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Effect of cold treatment on the amino acid composition of veal

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Abstract. Veal is a promising raw material for use in the daily diet, as well as for production of functional and dietary foods. However the effect of cold treatment on the amino acid composition of veal has not been sufficiently studied. The aim of this study was the amino acid composition analysis of veal subjected to various variants of cold treatment.

The selected material under research was muscle tissue of hip parts from calves, grown in the Leningrad Region, Russia and aged no more than 3 months. Cooling to 4 ± 1°C and rapid freezing to the temperature of minus 18°C at the cooling air temperatures of minus 24°C and minus 35°C were used as variants of cold treatment. Amino acid composition analyses were carried out using precolumn derivatization with phenylisothiocyanate and reversed-phase gradient HPLC on the Shimadzu 20-AD chromatograph with spectrophotometric detection at 254 nm.

The results show the effect of cold treatment on the content of free amino acids and total amino acid composition of veal. In many respects changes in amino acid composition are concerned with moisture losses during the refrigerating treatment. The dependence between the change in amino acid content and the structure of its side chain group type is shown. Amino acid score for essential amino acids was calculated and conclusions about changes in biological value of veal protein were made.

The obtained data can be used in biological value calculation of the multi-component products and food rations with veal subjected to refrigerating treatment.

Key words: cooling, freezing, HPLC, protein, meat.

INTRODUCTION

The main sources of protein for humans are products of animal origin and some legumes. Protein biological value depends strongly on the type of raw material, and it determines formation of the daily diet and reasoning of the consumption norms for animal and vegetable proteins. The physiological average daily requirements in proteins are systematically studied and analyzed in the decisions of the FAO/WHO and national organizations of different countries, including the Russian Federation (FAO 1970; FAO/WHO 1991, 2007, 2011). However the effect of cold treatment on the amino acid composition of meat has not been sufficiently studied.

Because of its chemical composition, veal is a promising raw material for use in the daily diet, as well as for production of functional and dietary foods. Some of these products are characterized by high protein content, at that the share of complete proteins should be at least 60% of the total protein content. Furthermore, the internal organs of calves are used to obtain a large number of biologically active substances, it

is necessary to store and rationally process the rest of the carcass. In this regard, a study of veal is of particular interest.

In chicken muscle proteins after freezing at -30°C and frozen storage at -5°C for 10 weeks, the changes occurring in myofibrillar proteins as a result of frozen storage were indicated by the loss of -SH groups, ATPase activity, solubility, and water-holding capacity (Khan et al., 1968). Also the results show that rapid freezing preserves the integrity of muscle proteins to a greater extent than slow freezing (Khan & Berg, 1967). Freezing of bovine muscle has a denaturating effect on myofibrillar proteins; the lower the freezing rate the greater the loss (Wagner & Anon, 1985).

Disulfide bond formation with the concomitant decrease in sulfhydryl group was found in all species of some tropical fish during frozen storage (Benjakul et al., 2003). It was found that the denaturation of proteins during freezing is closely related to surface-induced denaturation (Chang et al., 1996). The review was made to describe the various stages of freezing of freeze-dried therapeutic proteins and examine the consequences of the various stresses developing during freezing on protein stability; however, the study of changes of amino acid composition was not carried out (Bhatnagar et al., 2007). Decrease in the content of each of sixteen amino acids was found in *Lactobacillus bulgaricus* after freeze-drying (Mitić, 1976).

For other food products effect of freezing to -25°C on amino acid composition was studied for selected species of edible mushroom (Bernaś & Jaworska, 2012). After freezing at temperature of cooling air of -35°C and frozen storage mushrooms contained significantly higher levels (3–118% on average) of leucine, lysine, methionine, and phenylalanine, but lower levels (8–61% on average) of cysteine, histidine, isoleucine, tyrosine and valine, compared with canned products.

Nutritional composition of veal is studied in several works, including the amino acid composition of preruminant calves and special fed veal ribeyes (Williams, 1978; Riss et al., 1983; Williams, 2007). However, information about the changes in amino acid composition of veal depending on conditions of cold treatment could not be found.

The aim of this study was the amino acid composition analysis of veal subjected to different variants of cold treatment.

