QUALITY ASSESSMENT OF PROTEINS IN COOKED SAUSAGES WITH FOOD COMPOSITIONS

O. Fursik, postgraduate**, E-mail: fursikoksana@gmail.com
I. Strashynskiy, PhD, Associate Professor*, E-mail: sim2407@i.ua
V. Pasichnyi, Doctor of Technical Science, Professor**, E-mail: pasww1@ukr.net
O. Kochubei-Lytvynenko, PhD, Associate Professor, director**, E-mail: okolit@email.ua

Department of meat and meat products technology
"N" Educational-scientific institute of food technologies
National University of Food Technologies 68 Volodumurska str., Kyiv, Ukraine, 01601

Abstract. In the article, the data are given of research carried out in vitro to determine the amino acid composition and the degree of digestibility of the reference and experimental samples of cooked sausage, with the use of the protein-containing composition developed. The protein digestibility-corrected amino acid score (PDCAAS) has been calculated to clarify the assimilation of amino acids that enter the body as part of proteins in experimental cooked sausage samples.

It has been established that replacing a part of the meat raw material with the protein-containing composition in the formula of cooked sausages does not affect significantly the amino acid composition of the finished product. The addition of mechanically deboned poultry meat reduces the amount of such essential amino acids as isoleucine by 68%, compared with the control formula, leucine by 38%, and valine by 48%. At the same time, the content of lysine significantly increases by 1.5 times. The in vitro index of digestibility for an experimental sample of cooked sausages with protein-containing composition at the pepsinolysis stage is slightly reduced compared with the reference sample (by an average of 7%). At the second stage of hydrolysis (trypsin enzyme), this parameter does not differ from the reference one. During the two stages of hydrolysis, this parameter, with mechanically deboned poultry meat introduced, decreased by an average of 20%, compared with the reference sample.

Calculated PDCAAS has allowed establishing that the true efficiency of proteins in cooked sausages is different from the in vitro index of digestibility, which is due to the presence of limiting values of the essential amino acids content in the product.

Key words: protein preparations, protein-containing composition, cooked sausages, protein quality, amino acid composition, degree of digestibility in vitro.

ОЦІНКА ЯКОСТІ БІЛКІВ ВАРЕНИХ КОВБАС
з ХАРЧОVOЮ КОМПОЗИЦІЄЮ

О.П. Фурсік, аспірант**, E-mail: fursikoksana@gmail.com
I.М. Страшынський, кандидат технічних наук, доцент*, E-mail: sim2407@i.ua
V.М. Пасічний, доктор технічних наук, професор*, E-mail: pasww1@ukr.net
О.В. Кочубей-Литвиненко, кандидат технічних наук, доцент, директор**, E-mail: okolit@email.ua

Кафедра технології м’яса та м’ясних продуктів
"Південно-науковий інститут харчових технологій
Національний університет харчових технологій, вул. Володимирська, 68, м. Київ, Україна, 06106

Анотація. У статті наведено дані проведених досліджень по визначенню амінокислотного складу та ступені перетравлення в умовах ін вітру контролю та інших діяльних зразків варених ковбас із використанням розробленої білокомпонентної композиції.

Для уточнення засвоюваності амінокислот, що надходять в організм білоокислотний склад засвоєнності білків (PDCAAS).

Встановлено, що заміна білокомпонентної композиції частини м’ясної сировини в складі рецептури варених ковбас суттєво не впливає на амінокислотний склад готового продукту. Додаткове внесення м’яса птиці механічного обвалювання сприяє зменшенню кількості таких незамінних амінокислот як ізолейцин на 68% порівняно з контрольною рецептурою, лейцин – на 38% та валін – на 48%. Поряд з цим значно збільшується вміст лізину у 1,5 рази. Показник ступені перетравлення в умовах ін вітру для діяльного зразка варених ковбас з білокомпонентною композицією на етапі пепсинолізу суттєво зменшується в порівнянні з контролем зразком (в середньому на 7%), на другому етапі гідролізу (ферментом тріпсин) даний показник не відрізняється від контрольного. Протягом двох етапів гідролізу даний показник при внесені м’яса птиці механічного обвалювання зменшився в середньому на 20% в порівнянні з контрольним зразком.

