

ELEMENTS REGARDING THE LIPOLYSIS AND PROTEOLYSIS OF FROZEN MEATS FROM THE MANGALITA BREED

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Abstract

In the case of the present research, it was wanted to know and evaluate the transformations and changes of lipolytic and proteolytic type that intervene in the intimacy of the meat-raw material, obtained from the Mangalița pig breed and preserved by freezing, for different periods of time.

The need to determine such sensory, physico-chemical and microbiological transformations, which appeared during the conservation period in some animal products, is of great interest in the spectrum of evolution of quantitative and qualitative parameters and their correlation with the state of freshness and optimal storage time, in order to know as accurately as possible the term of validity, durability of a food product, as well as to ensure consumer protection.

To study the lipolytic and proteolytic changes in pork, which occurred during storage in the frozen state, we worked on a number of 25 carcasses, the samples needed for analysis being collected before freezing at -18 ° C and then at intervals of 2, 4, 6 and 8 months storage. The control sample and the experimental samples were taken from the muscles of the anterior thigh, the croup and the dorsal muscles.

Regarding the lipolytic changes, their nature was of hydrolytic and oxidative type, these transformations being highlighted by determining the free acidity, in the first case, and by determining the peroxide index (PV), the iodine index (IV), the content in malondialdehyde (MDA-TBARS), epihydric aldehyde and fatty acids, in the second case.

Such research has a special role to play because, for example, oxidizing products existing in food and absorbed in the body have a combined action on the enzyme system, vitamins and proteins.

Regarding proteolytic changes, they were initially in a beneficial proteolytic register, not exceeding certain limits, which is characterized by improved nutritional-biological properties but later developed harmful forms for the consumer, appearing factors such as biogenic amines (histamines, betaines, etc.) or toxic compounds, such as iodine, hydrogen sulfide, phenols, mercaptans and ammonia.

In conclusion, we can argue that lipolytic changes depend on the morphological structure of the meat, the presence of marbling and perselation, the content of saturated and unsaturated fatty acids and the ratio between them, the duration and storage conditions, the type of salting, the presence of heavy metals, pesticides, the presence of hemoglobin and the intensity of enzymatic activity (the action of lipoxidases).

Regarding the dynamics of proteolysis in frozen pork, it was influenced by the age of the animal, the fineness of the muscle fiber, the ratio between the interfibrillar and interfasciolar connective tissue, the freezing temperature, the degree of biotic pollution of the meat, the nature of the biota, etc.

Keywords: lipolysis, proteolysis, frozen meat, Mangalita

INTRODUCTION

The various proteolytic and lipolytic reactions that occur in frozen pork, influence

and accelerate the occurrence of changes that may lead to restrictions on how to process and consume meat.

The knowledge and evaluation of the changes, associated with the identification of the determining factors, during the meat storage allows an optimization of the storage

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time and the programming of the optimal microclimate parameters, through which to avoid the occurrence of the depreciation of frozen meat.

MATERIAL AND METHOD

In order to establish the lipolytic and proteolytic changes in pork, which appeared during the frozen storage period, we worked on a number of carcasses, 25 males and females belonging to the Mangalita breed, red-brick variety, with slaughter weights over 130 kg, the samples required for analysis being collected before the carcasses are frozen at -18°C and then at intervals of 2, 4, 6 and 8 months of storage. The control sample and the experimental samples were taken from the muscles of the anterior thigh (back region), the croup and the dorsal muscles.

For lipids, in order to assess the hydrolytic transformations, the free acidity was determined, and the oxidative processes were highlighted by determining the peroxide index (PV), the content in malondialdehyde (MDA-TBARS), in epihydric aldehyde and the content of fatty acids, and for proteins were determined sample pH, humidity, total

nitrogen, ammoniacal nitrogen and amino acids were dosed.

RESULTS AND DISCUSSION

a). Data on lipolytic transformations

According to the literature, the essential lipolytic transformations that occur in the case of frozen pork carcasses refer to those of the hydrolytic and oxidative type. The hydrolysis of triglycerides, in particular, results in glycerin and fatty acids, which leads to an increase in the acidity of the frozen raw material, with an influence on its further processing, and the oxidation of fats leads to the appearance of products responsible for the taste and smell of rancid. related toxicological consequences.

