structure, improving the juiciness and tenderness of the product. The most pronounced effect was observed in samples treated with papain, while ficin had the greatest effect on mutton and beef. The study of pH dynamics showed that without enzyme treatment, pH increased to 6.67 after 5 days, indicating initial signs of spoilage. At the same time, the combined application of enzymes kept the pH at a stable level (6.20), which helped to extend the shelf life of the product. Thus, the use of proteolytic enzymes in combination with multicomponent brine improves the texture, organoleptic characteristics and functional and technological properties of meat products, which can be recommended for industrial production.

Keywords: enzyme preparations, goji berry extract, dietary fibre, camel meat, inoculation.

ПРОТЕОЛИТИЧЕСКИЕ ФЕРМЕНТЫ ДЛЯ УЛУЧШЕНИЯ СВОЙСТВ МЯСНЫХ ПРОДУКТОВ ИЗ НЕТРАДИЦИОННОГО СЫРЬЯ

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В данной научной статье изучено влияние ферментативной обработки на качество мясных продуктов из нетрадиционного мясного сырья (верблюжатина, конина, баранина, говядина). В качестве ферментов использовали бромелаин (0,2–0,5%), папаин (0,1–0,3%) и фицин (0,1–0,5%), а также их комбинацию. Для улучшения функционально-технологических характеристик применяли многокомпонентный рассол, содержащий растительные компоненты (порошок облепихи, тыквенный порошок и экстракт ягод годжи, экстракт розмарина), растительные ферменты, фосфаты и белковые гидролизаты. Результаты показали, что использование ферментов способствовало размягчению

структуры мяса, улучшению сочности и нежности продукта. Наиболее выраженный эффект наблюдался у образцов, обработанных папаином, тогда как фицин оказывал наибольшее влияние на баранину и говядину. Исследование динамики pH показало, что без ферментной обработки pH увеличивался до 6,67 через 5 дней, что свидетельствовало о начальных признаках порчи. В то же время комбинированное применение ферментов позволяло удерживать pH на стабильном уровне 6,20, что способствовало продлению срока хранения продукта. Таким образом, применение протеолитических ферментов в сочетании с многокомпонентным рассолом улучшает текстуру, органолептические показатели и функционально-технологические свойства мясных продуктов, что может быть рекомендовано для промышленного производства.

Ключевые слова: ферментные препараты, экстракт ягод годжи, пищевые волокна, верблюжатина, инъецирование.

ДӘСТҮРЛІ ЕМЕС ШИКІЗАТ НЕГІЗІНДЕ ЕТ ӨНІМДЕРІНІҢ ҚАСИЕТТЕРІН ЖАҚСАРТУ МАҚСАТЫНДА ПРОТЕОЛИТИКАЛЫҚ ФЕРМЕНТТЕРДІ ПАЙДАЛАНУ

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Бұл ғылыми мақалада ферментативті өңдеудің дәстүрлі емес шикізаттан (түйе еті, жылқы еті, қой еті, сиыр еті) алынған ет өнімдерінің сапасына әсері зерттелді. Фермент ретінде бромелаин (0,5–0,2%), папаин (0,1–0,3%) және фицин (0,1–0,5%), сондай-ақ олардың комбинациясы қолданылды. Функционалды-технологиялық сипаттамаларын жақсарту үшін өсімдік компоненттері (шырғанақ ұнтағы, асқабақ ұнтағы және годжи жидектерінің сығындысы, гүлшетен сығындысы), өсімдік ферменттері, фосфаттар және ақуыз гидролизаттары бар көпкомпонентті тұздық қолданылды. Зерттеу нәтижелері ферменттерді қолдану еттің құрылымын жақсартып, шырындылығы мен жұмсақтылығын арттыруға ықпал ететінін көрсетті. Ең айқын әсер папаинмен өңделген үлгілерде байқалды, ал фицин қой еті мен сиыр етіне анағұрлым күшті әсер етті. pH динамикасын зерттеу ферменттік өңдеусіз pH 5 күн ішінде 6,67-ге дейін артқанын көрсетті, бұл еттің бұзылуының бастапқы белгілеріне сәйкес келеді. Сонымен қатар, ферменттердің комбинациясы pH деңгейін тұрақты деңгейде (6,20) ұстап тұруға мүмкіндік берді, бұл ет өнімінің сақтау мерзімін ұзартуға ықпал етті. Осылайша, протеолитикалық ферменттерді көпкомпонентті тұздықпен бірге қолдану ет өнімдерінің құрылымын, органолептикалық көрсеткіштерін және функционалды-технологиялық қасиеттерін жақсартады, өнеркәсіптік өндірісте қолдануға ұсынылады.