Розрахунок показника скорогована на амінокислотний склад засвоєнності білків (PDCAAS) дозволив встановити, що істинна ефективність білків у складі варених ковбас відрізняється від показника перетравлення in vitro, що обумовлено наявністю німітуючих значень вмісту незамінних амінокислот у складі продукту.

Ключові слова: білкові препарати, білокомпонентна композиція, варені ковбаси, якість білка, амінокислотний склад, ступінь перетравлення in vitro.
**Introduction. Formulation of the problem**

A comprehensive solution of shortage and quality problems of raw materials, increase of economic efficiency of production, prevention of technological errors and product quality defects by the reasonable use of food additives and the creation of a composition based on them are relevant tasks that contribute to providing people with high-quality food products. Modern concepts of quantitative and qualitative human needs for nutrients are reflected in the concepts of balanced and adequate nutrition. According to the first concept, in the process of normal activity, a person needs a certain amount of energy and a complex of nutrients: proteins, amino acids, carbohydrates, fats, fatty acids, mineral elements, vitamins. Many of them are irreplaceable, namely, they are not produced in the body, but are necessary for vital activity. The second concept proves that a strict proportion of the components of nutrition should be observed. It is the decisive factor that determines food assimilability and regulates the metabolism at the level of homeostasis [1].

For adequate nutrition and health maintenance, it is necessary to receive essential amino acids which form the basis of the protein. They are not synthesised by the body, that is why their presence in the diet is so important. Replacing meat raw material with proteins of plant and animal origin in meat products formulae makes it necessary to study the problem of their influence on the assimilability of finished products.

**Analysis of recent research and publications**

Proteins are the main structural unit of meat products mince. The quantitative content of proteins in the system, their qualitative composition, the environmental conditions determine the degree of stability and balance of the meat systems obtained and affect the functional and technological (FTP) as well as structural and mechanical properties (SMP) [2].

When combining meat ingredients with each other, and with raw material of plant and animal origin, the structure of the product (the internal structure and the nature of interaction between individual elements) changes. This change is determined by the chemical composition, biochemical parameters, temperature, dispersion, technological factors. Depending on the proportion of the recipe components, the water and fat content, the nutritional and biological value changes, too, as well as the technological, organoleptic and rheological characteristics of the final product [3,4]. The production of combined meat products containing proteins of plant (soya, peas, lentils) and animal origin, allows us to use raw materials rationally, to prevent the complete protein deficiency in the diet, to improve the nutritional and biological value of products [5].

On analysing the work of Haili Niu [6], it was determined that the use of a complex of myofibrillar proteins with soy isolates (in the amount of 0.5% and 0.75%) provides a synergistic interaction between these protein molecules. This, in turn, contributes to the increase of forces that form the protein gel matrix (with the improvement of the gel structure) and determine the thermal stability of the protein while reducing the loss of moisture.

The use of protein filler in the technology of minced semifinished meat products makes for an increase in the total moisture and a slight decrease in the mass fraction of proteins, fats, and ash elements. It has been determined that limiting amino acids are absent in the experimental samples. The coefficients of the difference in the amino acid score (CDAAS) and the utility coefficient indicate an improvement in the balance of essential amino acids [7].

By the amino acid composition and analytical calculations of biological value, one can only get an idea of the potential value of the product’s protein component, since not everything that comes with food is utilised by the human body, but only what can be absorbed through the walls of the intestine into the bloodstream after digestion in the digestive tract. The degree of proteins digestibility by proteolytic enzymes of the gastrointestinal tract is one of the main indicators that determines the biological value of food products. That is why, the results of determining the ability to digest proteins in vitro by digestive enzymes can be used to predict the degree of their utilisation by the body [8-9].

In vitro determination of the digestive ability is a faster, more available, and no less effective method than in vivo methods of analysis [10].