In order to perform the required analyzes and determinations, samples were taken from the carcass muscles before freezing, immediately after freezing, at 2 months, 4 months, 6 months and 8 months, the carcasses being stored frozen at -18°C .

From the 25 carcasses, a number of 75 samples were taken, for each date and control period, the results obtained being presented in table 1.

Table 1 Dynamics of lipolytic transformations in frozen Mangalita pork fat

N= 75

Sample code	Harvest time	Statistical estimators	Fat humidity (%)	Total fat (%)	Acidity (g% oleic acid)	Iodine content (g%)	Peroxide index (meq O ₂ /kg)	MDA (mg/kg)	Aldehydes
G0	Before freezing	$\bar{X} \pm S_{\bar{X}}$	42.8 ± 0.20	37.4 ± 0.06	0.46 ± 0.004	67.5 ± 0.14	0.85 ± 0.07	0.25 ± 0.013	-
G1	After freezing	$\bar{X} \pm S_{\bar{X}}$	41.0 ± 0.68	37.0 ± 0.07	0.58 ± 0.005	66.4 ± 0.14	1.2 ± 0.06	0.50 ± 0.014	-
G2	After 2 months of freezing	$\bar{X} \pm S_{\bar{X}}$	39.6 ± 0.43	36.8 ± 0.05	0.75 ± 0.006	66.2 ± 0.15	1.8 ± 0.012	1.65 ± 0.017	-
G3	After 4 months of freezing	$\bar{X} \pm S_{\bar{X}}$	39.1 ± 0.76	35.4 ± 0.08	0.86 ± 0.007	65.8 ± 0.14	1.9 ± 0.014	1.93 ± 0.008	-
G4	After 6 months of freezing	$\bar{X} \pm S_{\bar{X}}$	40.0 ± 0.31	34.7 ± 0.04	0.86 ± 0.007	65.8 ± 0.14	2.1 ± 0.014	2.53 ± 0.008	traces
G5	After 8 months of freezing	$\bar{X} \pm S_{\bar{X}}$	38.2 ± 0.87	34.2 ± 0.04	1.15 ± 0.014	64.5 ± 0.13	1.9 ± 0.013	3.5 ± 0.007	traces

During the freezing period, according to the data in table 1, there are quantitative depreciations of the carcasses, the percentage of water in fat decreasing to a maximum of $38.2 \div 0.87\%$ (at 8 months of storage), and that of fats was between $35.4 \div 0.08\%$ at 4 months and $34.2 \div 0.04\%$ at 8 months of storage, under controlled environmental conditions.

The acidity of the meat increased during storage in the frozen state, from $0.46 \div 0.004\text{ g\%}$ (before freezing) to $1.15 \div 0.014\text{ g\%}$ (after 8 months of freezing), between the freezing time factor and the value of acidity being noticed a positive correlation.

Technologically, changes in acidity are considered to be those of more than 1.0 g\%

oleic acid, when the fat enters an advanced process of hydrolysis, sensory noticing a slightly acidic smell and a sour taste.

The iodine index, which characterizes the degree of neutralization of lipids, respectively the proportion of unsaturated higher fatty acids, decreased over time, from a value of $67.5 \div 0.14$ g I%, recorded before freezing, to $64.5 \div 0.13$ g I%, due to the reduction of unsaturation by oxidation of unsaturated fatty acids.

According to the recorded data, there is an inverse correlation between the storage time of frozen carcasses and the iodine index, a fact confirmed by the literature consulted.

The peroxide index increased during freezing, ranging from a minimum of $0.85 \div 0.07$ meq O₂ / kg before carcass freezing to a maximum of $1.90 \div 0.013$ meq O₂ / kg at 8 months of freezing. Upon recording values of more than 2.0 meq O₂ / kg of the peroxide index, severe oxidative processes are installed, with sensory changes of smell and special taste, rancid, the stage that coincided, in our experiments, with the 6th month of storage of the carcasses.

The recorded data establish that, between the 4th and 6th month of storage, a period of oxidation propagation is installed, followed by a phase of decline, recorded after the 6th month, when it is already formed. by-products of oxidation, the oxidative status

passing from the primary state to the secondary state.

Malondialdehyde (MDA) provides information on lipid peroxidation, which can be accounted for by analyzing the resulting products. According to the data obtained, at values above 2.5 MDA mg / kg of the TBARS test, we are already witnessing noticeable and organoleptic changes.