Негізгі сөздер: ферменттік препараттар, годжи жидегі экстракты, тағамдық талшықтар, түйе еті, инъекциялау.

Introduction

The growing standard of living of the population in conditions of deficiency of proteins of animal origin has caused intensive development of a new direction in the technology of meat products, which consists of the optimal combination of both meat and non-meat (primarily vegetable) protein-containing food components to obtain high-quality and biologically nutritious food products [1].

Plant-protein compositions provide the formation of organoleptic characteristics and structure formation of meat and vegetable products; they are good surfactants and reduce the interfacial tension of minced meat. Functional properties of high-protein vegetable ingredients are reduced to their stability during heat treatment, ability to form gels and increase moisture- and fat-binding ability of the meat system as a whole. These properties should be taken into account when selecting certain protein plant ingredients and brought in line with the properties of the raw materials used [1].

World experience shows the advisability of using enzyme preparations of plant, animal and microbiological origin, which have proteolytic activity and are able to partially hydrolyse meat proteins with an increased content of connective tissue [2].

Improvement of taste, aroma and consistency of meat, stabilisation of its colour, acquisition of specific properties in the process of technological processing largely depend on the content of enzymes in meat. Meanwhile, muscles of farm animals are characterised by low concentration of intracellular enzymes. Some anatomical parts of the carcass are characterised by an increased content of connective tissue. This causes the toughness of such meat and its slow ripening [3].

Enzyme preparations introduced into the raw material provide an analogous effect of protein structure transformation to the autolytic one, while the meat ripening process under their influence is 3-5 times faster and ends in a shorter time. Enzyme preparations differ in specificity of influence on the basic proteins of meat - myosin, collagen and elastin. Under the influence of enzymes there are significant changes in meat proteins and, accordingly, the system of extractive substances, which ultimately predetermines the formation of the required consistency (tenderness), the level of water-binding and adhesive ability, taste and odour.

This article reviews the main enzyme preparations of plant origin used in meat processing, as well as the influence and impact on the quality of meat product from non-traditional raw materials.

Bromelain, papain and ficin (also spelled fikain) are cysteine proteases (EC 3.4.22), often referred to as thiol or sulfhydryl proteases. They are endopeptidases with low substrate specificity and are able to catalyse the hydrolysis of a wide range of chemical bonds (peptide, amide, ester) [4].

Bromelain is a mixture of cysteine proteases isolated from the stem (EC 3.4.22.32, 24.5 kDa) and fruit (EC 3.4.22.33, 25 kDa) of pineapple *(Ananas comosus)* [4, 5] are pH 5.0-7.0 and 50 °C [4,5]. It should be noted that a wider range (optimum at pH 6.0-8.5 and 50 to 60 °C) was described by Grzonka et al [3]. The proteolytic activity of bromelain is of great interest for numerous applications, mainly in food products [6]: in the baking industry - to improve dough relaxation and uniform rise, and to produce hypoallergenic flour [6, 7], [8], [9]; in tenderisation - as a hydrolysing agent for meat, oysters, chicken, fish and squid [4]; anti-browning agent to prevent fruit browning and phenol oxidation [4]; in alcohol production - to increase the protein stability of beer [4]; in animal feed production - to assess protein degradation in ruminant feeds [4]; in winemaking - to stabilise white wine (including immobilised bromelain) [4, 5, 6, 7, 8]. Bromelain cleaves myofibrillar proteins and collagen and causes excessive tenderness in meat [9]. It is estimated that 95% of enzymes used in the USA are derived from plant proteases such as papain and bromelain, while microbially derived tenderisers are not widely used [10].