Studies by Wen Siying and others [11] to determine the ability to digest meat raw material, showed that pork and beef have a certain similarity in peptide hydrolysis of proteins by pepsin and trypsin, but are significantly different from poultry and fish. This is due to the difference in the amino acid composition of these raw materials types and the peculiarity of enzyme preparations’ effect.

Operating with indicators of amino acid score and digestibility in vitro only, you do not take into account the degree of assimilation of human proteins, that is, their effectiveness [12]. Thus, the parameter of protein digestibility-corrected amino acid score (PDCAAS) is recognised and approved as a method of assessing the quality of the protein, taking into account the amino acid composition and the index of assimilation of the food matrix. This parameter is derived from the amino acid score and corrected on the basis of the analysis of protein digestibility in vitro [13].

This indicator is calculated for all types of products, which allows determining more accurately the value and benefits of their use by a person. Studies by Khattab et al. [14] to determine the effects of different ways of processing on the digestibility of plant proteins and the PDCAAS index found out the following. For asparagus beans, peas, and red beans, as a result of this or that type of treatment – soaking, microwave processing, boiling, or boiling under pressure (in an autoclave) – the amount of essential amino acids and the
proteins assimilability in vitro increases (in the above sequence of processing methods). The PDCAAS index decreases in the following order of the heat treatment methods used: boiling under pressure (in an autoclave), microwave processing, boiling, soaking, – and varies for asparagus beans in the range of 97–64%, for peas 92–64%, for red beans 77–68%, respectively.

Other studies compare PDCAAS data for different types of proteins. Thus, in the works [15,16], the values of this parameter for eggs, cow’s milk, beef, soy (concentrate), whey flour are found to be 118%, 121%, 92%, 91% and 42%, respectively. In the articles [17-19], the PDCAAS parameter is given for: whey concentrate – 100%, soy flour – 80%, soybean isolate – 100%, and textured soy protein – 65%. The difference in the parameter’s value is due to many factors. The main ones are the difference in the amino acid composition, the presence of inhibitors of enzymes, and the methods of treatment applied to the sample under study, which determines the degree of assimilation and the consumption value of a product.

**The aim of this work** is to investigate the amino acid composition and amino acid score of the experimental samples, and the digestibility of cooked sausages in the human body, on the basis of the data obtained on the degree of digestion of proteins in vitro by the pepsin-trypsin enzyme system. Another aim was calculation of the protein digestibility-corrected amino acid score (PDCAAS).

The subject of the research is cooked sausages, in which part of the meat raw material was replaced with the nutritional composition developed, and mechanically deboned poultry meat was used (MDPM).

To achieve this goal, the following tasks have been solved:

- to prove, basing on the literary sources analysis and on our own studies, the prospects of this line of work that consists in studying of how the composition of sausage products affect their digestibility and how this indicator changes depending on the amino acid score of the product;
- to investigate the effect of a protein-containing composition and MDPM on the amino acid composition of the finished product;
- to study the effect of the additive developed and MDPM on the in vitro digestibility of cooked sausages;
- to calculate the index of protein digestibility-corrected amino acid composition of cooked sausage products with protein-containing composition and MDPM;
- make conclusions about the results obtained and the prospects for further research.

**Research Materials and Methods**

To solve these tasks in the cooked sausages technology, a hydrated protein-containing composition was used, which included: pork skin protein Belkoton-95, soy isolate Pro-Vo 500 U, guar gum, xanthan gum, carboxymethylcellulose, dry milk whey [20].

The studies [21,22] established the rational degree of hydration of the composition at the level 1:2, which provides the necessary properties of gels. The hydration was carried out with water at the temperature 10±2°C with nanocomposite introduced in the amount 0,3% of the gel mass.

As the nanocomposite, silica was used, synthesised by the specialists of the Amorphous Structures and Structurally Ordered Oxides Department of A.A. Chuyko institute at NAS of Ukraine. The specific area of the surface of the silica was S\text{tot} = 232 m^2/g, with the correspondent mean radius of primary nanoparticles 5,88 nm and the poured density \( \rho_\text{p}=22 \, \text{g/cm}^3 \) [23,24]. The usefulness of this food additive (E551) is proved by the researches of the effect it has on the properties of milk proteins [12] and protein preparations [8,9,13].