There is an inverse correlation between the MDA and the peroxide index, ie when the peroxide index decreases, the MDA value increases, which indicates the presence of oxidative secondary compounds in meat, with toxic effects. Practically, relatively fresh pork meat values of 2.5 MDA mg / kg (6-7 months), beyond these values appearing alteration changes, characterized by sensitive smell and strong rancid taste.

The action of proteases that degrade proteins also competes with the release of volatile amines, recognized by their pungent odor, and putrefaction compounds.

In order to highlight the fatty acid composition and the dynamics of the transformations during the freezing, samples were taken in the same periodic regime mentioned before, respectively before freezing (G0), at the installation of freezing (G1), at 2 (G2), 4 (G3), 6 (G4) and 8 (G5) months of carcass freezing at -18°C.

The obtained results are presented in table 2.

Table 2 Dynamics of fatty acid fat composition in frozen Mangalitzka pork

Specification	G0	G1	G2	G3	G4	G5
Acid oleic C18:1 (%)	48.50	47.94	45.12	45.62	46.18	47.20
Acid linoleic C18:2 (%)	6.75	7.26	8.03	9.20	10.72	12.23
Acid linolenic C18:3 (%)	2.60	2.83	3.12	4.37	5.08	4.83
Acid palmitic C16:0 (%)	25.05	23.50	24.55	25.17	26.48	26.56
Acid palmitoleic C16:1 (%)	1.50	1.10	1.20	1.50	1.59	1.65
Acid meristic C14:0 (%)	0.70	0.65	0.70	0.95	1.08	1.20
Acid arahidic C20:0 (%)	0.26	0.52	1.35	1.81	2.70	1.35
Acid stearic C18:0 (%)	12.31	14.5	18.60	20.50	21.60	20.80
Acid arahidonic C20:4 (%)	0.95	3.20	4.05	4.52	4.80	3.84
SFA	34.59	36.25	38.16	39.53	40.14	43.27
MUFA	55.96	54.12	54.70	55.08	58.17	59.25
PUFA	9.41	10.72	11.27	14.30	15.13	17.08

Note: SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids

With regard to saturated fatty acids (SFA), the first determinations show that oleic acid (48.50%), palmitic acid (25.05%) and stearic acid (12.31%) are found in higher

proportions, and in smaller quantities myristic acid (0.70%), palmitoleic acid (1.50%) and arachidonic (0.95%) and arachidic (0.26%) acids because, in the last

determination of during the storage period, to register important increases, especially for palmitic acid (26.56%) and stearic acid (20.80%).

During storage, the percentage of SFA (saturated fatty acids) increases from 34.59% (before freezing) to 43.27% at the end of the freezing period, and the percentage of MUFA (monounsaturated fatty acids) increases from 55.96% to 59.25%, in the same experimental period.

Regarding the content in polyunsaturated fatty acids (PUFA), the values recorded are between 9.41% before freezing and 17.08% at the end of the experiment.

b). Data on proteolytic transformations

Knowing the level of proteolytic changes allows the optimization of the storage period of raw meat (carcasses, carcass halves) and finished products, in order to prevent changes in spoilage, which can affect the health and safety of meat for consumption. Also, the knowledge of proteolytic changes allows the generation and use of the maturation factor, which has an important role in the sensory-nutritional and technological qualities of meat, as well as in digestion, by increasing the digestive utilization coefficient of meat, as the amino acid content increases.

If the proteolysis exceeds the maturation phase, harmful compounds may appear in the products, either of intermediate type such as mercaptans, iodine, hydrogen sulfide, phenols, ammonia, etc. or final type, such as biogenic amines.

Samples for determinations were taken from a number of carcasses (75 samples), of males and females belonging to the Mangalitzza breed, the brick variety, harvested before the carcasses were frozen at -18°C and then at intervals of 2, 4, 6 and 8 months storage. The control sample and the experimental samples were taken from the muscles of the anterior thigh (back region), the croup and the dorsal muscles, the results obtained being detailed in tab.3.

The analysis of the data in table 3 highlights the fact that during storage in the frozen state, the pork from the Mangalitzza

breed registers an increase in pH, from $5.8 \div 0.15$ to $6.3 \div 0.03$ units, a decrease in the humidity, from $72.70 \div 0.23\%$ to $61.37 \div 0.87\%$, of the protein substances, from $15.50 \div 0.35\%$ to $13.80 \div 0.07\%$ and of total nitrogen, from $2.45 \div 0.12\%$ to $2.10 \div 0.07\%$.