MATERIALS AND METHODS

The selected material under research was muscle tissue of hip parts from calves, grown in the Leningrad Region, Russia and aged no more than 3 months. The meat was placed in a refrigerator at a temperature of 4 ± 1°C in 2 h after slaughter. After three days of refrigerated storage, the samples with a thickness of 2.0 ± 0.3 cm and a weight of 200 ± 5 g were isolated from the *musculi biceps femoris et gluteus superficialis* and subjected to rapid freezing to -18°C. Freezing at the temperatures of the cooling air of -24°C and -35°C were used as variants of cold treatment. Storage of frozen meat at -18°C held no longer than 24 h. Prior to analysis, each sample was placed in an individual plastic container and defrosted for 4 h at 5°C under natural convection.

Standard samples of amino acids (Sigma), phenylisothiocyanate (Sigma), acetonitrile (HPLC-grade, J.T.Baker) were used for the research. Isopropyl alcohol (HPLC-grade), sodium acetate (*puriss.*), hydrochloric acid (*puriss.*), sodium hydroxide

(*puriss. spec.)* were from Vekton, Russia. Water for analysis was produced with a Milli-Q purification system (Millipore) from twice-distilled water.

Amino acid composition analyses were carried out using precolumn derivatization with phenylisothiocyanate and reversed-phase gradient HPLC. The method is a combination and modification of methods described in Heinrikson & Meredith (1984), Gunawan et al. (1990), Fierabracci et al. (1991), González-Castro et al. (1997). The used method is certified by All-Russian Research Institute of Metrology and has a number of M-02-902-142-07.

Analyses of amino acids derivatives were performed by liquid chromatograph Shimadzu LC-20 Prominence with spectrophotometric detector (254 nm); column with a reverse phase C18 (250 x 4.6 mm, 5 μm, manufactured by Supelco) and a corresponding precolumn; mobile phase – a mixture of 6 mM sodium acetate, pH = 5.5 (component A), 1% isopropyl alcohol solution in acetonitrile (component B), and 6 mM sodium acetate pH = 4.05 (component C). Chromatographic analyses were carried out in a gradient mode. Mobile phase flow rate was 1.2 ml min^{-1}.

Standard samples of amino acids were dissolved in 1 M hydrochloric acid solution. Aliquots of standard solution of 15, 25, 50, 100 and 150 μl were placed in five test-tubes. Hydrochloric acid was removed from aliquots by drying on a water bath at 60°C in a stream of air through the capillary by suction created by an electric vacuum pump. Sodium hydroxide solution (0.10 ml of 0.15 M) was added to the dried amino acids, mixture was stirred, and then 0.35 ml of phenylisothiocyanate in isopropyl alcohol and 0.05 ml of water were added. The solution was thoroughly stirred and left for 20 min at room temperature, then evaporated to dryness at a temperature of 60°C. The dry residue was dissolved in 1 ml of water. The resulting solution was subjected to chromatographic analysis.

Sample preparation for determining the total contents of amino acids included the acid hydrolysis with 6 M hydrochloric acid at 110°C for 16–18 h. Samples (~ 0.2 g) were placed in vials, that were filled with a solution of hydrochloric acid and hermetically sealed. Before sealing the vapor phase was purged for 3 min with nitrogen to prevent oxidation of amino acids by atmospheric oxygen. After cooling, the hydrolyzates were filtered; aliquots (0.2–0.3 ml) were placed in test-tubes and evaporated to dryness on a water bath at 60°C in a stream of air similar to the standard solutions. Sodium hydroxide solution (0.10 ml of 0.15 M) was added to the dried aliquots, mixture was stirred, and then 0.35 ml of phenylisothiocyanate in isopropyl alcohol and 0.05 ml of water were added. The solution was also thoroughly stirred and left for 20 min at room temperature, then evaporated to dryness at a temperature of 60°C. The dry residue was dissolved in 1 ml of water. The resulting solution was subjected to chromatographic analysis.