Papain (EC 3.4.22.2) is a cysteine protease derived from the latex of papaya (*Carica papaya*), which is believed to play a physiological role in plant defence against insects [11]. Papain has been shown to have high enzymatic activity over a wide range of pH (5.0-8.0) and 65 °C; its molecular mass is 23 kDa. In the active site, Cys25 and His159 are two important residues for protease activity [11]. Papain is inactivated at 90 °C. The enzyme activity is reduced by pressure, especially at high pressure (800 kPa) and temperature (60 °C), possibly due to oxidation of the thiolate ion in the active site [12]. Papain is widely used as a common ingredient in brewing and meat processing [11]. As a protein digesting agent, papain is used to combat dyspepsia and other gastrointestinal disorders [11, 12]. The addition of ginger and papain powder improved the physicochemical and sensory properties of camel patties [11]. The recommended dosage of papain for meat tenderisation is 0.6%, which results in improved texture and meat quality. Exceeding the dose may affect the quality and texture of meat [11]. Some studies have reported the effect of different concentrations of papain on the development of proteolysis and sensory characteristics of dry sausage [11].

Ficin (EC 3.4.22.3, 26 kDa) is obtained from the latex of Ficus glabrata, Ficus anthelmintica, and Ficus laurifolia [4, 12]. The maximum activity of ficin is achieved in the pH range of 4.5-8.0 and temperature range of 45-65 °C [4]. Like other cysteine proteases, ficin requires cysteine or other reducing agents for activation. The enzymes have a broad specificity towards hydrophobic amino acid residues. Ficin is irreversibly inactivated by strong oxidising agents and iodoacetate. The enzyme is inhibited by weak oxidising agents and divalent metals, but the inhibition can be reversible [13]. Ficin is a well-known plant protease used for meat tenderisation [14], milk coagulation in cheese production or peptide synthesis [15].

Thus, the aim of this study was to investigate the effect of treatment with special enzyme preparations of plant origin (bromelain, papain, ficin) as part of a multicomponent brine of goji berry extract, rosemary extract, pumpkin powder, sea buckthorn powder on organoleptic and functional-technological properties of meat raw materials.

Materials and research methods

The objects of the study were selected as follows: camel, horse, beef, and mutton. Enzymes (bromelain, papain, ficin) were used to accelerate meat ripening.

For the multi-component brine the following were used:

Main components:

Chilled water (2-4°C), nitrite salt (0.5-0.6%), sugar.

Functional components:

Phosphates (sodium tripolyphosphate, pyrophosphate) (0.3-0.5%), protein hydrolysates (collagen, whey protein) (0.5-1%).

Plant Ingredients:

Pumpkin powder (source of dietary fibre, retains moisture), sea buckthorn powder (antioxidant, improves taste and colour), goji berry extract (liquid) and rosemary extract (liquid).

Enzymes to improve texture and maturation of meat:

1. Bromelain (0.2-0.5%) - enzyme from pineapple, softens muscle fibres, improves juiciness.

2. Papain (0.1-0.3%) - an enzyme from papaya, accelerates meat ripening, improves tenderness.

3. Ficin (0.1-0.5%) - enzyme from figs, improves softness and loosens connective tissue.

Technology of brine preparation and application:

1. Brine preparation

Brine preparation includes heating 30% of water to 40-45°C and dissolving phosphates, salt, sugar. In the prepared solution we add enzyme, according to the recipe brine, protein hydrolysate, pumpkin powder, sea buckthorn powder, goji berry extract, rosemary extract, according to the developed recipes. Then to this brine bring the remaining amount of cold water 70%, brine to homogeneity, until the complete dissolution of the auxiliary ingredients, and cooled to 2-4 ° C, brine filtered.

2. Salting of raw meat

Prepared pieces of meat raw material were injected with brine, using a single-needle syringe, in the amount of 15-20% of the weight of meat raw material.

3. Maturation of meat raw material in brine

Further injected pieces of meat raw material were placed in containers and poured the remaining brine in the amount of 70%. The salted meat raw material is kept in various containers, allowed by the current normative documents, in a refrigerated chamber, at that the temperature of meat raw material should not exceed the temperature of 0-40C, during 24-48 hours.

4. Massaging of salted meat raw material

Massaging of salted meat raw material was carried out under vacuum in a tenoriser for 3 hours with the addition of 10% of multi-component brine in 2 stages, according to the following mode of rotation: stage 1 (1 hour) - rotation for 20 minutes at 16 rpm, 20 minutes of rest; stage 2 (2 hours) - rotation for 20 minutes at 10 rpm, 20 minutes of rest).

Methodology for the determination of pH in meat products:

Before starting the measurement, it was taken into account that enzymes can affect the pH level, as they partially hydrolyse proteins and release amino acids. Further, after meat treatment with multi-component brine, meat samples (50-100 g) were taken from different areas, as brine distribution can be heterogeneous. We divide the samples into surface and deep layers. The cooled meat was incubated at 20°C for 30 min before measurement.