As a consequence of proteolytic enzymatic activity, ammonia increased from $18.3 \div 0.12$ to $31.7 \div 0.26$ mg%, and amino nitrogen from $125 \div 3.10$ mg to 156 mg $\div 5$, 35%.

The results of our research are based on data from specialized studies, which certify that temperature is a determining factor that influences the proteolytic changes in meat; the lower the temperature, the slower the proteolysis, without stopping completely.

Proteolytic changes may also be influenced by other factors, such as breed specialization, age, fattening status, morphological structure, and hygienic quality of the meat.

Highlighting the dynamics of the amino acid content of Mangalitzza pork, during the frozen storage period, shows that there is a change and a reduction in the overall weight of some amino acids, compared to the total amino acid content of the samples collected, due to both inhibition of some enzymatic systems, as a result of freezing, as well as an exacerbation of the activity of other amino acids, as a result of creating new relationships between enzymes and the degraded substrate (tab. 4).

According to table 4, frozen meat stored at the end of storage has higher amounts of Lysine (13.14 mg / g protein), Arginine (11.08 mg / g protein), Glycine (9.02 mg / g protein), Tyrosine (8.20 mg / g protein), Leucine (8.20 mg / g protein), Aspartic acid (19.08 mg / g protein), Cystine (8.03 mg / g protein), and lower amounts of Isoleucine (6.60 mg / g protein), Valine (7.06 mg / g protein) and Threonine (4.07 mg / g protein).

In the pre-freeze state, the total free amino acid content was 72.07 mg / g crude protein, so that at 6 months it reaches 120.09, and at 8 months this content increases to over 130 mg amino acids (137.09 mg/mg/g protein).

Table 3 Dynamics of physico-chemical transformations in frozen Mangalitzza pork

N= 75

Sample code	Harvest time	Statistical estimators	Humidity (g%)	Proteins (g%)	pH	Total Nitrogen (g%)	Amino Nitrogen (mg%)	Ammonia (mg%)	Total amino acid content (mg/g)
P0	Before freezing	$\bar{X} \pm S_{\bar{X}}$	72.70 ±0.23	15.50 ±0.35	5.8 ±0.15	2.45 ±0.12	125.0 ±3.10	18.3 ±0.12	86.17 ±2.40
P1	After freezing	$\bar{X} \pm S_{\bar{X}}$	71.12 ±0.68	15.23 ±0.44	5.9 ±0.05	2.38 ±0.20	127.0 ±2.20	18.9 ±0.60	89.22 ±3.41
P2	After 2 months of freezing	$\bar{X} \pm S_{\bar{X}}$	68.04 ±0.43	14.70 ±0.67	6.2 ±0.30	2.28 ±0.32	132.0 ±4.15	19.8 ±0.12	94.58 ±3.40
P3	After 4 months of freezing	$\bar{X} \pm S_{\bar{X}}$	65.73 ±0.15	14.23 ±0.15	6.2 ±0.14	2.23 ±0.07	140.0 ±3.28	24.6 ±0.81	98.18 ±2.83
P4	After 6 months of freezing	$\bar{X} \pm S_{\bar{X}}$ Semnificație "t"	63.14 ±0.76 *	14.05 ±0.67 *	6.4 ±0.07 *	2.16 0.04 *	147.0 ±3.60 *	28.4 ±0.30 *	116.25 ±4.80 *
P5	After 8 months of freezing	$\bar{X} \pm S_{\bar{X}}$ Semnificație "t"	61.37 ±0.87 **	13.80 ±0.07 **	6.3 ±0.03 **	2.10 ±0.07 **	156.0 ±5.35 **	31.7 ±0.26 **	165.18 ±5.41 **