For the determination of free amino acids, the meat samples were homogenized; sample (5 g) was placed in a glass cup, filled with 50 ml of ethyl alcohol, mixed with a magnetic stirrer, and small amount of fluid was filtered through a membrane filter. Aliquots (0.2 ml) were collected, placed in vials and evaporated to dryness on a water bath at 60°C in a stream of air. Phenylisothiocyanate derivatisation was then performed; samples were dried and then dissolved in 1 ml of water similar to the standard solutions and the solutions after acid hydrolysis. The obtained samples were subjected to chromatographic analysis.

All experiments were performed with at least three replicates; data was processed by methods of mathematical statistics at theoretical frequency 0.95.

RESULTS AND DISCUSSION

Changes in free amino acids after freezing

Free essential and non-essential amino acids content change after veal freezing process is shown in Table 1. As can be seen from Table 1 the total amount of these acids is increased regardless of the freezing temperature. However, more free essential amino acids are accumulated in the meat frozen at a temperature of -24°C (1.92 times) than at -35°C (1.31 times).

Table 1. The contents of free amino acids in veal

Amino acid	Mass fraction of free amino acids in meat, mg g(protein)$^{-1}$		
	before freezing	after freezing at t, °C	
		-24	-35
Essential			
Histidine	1.47 ± 0.06	2.2 ± 0.2	1.15 ± 0.08
Isoleucine	0.17 ± 0.01	0.3 ± 0.03	0.25 ± 0.02
Leucine	0.31 ± 0.02	0.64 ± 0.04	0.44 ± 0.04
Lysine	0.18 ± 0.01	0.76 ± 0.08	0.63 ± 0.05
Methionine	0.021 ± 0.001	0.042 ± 0.004	0.038 ± 0.002
Cysteine + Cystine	trace amounts	trace amounts	trace amounts
Phenylalanine	0.2 ± 0.02	0.31 ± 0.02	0.24 ± 0.02
Tyrosine	0.19 ± 0.01	0.39 ± 0.03	0.29 ± 0.02
Threonine	0.29 ± 0.02	0.77 ± 0.05	0.59 ± 0.05
Tryptophan	trace amounts	trace amounts	trace amounts
Valine	0.24 ± 0.02	0.49 ± 0.03	0.4 ± 0.03
Total:	3.071	5.902	4.028
Non-essential			
Alanine	1.11 ± 0.08	1.7 ± 0.1	2.4 ± 0.2
Arginine	11.0 ± 0.8	15.2 ± 0.9	11.7 ± 0.6
Serine	0.34 ± 0.02	0.60 ± 0.04	0.41 ± 0.04
Aspartic acid & Asparagine	2.6 ± 0.2	1.9 ± 0.2	1.54 ± 0.08
Glutamic acid & Glutamine	2.4 ± 0.1	1.41 ± 0.08	1.2 ± 0.1
Glycine	0.61 ± 0.06	0.74 ± 0.05	0.76 ± 0.06
Proline	0.17 ± 0.01	0.24 ± 0.02	0.19 ± 0.01
Hydroxyproline	0.037 ± 0.003	0.042 ± 0.003	0.032 ± 0.002
Total:	18.267	21.832	18.232
In total:	21.338	27.734	22.26

The content of free non-essential amino acids changed in a lesser degree. Thus, their content in veal, frozen at -24°C increased 1.20 times and at -35°C remained almost unchanged.

Analysis of the data presented in Table 2 shows that the change in the amount of free amino acids after freezing depends not only on the temperature of the process, but also on the structure of their side chain group type. Content of certain amino acids is reduced. Thus, meat freezing at -24°C and -35°C reduced the amount of aspartic acid and asparagine 1.37 and 1.69 times, glutamic acid and glutamine 1.70 and 2.00 times, respectively. Possibly, this change is due to the fact that the polar amino acid with anionic radicals – aspartic acid and its monoamide glutamine have clearly marked hydrophilic properties. Reactions of these acids deamination with formation of nitrogen-free compounds – glutaric and succinic acids could take place during phase transformation of water into ice and freezing. The histidine content decrease in 1.28 times during veal freezing at -35°C was also noted, which can probably be explained by the reaction of decarboxylation and the formation of nitrogenous compound histamine.