The pH was measured by direct basic contact method. We insert the needle electrode directly into the meat to a depth of 1.5-2 cm. Then wait for the reading to stabilise (10-30 seconds).

It is recommended to measure pH at different stages of processing to assess how the acid-alkaline balance changes during the application of enzymes.

Results and discussions

The application of bromelain enzyme in concentration of 0.2-0.5%, papain in concentration (0.1-0.3%), ficin in concentration (0.1-0.5%) and in combination with multicomponent brine had a significant effect on the quality of the finished meat product. (Table 1). Proteolytic enzymes, contributed to the hydrolysis of muscle fibre proteins, which improved the texture of the meat. Due to the action of bromelain, the meat texture became softer, which improved the consistency of the meat product. This is especially important as we used tougher types of meat (camel meat, horse meat). The enzymes contributed to the destruction of connective tissues, which resulted in improved juiciness and softness of the meat. The greatest effect was observed in samples treated with papain, while ficin had a more pronounced effect on lamb and beef.

Table 1. Composition of investigated samples of meat products

№	Type of meat raw material	Plant components of the brine	Enzymes	Enzyme concentration (%)
1	Camel meat Horse meat Lamb Beef	Goji berries, rosemary, pumpkin powder, sea buckthorn powder	Bromelain	0,5–0,2
2	Camel meat Horse meat Lamb Beef	Goji berries, rosemary, pumpkin powder, sea buckthorn powder	Papain	0,1–0,3
3	Camel meat Horse meat Lamb Beef	Goji berries, rosemary, pumpkin powder, sea buckthorn powder	Ficin	0,1–0,5
4	Camel meat Horse meat Lamb Beef	Goji berries, rosemary, pumpkin powder, sea buckthorn powder	Bromelain Papain Ficin	0,1–0,5

The use of bromelain also affected the shelf life of meat products, reducing the risk of microbiological contamination due to accelerated penetration of salt and other preservatives, which increased the stability of the product under storage conditions. The use of papain and ficin in combination with a multi-component brine with plant extracts and powders has been shown to be effective in improving the quality of meat products from non-traditional raw materials. The main effects include improvement in texture and juiciness of meat as well as increase in its functional value. The technology, including enzymatic treatment and the use of plant extracts, can be the basis for the creation of new types of meat products with improved flavour and nutritional characteristics, which opens up prospects for expanding the range and improving the quality of products in the meat industry.

The study determined pH in meat products without enzymes and with bromelain+fatin+papain enzymes (Table 2). At the initial stage (0 hours), the pH of all samples is between 6.00-6.28, which is slightly above the normal range (5.4-6.2) but acceptable for fresh meat. After 12 and 24 hours, the pH gradually increases in all samples, which is due to enzymatic hydrolysis of proteins and accumulation of free amino acids. After 5 days, pH continues to increase, with the most pronounced increase observed in the second sample (6.67), which exceeds the normal level and may indicate the initial signs of spoilage.

Table 2. Determination of meat product pH

№	Type of meat raw material	pH after 0 hours	pH after 12 hours	pH after 24 hours	pH after 5 days	Normal
1	Camel meat Horse meat Lamb Beef	6,03	6,13	6,29	6,39	
2	Camel meat Horse meat Lamb Beef	6,28	6,37	6,47	6,67	
3	Camel meat Horse meat Lamb Beef	6,02	6,12	6,31	6,39	
4	Camel meat Horse meat Lamb Beef + Bromelain Papain Ficin	6,0	6,11	6,15	6,20	***5,8-6,2***

In the fourth sample (with bromelain, papain and ficin), pH remains the most stable and closer to the normal range after 5 days (6.20). This indicates a controlled process of enzymatic degradation of proteins without excessive accumulation of alkaline products. In the second sample (without enzymes), the pH reaches 6.67, which is much higher than normal. This may indicate the initial processes of microbiological decomposition, especially during long-term storage. The optimal option is to use a combined enzyme preparation (bromelain, papain, ficin) to keep the pH stable and avoid a sharp increase in acid-base balance, potentially extending the shelf life. Without enzyme treatment, there is a tendency for the pH to increase significantly, which can negatively affect the texture, flavour and safety of the meat product.