To make a reference sample, the recipe of first grade cooked sausages complying with TU U 15.1-20021369-005:2007 was chosen. The ingredients are: second class beef, medium-fat pork, poultry meat (red chicken meat), lard (brisket), flour, melange, salt, and spices. On its basis, the formulae were developed for the experimental samples of cooked sausages. In experimental sample No. 1, 10% of lard, 10% of second grade beef, and 10% of red chicken meat were replaced by 30% of the hydrated composition. In experimental sample No. 2, 30% of the MDPM was added instead of 10% of the medium-fat pork and 20% of red chicken meat. The amount of MDPM and protein-containing composition to be added to cooked sausages was experimentally determined to be 30%, which provides high levels of FTP and SMP [26,27]. The protein-containing composition developed was introduced in the hydrated form at the chopping stage after the low-fat raw material, sodium nitrite, and phosphates, with the required amount of salt and with 20% of water added to the main raw material. The experimental samples of cooked sausages were made in accordance with the standard technology [28].

For the reference sample and the selected experimental samples, the amino acid composition of the proteins was determined by the method of ion exchange liquid-column chromatography [29], and the calculation method helped determine the amino acid score by the formula [30] with some modification:

\[
\text{SCORE} = \frac{x}{y} \times 100\% \tag{1}
\]

where, \( x \) – mg of the essential amino acid in 1 g of the tested protein, \( y \) – mg of the same amino acid of protein in the etalon (standard).

In the finished sausage samples, the parameter of protein digestibility in vitro has been studied [8]. The method consists in affecting gradually the protein substances of the objects of the proteinase system composed of pepsin and trypsin. The method is based on enzymatic hydrolysis of proteins (the enzymes pepsin
and trypsin) in the product under study, in which the availability of attacked peptide bonds is determined not only by the properties of the protein, but also by additional factors associated with the structure and chemical composition of the food product. Hydrolysis is carried out in a special device that provides continuous mixing and dialysis. Fermentation is carried out in two stages, each lasting 3 hours. The first stage is fermentation with pepsin, the second is with trypsin. The content of proteolytic enzymes corresponds to their average concentration in the human gastrointestinal tract. The products of hydrolysis are determined by the Lowry method [31].

Determination of the protein mass fraction by the Lowry method [31] consists in establishing the amount of protein in the product by the content of a free amino acid, tyrosine, in the experimental solutions. The latter was determined with a calibration graph in accordance with the optical density of the prepared solutions. The optical density is determined at a wavelength of 750 nm. The calibration graph is constructed using tyrosine solutions with a recommended mass concentration. The digestibility was calculated according to the formula of Siying, Wen et al. (2015) [11] with some modifications:

\[ DT = \frac{W_t}{W_i} \times 100\% \quad (2) \]

where, DT is digestibility,%; W_t is tyrosine content in the experimental sample after digestion; W_i is the total tyrosine content in the experimental product.

To determine accurately the digestibility degree of the experimental cooked sausages, the obtained in vitro data on the digestibility was recalculated, taking into account the values of amino acid score for the essential amino acids of the products obtained. The PDCAAS calculation method is based on such parameters as protein content, amino acid composition, and digestibility. A limit estimation of the amino acid composition (i.e., the ratio of the first limiting amino acid content in 1 g of protein to the content of the same amino acid in the reference protein or in the reference sample) multiplied by the protein digestibility allows obtaining information on the digestibility-adjusted quality of the protein. The first limiting amino acid is the essential amino acid contained in the product in the lowest concentration. Depending on the concentration of other essential amino acids, you can calculate and describe PDCAAS for the second, third, fourth, etc. limiting amino acids [32].

After analysing literary sources [33,34], the protein digestibility-corrected amino acid score was calculated by the formula:

\[ PDCAAS = \frac{a}{b} \times DT \quad (3) \]

where, a is mg of limiting amino acid in 1 g of the experimental protein, b is mg of the same amino acid in 1 g of the control, or ideal, protein. DT is the index of protein digestibility of this product (%).