However, the content of most free essential and non-essential amino acids has increased in meat frozen at -24°C and at -35°C. It should be noted that the greatest changes take part in the polar amino acids with hydrophilic nonionogenic and cationic radicals (Table 2). Thus, the amount of polar amino acids with hydroxyl nonionogenic radicals increased 2.15 and 1.57 times in meat frozen at -24°C and -35°C, respectively. The biggest changes from the polar amino acids with cationic radicals underwent lysine, its content significantly increased 4.22 and 3.50 times in meat, frozen at -24°C and -35°C. The amount of arginine in meat frozen at -35°C mostly unchanged.

The freezing temperature has no significant effect on the content of non-polar amino acids with hydrophobic side chains. Thus, the amount of amino acids having aliphatic hydrocarbon radicals increases 1.57 and 1.69 times in meat frozen at -24°C and -35°C respectively. The exceptions are the amino acids alanine, leucine and isoleucine. The amount of alanine increased 1.53 and 2.16 times, valine – 2.04 and 1.67 times, isoleucine 1.76 and 1.47 times in meat frozen at -24°C and -35°C, respectively.

The amount of phenylalanine is increased 1.55 and 1.20 times, methionine – 2.00 and 1.81 times in meat frozen at -24°C and -35°C respectively.

Accumulation (content increase) of free amino acids during meat freezing obviously is due to the proteolysis of muscle and connective tissue proteins. It is known that proteins ability to be attacked by proteolytic enzymes is greatly enhanced during freezing (Lawrie, 1968).

Meat protein denaturation takes place during a freezing process, it is accompanied by conformational changes in a structure of a protein molecule. Since denaturation ruptures large number of weak hydrogen and then hydrophobic and ionic bonds, it should be assumed that cryodenaturation destroys mainly secondary structure of proteins. This structure is formed by an interaction between functional groups of amino acids by hydrogen bonds between the oxygen atoms and amino groups nitrogen atoms.

Table 2. Changes in the free amino acids contents in veal after freezing depending on the polarity of radicals

Amino acid	Functional group	Mass fraction of free amino acids in meat, mg g(protein)$^{-1}$		
		before freezing	after freezing at t, °C	
			-24	-35
Nonpolar amino acids with hydrophobic side chains				
Alanine	Aliphatic hydrocarbon R-groups	1.11 ± 0.08	1.7 ± 0.1	2.4 ± 0.2
Glycine		0.61 ± 0.06	0.74 ± 0.05	0.76 ± 0.06
Valine		0.24 ± 0.02	0.49 ± 0.03	0.4 ± 0.03
Leucine		0.31 ± 0.02	0.64 ± 0.04	0.44 ± 0.04
Isoleucine		0.17 ± 0.01	0.3 ± 0.03	0.25 ± 0.02
Proline		0.17 ± 0.01	0.24 ± 0.02	0.19 ± 0.01
Hydroxyproline		0.037 ± 0.003	0.042 ± 0.003	0.032 ± 0.002
Total:		2.65	4.15	4.47
Phenylalanine	Aromatic, heterocyclic hydrocarbon R-groups	0.2 ± 0.02	0.31 ± 0.02	0.24 ± 0.02
Tryptophan		trace amounts	trace amounts	trace amounts
Total:		0.20	0.31	0.24
Methionine	-SH	0.021 ± 0.001	0.042 ± 0.004	0.038 ± 0.002
Total:		0.021	0.042	0.038
In total:		2.87	4.50	4.75
Polar amino acids with hydrophilic non-ionogenic radicals				
Tyrosine	-OH	0.19 ± 0.01	0.39 ± 0.03	0.29 ± 0.02
Serine		0.34 ± 0.02	0.60 ± 0.04	0.41 ± 0.04
Threonine		0.29 ± 0.02	0.77 ± 0.05	0.59 ± 0.05
Cysteine + Cystine	-SH	trace amounts	trace amounts	trace amounts
Total:		0.82	1.76	1.29
Polar amino acids with anionic radicals				
Aspartic acid & Asparagine	-COOH	2.6 ± 0.2	1.9 ± 0.2	1.54 ± 0.08
Glutamic acid & Glutamine		2.4 ± 0.1	1.41 ± 0.08	1.2 ± 0.1
Total:		5.0	3.31	2.74
Polar amino acids with cationic radicals				
Histidine		1.47 ± 0.06	2.2 ± 0.2	1.15 ± 0.08
Lysine		0.18 ± 0.01	0.76 ± 0.08	0.63 ± 0.05
Arginine		11.0 ± 0.8	15.2 ± 0.9	11.7 ± 0.6
Total:		12.65	18.16	13.48