Conclusion

The results of the study showed that the use of enzyme preparations (bromelain, papain, ficin) in combination with multicomponent brine has a significant effect on the quality, texture and functional and technological indicators of meat products from non-traditional raw materials (camelina, horse meat, mutton, beef). Enzymatic treatment promoted the destruction of connective tissues, which led to an increase in softness and juiciness of meat. A particularly pronounced effect was observed with bromelain, which softened muscle fibres and improved consistency. Papain was most effective in improving meat tenderness, while ficin had a more pronounced effect on the texture of lamb and beef.

pH stability and shelf life extension. Without enzyme treatment, there was a tendency for a significant increase in pH (up to 6.67 after 5 days), which could indicate initial spoilage processes. The combined use of enzymes (bromelain, papain, ficin) allowed to keep the pH within the normal range (6.20), preventing an abrupt change in the acid-alkaline balance.

The enzymes promoted accelerated penetration of salt and functional components, which improved the stability of the product under storage conditions. Increase of functional and technological characteristics. The introduction of enzymes in the formulation of meat products promoted uniform distribution of multi-component brine, which improved moisture retention and increased the yield of the finished product. Plant extracts (goji, rosemary, sea buckthorn, pumpkin powder) enhanced antioxidant properties and stabilised the colour of the meat, which had a positive effect on the appearance and perception of the product by consumers.

Thus, enzymatic treatment in combination with multicomponent brine is an effective method of improving the texture, organoleptic and functional-technological characteristics of meat products from non-traditional raw materials. This technology opens prospects for the development of new products with improved consumer properties, and also contributes to the extension of shelf life and preservation of the quality of meat products.

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ЖЕМДІК ДӘНДІ ДАҚЫЛДАРДЫҢ ЖАҢА СҰРЫПТАРЫН ІРІ ҚАРАҒА АРНАЛҒАН ҚҰРАМА ЖЕМ ӨНДІРІСІНДЕ ТИІМДІ ҚОЛДАНУ

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Мақалада жергілікті дәнді дақылдардың жаңа сұрыптары: «Казахстанская 20» қонақжүгерісі, «КазНИИЗиР 80» қонақтарыны құрама жем өндірісінде қолданудың зерттеу нәтижелері берілген. Әдебиет деректерін сараптау нәтижесі Отандық қонақжүгері сұрыпын құрама жем өндірісінде қолдану туралы деректердің аздығын, ал қонақтарыны құрама жем өндірісінде, мал азығында қолдану туралы деректердің жоқтығын көрсетті. Зерттеу нәтижелері елімізде қонақжүгері сұрыптарын өсіру қолға алынғанын көрсетеді. Жемдік қонақжүгері, қонақтары сұрыптарының минералдық және аминқышқылдық құрамының құрама жем өндірісінде кең қолданылатын жүгері мен арпа дақылдарының құрамына жуық екендігі анықталды. Дәнді дақылдардың физикалық-технологиялық қасиеттерін анықтау нәтижесі дәнді дақылдардың сусымалдығы жақсы дақылдарға жататынын көрсетті. Дәнді дақылдардың химиялық, аминқышқылдық және минералдық құрамы анықталды. Зерттеу нәтижесі: қонақжүгерідегі ақуыз мөлшері жүгерімен салыстырғанда 21,22%, май мөлшері 5,5% көп екендігін, клетчатка мөлшері 1,5 есе көп екендігін көрсетті. Қонақжүгерідегі ақуыз мөлшері тарымен салыстырғанда 22,8%, май мөлшері 1,53% көп, ал клетчатка мөлшері қонақтарыда тарымен салыстырғанда 1,38 есе көп екендігін көрсетті. Дәнді дақылдардың аминқышқылдық құрамын талдау нәтижесі қонақтарының аминқышқылдық құрамы қонақжүгеріге қарағанда құнды екенін көрсетті, яғни ауыстырылмайтын аминқышқылдары: лизин мөлшері қонақтарыда қонақжүгеріге қарағанда 4,62 есе, метионин 2,05 есе, треонин 1,53 есе көп сақталады. Зерттеу нәтижесі жергілікті «Казахстанская 20» қонақжүгерісі, «КазНИИЗиР 80» қонақтарыны құсқа, жас малға арналған құрама жемге қолданғанда, оның сіңімділігін жақсарту үшін жылумен өңдеу түрлерін қолданудың қажеттігін көрсетеді.

Негізгі сөздер: қонақжүгері, қонақтары, физикалық-технологиялық қасиеттері, химиялық құрамы, минералдық құрамы, аминқышқылдық құрамы.