Table 4 Dynamics of physico-chemical transformations in frozen Mangalitzza pork

N= 75

Nr.crt.	Amino acid type	Free amino acid content (mg / g protein)			
		Before freezing	Before freezing	After 6 months of storage	After 8 months of storage
1.	Leucine	7.25	7.80	8.12	8.60
2.	Isoleucine	4.70	4.80	5.06	6.60
3.	Tirosyne	3.56	3.90	7.13	8.20
4.	Methionine	2.35	2.80	4.28	5.70
5.	Proline + Alanine	1.90	2.00	6.69	8.03
6.	Threonine	2.62	2.65	3.40	4.07
7.	Phenylalanine	3.05	3.80	4.65	5.17
8.	Histidine	3.15	3.45	7.28	8.25
9.	Arginine	6.26	6.40	10.65	11.08
10.	Lisyne	7.86	8.10	10.53	13.14
11.	Systina	2.10	2.23	6.14	8.03
12.	Serin	4.25	4.60	5.93	7.01
13.	Glycine	3.22	3.60	7.82	9.02
14.	Valine	5.02	5.13	6.54	7.06
15.	Glutamic acid	4.56	4.80	7.12	8.05
16.	Aspartic acid	10.2	15.10	18.75	19.08
Total		72.07	81.16	120.09	137.09

CONCLUSIONS

a). Regarding lipolytic changes in meat

The data of our research and observations show that the physico-chemical transformations of fat in frozen meat depend on a number of factors, including the specialization of the breed, the presence of meat and marbling of meat, its fatty acid content, their type (saturated and unsaturated) and the ratio between them, storage conditions and duration, storage time, temperature, air currents, humidity, etc.

To assess the hydrolytic changes, the free acidity was determined, and to identify the

oxidative processes, the peroxide index (PV), the iodine index (IV), MDA-TBARS, the presence of epiphydrin aldehyde and the fat content in fatty acids were determined.

The acidity of the meat increased during storage in the frozen state, from $0.46 \div 0.004$ g% (before freezing) to $1.15 \div 0.014$ g% (after 8 months of freezing), between the freezing time factor and the value of acidity being noticed a positive correlation.

Technologically, changes in acidity are considered to be those of more than 1.0 g% oleic acid, when the fat enters an advanced

process of hydrolysis, sensory noticing a slightly acidic smell and a sour taste.

The iodine index, which characterizes the degree of neutralization of lipids, respectively the proportion of unsaturated higher fatty acids, decreased over time, from a value of $67.5 \div 0.14$ g I%, recorded before freezing, to $64.5 \div 0.13$ g I%, due to the reduction of unsaturation by oxidation of unsaturated fatty acids.

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The peroxide index increased during freezing, ranging from a minimum of $0.85 \div 0.07$ meq O₂ / kg before carcass freezing to a maximum of $1.90 \div 0.013$ meq O₂ / kg at 8 months of freezing. Upon recording values of more than 2.0 meq O₂ / kg of the peroxide index, severe oxidative processes are installed, with sensory changes of smell and special taste, rancid, the stage that coincided, in our experiments, with the 6th month of storage of the carcasses.

The recorded data establish that, between the 4th and 6th month of storage, a period of oxidation propagation is installed, followed by a phase of decline, recorded after the 6th month, when it is already formed. by-products of oxidation, the oxidative status passing from the primary state to the secondary state.

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b). Regarding proteolytic changes in meat

The analysis of the data in table 3 highlights the fact that during storage in the frozen state, the pork from the Mangalitzza breed registers an increase in pH, from $5.8 \div 0.15$ to $6.3 \div 0.03$ units, a decrease in the humidity, from $72.70 \div 0.23\%$ to $61.37 \div 0.87\%$, of the protein substances, from $15.50 \div 0.35\%$ to $13.80 \div 0.07\%$ and of total nitrogen, from $2.45 \div 0.12\%$ to $2.10 \div 0.07\%$.

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Frozen pork stored at the end of storage has higher amounts of Lysine (13.14 mg / g protein), Arginine (11.08 mg / g protein), Glycine (9.02 mg / g protein), Tyrosine (8.20 mg / g protein), Leucine (8.20 mg / g protein), Aspartic acid (19.08 mg / g protein), Cystine (8.03 mg / g protein), and lower amounts of Isoleucine (6.60 mg / g protein), Valine (7.06 mg / g protein) and Threonine (4.07 mg / g protein).

In the pre-freeze state, the total free amino acid content was 72.07 mg / g crude protein, so that at 6 months it reaches 120.09,

and at 8 months this content increases to over 130 mg amino acids (137.09 mg / mg / g protein).

In conclusion, according to the data on lipolytic and proteolytic changes during the study, we recommend keeping Mangalitzsa pork, frozen, in the form of carcass, for 8-9 months, during which time the meat and fat are kept in good nutritional conditions, generating a valuable raw material for processing in the form of meat preparations and derivatives.

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