The absolute measurement error was determined using Student’s criterion. The reliable interval P=0.95, the number of reiterations in determinations is 3-4, the number of parallel tests of the studied samples is 3.

### Research Materials and Methods

Biological value, as a criterion for evaluating protein, is very important for determining the effectiveness of its use by the human body. The amino acid composition of a product is one of its biological value indicators.

The amino acid analysis of the reference and experimental samples of cooked sausages was carried out to determine the change in the amino acid composition, depending on the level of replacing the meat raw material with a protein-containing composition and MDPM. The results of the research are shown in Figure 1.

![Graph showing amino acid content](image)

**Fig. 1.** The essential amino acids content of cooked sausage proteins.

According to these data, in the reference sample and experimental sample No.1 of cooked sausages, the score of essential amino acids (such as cystine and methionine, leucine, tyrosine and phenylalanine, lysine, threonine) is more than 100%, so it fully provides body with them. In this case, the limiting amino acids are valine and isoleucine, the score of which is 69% and 70%, respectively, for the reference sample, and 62% and 68% for experimental sample No.1.
Comparing the data obtained with the reference sample, one can note an 8% increase in the amount of tyrosine and a slight decrease in the number of such amino acids as lysine by 9%, threonine by 2%, cysteine by 4%, leucine by 3%. However, their score values exceed 100%, while for valine and isoleucine, this index decreased by 7% and 2%, respectively, and is less than 100%.

In experimental sample No.2, the limiting amino acid is isoleucine. Its score is 18%, which is lower by 52% than the reference, and lower by 50% than experimental sample No. 1. Deficient are also such essential amino acids as valine (32%) and leucine (36%). For other amino acids, the score is high.

The important indicator of the quality of a high-protein product is the depth and rate of digesting proteins in the gastrointestinal tract under the action of digestive enzymes. This parameter characterises the biological value. It is evaluated by means of digesting proteins by digestive enzymes in vitro, and used to predict the degree of their utilisation by the organism.

The article presents the research of determining the effect of a hydrated protein-containing composition and MPMO on the degree of cooked sausages digestion by digestive enzymes that catalyse cleavage of proteins (pepsin and trypsin). Based on the results obtained, a graph was constructed of enzymatic hydrolysis of proteins by proteolytic enzyme pepsin. The graph is shown in Figure 2.

The analysis of digestibility of the experimental samples of cooked sausage shows that at the pepsinolysis stage, the parameter of protein cleavage for the sample with the composition added is slightly reduced (by an average of 7–9%), compared with the reference sample. This variation in the values is due to the constituents of the protein-containing composition that includes food hydrocolloids and pork skin protein with a high content of collagen fibres, and is introduced instead of meat raw material.

Adding to the recipe more MDPM instead of red poultry meat and pork leads to a marked decrease (by an average of 34%) in the proteins digestibility by pepsin, compared with the reference sample. These results can be explained by the ability of proteins to form strong bonds, by their number and qualitative composition, as well as by the structure and chemical composition of the food product. In particular, other researchers mention [35] that for pepsin, higher activity is observed during the cleavage of peptides containing such amino acids as phenylalanine, tyrosine, or leucine.

Figure 3 shows the results of the study of the effect a hydrated protein-containing composition and MDPM have on the degree of digesting cooked sausages by the digestive enzyme trypsin.

The analysis of the above data on the degree of digesting the experimental cooked sausage samples indicates that at this stage of hydrolysis, the rate of proteins cleavage for experimental samples is slightly different from that of the reference sample. The values obtained are within the error limits, which indicates
high attackability of the protein. A certain deviation in the values is explained (as in the period of pepsinolysis) by constituents of the protein-containing composition that includes food hydrocolloids and pork skin protein. The obtained result can also be substantiated by the peculiarity of trypsin action – it cleaves better the peptide bonds formed by carboxyl groups of lysine and arginine [11], whose content increases in the experimental samples. The peculiarity of trypsin with regard to positively charged substrates is determined by the presence of the aspartic acid carboxyl group contained in the sorption site (active center) of the enzyme. The obtained values of the digestibility of the finished product are, in general, equivalent to the characteristics of full-value food products.