Hydrogen bonds are formed between uncharged hydrophilic groups (-OH, -CO-NH2, SH-groups) and any other hydrophilic groups. Secondary structure of proteins has regular structures of two types: α-helix or β-structure. α-helical structure is formed by a great amount of hydrogen bonds and it is among the most stable conformation of the peptide backbone corresponding to the free energy minimum.

As a result of α-helix formation the polypeptide chain is shortened, but during cryodenaturation due to rupture of hydrogen bonds, the polypeptide chain elongates and becomes more accessible to the action of enzymes, as evidenced by the increase in free amino acids content (Table 1, 2).

In contrast to α-helices, breaking of hydrogen bonds that form β-structure by lots of hydrogen bonds between peptide groups linear regions of a single polypeptide chain or between different polypeptide chains does not cause elongation of the latter. Consequently the action of enzymes in β-structure of the denatured protein molecules will be hindered.

It should be noted that a possible cryodenaturation result is violation of the tertiary structure of proteins formed by hydrophobic, ionic and hydrogen bonds, but not covalent ones. Hydrophobic interactions occur between hydrophobic amino acid radicals, as well as Van der Waals forces between the closely spaced to each other atoms. As a result, hydrophobic core is formed inside of the protein globule. In the denatured protein hydrophobic radicals that in the native molecule structure are hidden within a hydrophobic core appear on the surface. In absence of strong repulsive charge molecules associate with each other by hydrophobic bonds that result in decreased proteins solubility. In addition, the compact dense spatial structure of the native protein after cryodenaturation is considerably increased in size and becomes also easily accessible to the action of enzymes.

Hydrophilic groups of amino acid radicals are also involved in the formation of the protein molecules tertiary structure. They tend to form hydrogen bonds with water, and therefore, they are mainly located on the surface of the protein molecule.

All hydrophilic amino acid group radicals trapped within the hydrophobic core interact with each other through ionic and hydrogen bonding. Ionic bonds occur generally between charged (anionic) carboxyl groups of aspartic and glutamic acids and the positively charged (cationic) groups of lysine, arginine or histidine.

Changes in proteins amino acids after freezing

The total amount of essential and nonessential amino acids and amount of each amino acid of veal protein are reduced during the freezing process regardless of temperature. However, such changes depend considerably on the structural characteristics of amino acids, chemical structure of their radicals and their solubility in water as well as on the freezing temperature.

Thus, the amount of essential amino acids of veal frozen at temperatures of -24°C and -35°C decreased by 10% and 7%, non-essential – by 17% and 9%, respectively (Table 3).

Table 3. Changes of essential and non-essential amino acids contents in veal proteins after freezing

Amino acid	Mass fraction of amino acids in meat, mg g(protein)$^{-1}$		
	before freezing	after freezing at t, °C	
		-24	-35
Essential			
Histidine	17.7 ± 1.8	13.2 ± 0.9	14.6 ± 1.3
Isoleucine	49 ± 5	40 ± 4	43.4 ± 3.1
Leucine	48 ± 4	46.6 ± 2.2	47 ± 4
Lysine	102 ± 7	91 ± 10	90 ± 5
Methionine	40 ± 4	36.4 ± 3.0	36.7 ± 3.2
Cysteine + Cystine	21.0 ± 2.0	20.4 ± 0.9	20.8 ± 1.9
Phenylalanine	44.3 ± 3.9	40 ± 4	43.1 ± 3.5
Tyrosine	32.8 ± 1.4	30.6 ± 3.1	31.4 ± 1.7
Threonine	33.3 ± 1.9	24.6 ± 2.2	28.7 ± 2.8
Tryptophan	22.4 ± 2.4	19.7 ± 1.2	21.0 ± 1.7
Valine	67 ± 4	65 ± 5	66 ± 6
Total:	477.5	427.5	442.7
Non-essential			
Alanine	32.3 ± 2.6	28.7 ± 1.6	30.4 ± 2.8
Arginine	52 ± 5	38.5 ± 1.6	43.4 ± 2.7
Serine	16.2 ± 1.0	13.5 ± 1.1	14.2 ± 1.4
Aspartic acid & Asparagine	47.4 ± 3.8	36.5 ± 1.8	39 ± 4
Glutamic acid & Glutamine	39.1 ± 3.5	28.7 ± 2.8	32.6 ± 1.5
Glycine	72 ± 7	68.3 ± 3.7	70.4 ± 3.8
Proline	26.1 ± 1.8	22.5 ± 1.4	24.6 ± 1.6
Hydroxyproline	62 ± 4	50.6 ± 3.7	60 ± 5
Total:	347.1	287.3	314.6
In total:	824.6	714.8	757.3