Figure 4 summarises the results of studying in vitro the digestibility of the reference and the experimental samples of cooked sausage with the protein-containing composition and MDPM, by the pepsin-trypsin system.

![Image of Figure 4]

**Fig.4. The digestibility of proteins in cooked sausages by the pepsin-trypsin system.**

In the experimental samples at the trypsinolysis stage, the digestibility of cooked sausages proteins by the proteases of the gastrointestinal tract is deeper, compared with that in the pepsinolysis stage. Thus, the protein cleavage values for the reference and experimental sample No.1 are almost the same – the difference is within error limits, indicating high protein attackability.

The results of studying how cooked sausages with the protein-containing composition and MDPM are fermented in a pepsin-trypsin system confirm the effect of incorporated components on the proteolytic hydrolysis ability of proteins in the finished product. This indicator for experimental sample No.1 is slightly reduced (by an average of 4.5%) at the end of process. For experimental sample No.2, there is a marked decrease in the ability to cleave the protein (by an average of 20%) compared with the reference sample. After the action of pepsin, the sausage protein hydrolysis is accompanied by a release of the largest number of amino acids as for the reference sample. The marked increase in the concentration of amino acids for the experimental samples is observed at the second stage of hydrolysis, immediately after adding trypsin to the system, and keeps growing continuously and intensively.

The general results of hydrolysis of meat product proteins are based on the ratio of the ingredients. It is known that a high content of connective tissue proteins (collagen and elastin ones) worsens the digestion and digestibility of the proteins contained in a product. Also, presence of indigestible carbohydrates (gums, carboxymethylcellulose), which are introduced as part of the composition, reduces digestibility. In addition, the difference in the results is due to some specific features of the action of the enzymes towards the amino and carboxyl groups of different amino acids in the peptides and, accordingly, to the difference in the amino acid compositions of the reference and the experimental samples. Important factors that also determine the digestive ability of proteins are their modifications, such as: the oxidation of amino acids of side chains; coupling in a protein-protein system and changes in the conformation of an amino acid chain (base cleavage), which can negatively affect both the functional properties and the nutritional value and digestibility of the organism [36].

On the basis of the experimental data, a functional relationship has been established between the index of the proteins digestibility degree \( y \), and the duration of hydrolysis \( x \), which is described by the following equations:

\[
\begin{align*}
y_1 &= 0.3458x^3 - 7.7121x^4 + 64.248x^3 - 245.92x^2 + 436.03x - 247.29 \\
R^2 &= 0.9869;
\end{align*}
\]

\[
\begin{align*}
y_1 &= 0.3667x^5 - 8.1061x^4 + 66.864x^3 - 252.83x^2 + 440.83x - 247.43 \\
R^2 &= 0.9846;
\end{align*}
\]

\[
\begin{align*}
y_2 &= 0.3208x^3 - 7.0455x^4 + 57.54x^3 - 214.08x^2 + 364.26x - 201.29 \\
R^2 &= 0.9812.
\end{align*}
\]

The approximation coefficients \( R^2 \) indicate high reliability of the equations that characterise the ability of protein components of cooked sausages to hydrolyse.

This information about the amino acid composition of proteins, their score, and digestibility in vitro is not informative enough to assess the human body’s ability to digest. It does not allow, either, asserting that the proteins of a product are assimilated in that very amount in which they enter the organism, since the degree of benefit from the protein sup-
The research results presented in the article are within the framework of research topics performed at the expense of the state budget: the number of state registration is 0118U003557 “Scientific and practical substantiation technologies of meat and meat products with extended shelf life” and the number of state registration is 0117U001243 “Scientific principles of developing resource-saving technologies of protein-containing polyfunctional concentrates for food products of special purpose.”


In vitro determination of the release kinetics of peptides and free amino acids
Joyce Boye, Ramani Wijesinha
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