Table 4 shows that the freezing process and its temperature have least effect on changes of amino acids with a nonpolar (hydrophobic) side chain group type compared to other studied amino acids. Their content decreases in veal frozen at -24°C and -35°C by 10% and 4%, respectively.

It is known that non-polar radicals having aliphatic hydrocarbon chains (radicals of alanine, valine, leucine, isoleucine, proline and methionine) and aromatic rings (radicals of phenylalanine and tryptophan) tend to each other or to other hydrophobic molecules in water, reducing their contact surface with water (Baynes & Dominiczak, 2009). So it can be assumed that freezing of moisture has no significant effect on deep proteolysis of proteins associated with the cleavage of amino acids with hydrophobic radicals.

Table 4. Changes in the amino acids contents in veal after freezing depending on the polarity of radicals

Amino acid	Functional group	Mass fraction of amino acids in meat, mg g(protein)$^{-1}$		
		before freezing	after freezing at t, °C	
			-24	-35
Nonpolar amino acids with hydrophobic side chains				
Alanine	Aliphatic hydrocarbon R-groups	32.3 ± 2.6	28.7 ± 1.6	30.4 ± 2.8
Glycine		72 ± 7	68.3 ± 3.7	70.4 ± 3.8
Valine		67 ± 4	65 ± 5	66 ± 6
Leucine		48 ± 4	46.6 ± 2.2	47 ± 4
Isoleucine		49 ± 5	40 ± 4	43.4 ± 3.1
Proline		26.1 ± 1.8	22.5 ± 1.4	24.6 ± 1.6
Hydroxyproline		62 ± 4	50.6 ± 3.7	60 ± 5
Total:		356.4	321.7	341.8
Phenylalanine	Aromatic, heterocyclic hydrocarbon R-groups	44.3 ± 3.9	40 ± 4	43.1 ± 3.5
Tryptophan		22.4 ± 2.4	19.7 ± 1.2	21.0 ± 1.7
Total:		66.7	59.7	64.1
Methionine	-SH	40 ± 4	36.4 ± 3.0	36.7 ± 3.2
Total:		40	36.4	36.7
In total:		463.1	417.8	442.6
Polar amino acids with hydrophilic non-ionogenic radicals				
Tyrosine	-OH	32.8 ± 1.4	30.6 ± 3.1	31.4 ± 1.7
Serine		16.2 ± 1.0	13.5 ± 1.1	14.2 ± 1.4
Threonine		33.3 ± 1.9	24.6 ± 2.2	28.7 ± 2.8
Cysteine + Cystine	-SH	21.0 ± 2.0	20.4 ± 0.9	20.8 ± 1.9
Total:		103.3	89.1	95.1
Polar amino acids with anionic radicals				
Aspartic acid & Asparagine	-COOH	47.4 ± 3.8	36.5 ± 1.8	39 ± 4
Glutamic acid & Glutamine		39.1 ± 3.5	28.7 ± 2.8	32.6 ± 1.5
Total:		86.5	65.2	71.6
Polar amino acids with cationic radicals				
Histidine		17.7 ± 1.8	13.2 ± 0.9	14.6 ± 1.3
Lysine		102 ± 7	91 ± 10	90 ± 5
Arginine		52 ± 5	38.5 ± 1.6	43.4 ± 2.7
Total:		171.7	142.7	148.0

Amino acids with uncharged hydrophilic radicals undergo more changes than ones with nonpolar radicals. Radicals of these acids better dissolve in water, since they are composed of polar functional groups (hydroxyl in serine, threonine and tyrosine, thiol in cysteine) that form hydrogen bonds with water. In this regard, water freezing and proteins denaturation followed by a break first of all of weak hydrogen bonds lead to a decrease of these acids amounts during freezing.

Amount of amino acids with polar non-ionogenic radicals is reduced by 14% and 8% in veal proteins during freezing at temperatures of -24°C and -35°C, respectively.

Significant content changes were noted for amino acids with polar anionic and cationic radicals. Amounts of amino acids with negatively charged polar radicals – aspartic acid and asparagine, glutamic acid and glutamine, after veal freezing at -24°C are reduced by 23% and 27%, after freezing at -35°C – by 18% and 17%, respectively.

The total content of amino acids with polar positively charged cations (histidine, lysine, arginine) in veal frozen at temperatures of -24°C and -35°C decreased by 17% and 14%, respectively.

The contents of amino acids such as tyrosine, leucine, valine and cysteine do not change at studied veal freezing temperatures, obviously due to their low solubility in water. In this regard, water freezing does not affect their structural changes.

Amino acid score of indispensable amino acids was calculated to estimate the changes in the biological value of veal after freezing (Table 5). It was achieved by a comparison of the content of the amino acid in the protein with its content in the requirement pattern (Eq 1) (FAO/WHO, 2007).

$$\text{Amino acid score} = \frac{\text{mg of amino acid in 1 g test protein}}{\text{mg of amino acid in requirement pattern}} \qquad (1)$$

Table 5. Changes in amino acid score of veal proteins after freezing

Amino acid	Adult requirements, mg g(protein)$^{-1}$ (FAO/WHO, 2007)	Amino acid score		
		before freezing	after freezing at t, °C	
			-24	-35
Histidine	15	1.18	0.88	0.97
Isoleucine	30	1.63	1.33	1.45
Leucine	59	0.81	0.79	0.80
Lysine	45	2.27	2.02	2.00
Methionine	16	2.50	2.28	2.29
Cysteine	6	3.50	3.40	3.47
Phenylalanine + tyrosine	38	2.03	1.86	1.96
Threonine	23	1.45	1.07	1.25
Tryptophan	6	3.73	3.28	3.50
Valine	39	1.72	1.67	1.69

CONCLUSIONS

The content of most free amino acids has increased in veal after freezing and defrosting. The greatest changes took part in the free polar amino acids with hydrophilic nonionogenic and cationic radicals. The amount of free amino acids with hydroxyl nonionogenic radicals increased 2.15 and 1.57 times after freezing at -24°C and -35°C, respectively. The content of free amino acids with cationic radicals increased 1.44 times in veal, frozen at -24°C. Accumulation of free amino acids during meat freezing is associated with the proteolysis of muscle and connective tissue proteins.

Freezing temperature has a weak effect on the total content of amino acids with hydrophobic (nonpolar) radicals in veal. Their content decreases by 10% and 4% after freezing at -24°C and -35°C, respectively. Significant changes are established for amino acids with polar anionic and cationic radicals during veal freezing at -24°C. The content of amino acids with polar anionic radicals decreased by 25%, with cationic radicals – by 17%. This can probably be explained by the reaction of decarboxylation and the formation of nitrogenous compounds.

It was found that the veal freezing process at temperatures -24°C and -35°C reduces amino acid score of all essential amino acids, although to varying degrees. Median amino acid score decrease of 10% occurred after freezing at -24°C (minimum decrease of 2% and maximum of 26%). After freezing at -35°C median amino acid score decrease was 7% (minimum–1%, maximum–18%). In cooled veal the only limiting amino acid was leucine with amino acid score of 0.81. There were two limiting amino acids in veal after freezing – leucine and histidine. Their scores after freezing at -24°C were 0.79 and 0.88, at -35°C – 0.80 and 0.97, respectively.

It is recommended to freeze veal rapidly at -35°C and below to slow the proteolysis and save contents of amino acids with polar nonionic, anionic and cationic radicals.

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