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PEPA ZIVKOVA NATSARIDOU

"INNOVATIVE TECHNOLOGY FOR PROCESSING MEAT AND MEAT PRODUCTS"

ABSTRACT

of a dissertation for the award of an educational and scientific degree

"DOCTOR"



Scientific field 5. Technical sciences,

with a professional direction, 5.12. Food technologies

Scientific specialty "Technology of meat products and fish"

Sofia, 2023

The dissertation work was discussed and directed for defense at a meeting of the Extended Scientific Council at the "Food Technologies Department", Institute of Cryobiology and Food Technologies, Sofia, held on 15.06.2023. The development includes the sections: introduction, literature review, materials and methods, results and discussion, general conclusions, literature, a total of 117 pages. Literary sources are cited (5 in Cyrillic and 175 in Latin). The thesis includes 29 tables and 29 figures.

The numbering of the figures and tables in the abstract not corresponds to those in the dissertation.

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"Innovative technology for processing meat and meat products"

Scientific field 5. Technical sciences,

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Supervisor:

Assoc. Prof. Daniela Petkova Miteva-Gyurova, PhD

Sofia, 2023

INTRODUCTION

FAO's vision is "A world without hunger and malnutrition, where food and agriculture in an ecologically sustainable way contribute to improving the economic and social standard of living of all, especially the poorest" (Term Plan 2018-21).

For modern society, meat as food is one of the main sources of food for the population. According to data from the United Nations and the World Health Organization, the world's population suffers from insufficient intake of complete proteins in food.

The trend for healthy eating, which began in recent years, has now gained even greater meaning, as foods must have an extended shelf life due to supply difficulties, but with improved qualities. Consumers are turning to vegetarianism and accepting meat as a carrier of toxic additives and saturated fat, making it harmful without considering the presence of essential amino acids in it. Therefore, its complete replacement with proteins of plant origin can provoke health problems. The food trend of the future is aimed at a more conscious and responsible consumption of a small amount of meat, but of very high quality.

Meat is a necessary part of the full nutrition of the population, the attention of scientists and technologists was directed towards finding a replacement: synthetic additives with natural sources of antioxidants and traditional processing technologies with new ones that spare and preserve the quality of meat and meat products.

Additives are an indispensable part of meat processing. Their use aims to achieve a characteristic taste and aroma, but also to prevent or delay oxidation changes and improve the overall quality of meat products. Many of the technological stages of processing accompanying the production of meat products can lead to the formation of compounds with toxic and carcinogenic effects in meat. Therefore, the most important priorities for the meat processing industry is the development and implementation of innovative technologies that allow the production of high-quality and safe meat products. The use of natural additives with a proven antioxidant and antimicrobial effect in combination with alternative technologies aims to obtain meat products that are safe for the health of consumers with an extended shelf life, which are characterized by high nutritional value, biological value and have a beneficial effect on human health. By consuming high-quality meat food, consumers can enjoy natural, safe, sustainable, attractive and appetizing food, which at the same time plays a prophylactic and preventive role against various diseases. The growing demand for minimally processed foods with preserved taste and nutritional characteristics, extended shelf life and guaranteed microbial safety have pushed the food industry to seek new methods and approaches to food processing (Considine et al., 2008).

HPP is proving to be a promising technology with the potential for industrial application in the meat industry. With it, without changing the quality characteristics, in combination with natural antioxidants and antimicrobial substances, an extension of the shelf life of the meat products treated in this way is achieved and their safety is guaranteed. High pressure processing or cold pasteurization, unlike conventional processing processes, does not depend on the size and shape of the product, which reduces preparation time.

The range of high-pressure processed food products available on the international market shows the future potential of this technology.

Extensive scientific research is needed regarding the benefits of applying HPP technology in the food industry to establish a processing regime and understand the full capacity of the technology and whether it can be applied commercially on a large scale. The destruction of microorganisms and the inactivation of enzymes at low or moderate temperatures without changing the organoleptic and nutritional properties shows that HPP technology has the possibility to be used in the development of a new generation of food with added value.

HPP is not likely to replace traditional processing methods, but may complement them or find niche applications.

The combination of HPP with other processing options such as heat, gamma irradiation, ultrasound, etc., can allow HPP to be integrated with other processes such as blanching, dehydration, osmotic dehydration, rehydration, frying, extraction, gelling, freezing and thawing. High capital costs may limit its initial application, which is offset by lower operating costs. The development of the technology and its commercialization will lead to a decrease in the cost of the equipment and a wide spread of harmless and affordable food products processed by HPP.

I. CONCLUSIONS FROM THE LITERATURE REVIEW

1.1. A critical review of existing meat preservation technologies.

Existing meat preservation technologies, such as heat treatment, microwave heating, low heat treatment, non-thermal technologies, controlling water activity by adding salting substances, chemical methods may be effective, but they are not useful for preserving the nutritional qualities of meat. The use of natural antioxidants is useful, but not sufficient in itself to preserve meat. The introduction of processing under high hydrostatic pressure, as a new method of cold pasteurization, makes it possible to preserve the natural qualities of the meat.

1.2. HPP technology - has an impact in the following areas:

- Impact of HPP against pathogenic microorganisms and those that cause meat spoilage. The main advantage of HPP technology is the effectiveness against microorganisms, as a pressure of 500 MPa and greater causes a reduction of CFU by almost 4 log. Therefore, pasteurization is possible at a pressure of 500 MPa and higher.
- Influence of the environment on the effectiveness of HPP for deactivating microorganisms - calcium, magnesium, citrate, phosphate, sucrose and others have been found to act as baroprotectors.
- Influence of packaging on the effectiveness of HPP for inactivating microorganisms - Soft polymeric: bags, boxes, trays and bottles are suitable, as a form, and PET, PE, PP and EVOH films, used individually or in combinations, are the most common types of packaging materials. Vacuum packaging is the most common type of packaging used for HPP products because a sealed package with no residual air in it has a higher compressibility.

- Influence of HPP on the main nutritional values of meat - HPP on the enzyme activity depends on the enzyme itself, the nature of the environment (substrate, ionic strength, pH, etc.), the processing conditions (pressure, temperature, time), which reflects the taste and texture of the meat product. Most of the examined and tested vitamins, such as group B, vitamins A, E, D did not show a significant change after HPP treatment in their concentration and activity at room temperature.
- Influence of HPP processing on the structure of muscle protein - Myofibril proteins, when applying a pressure of 300 MPa and higher, leads to their denaturation, agglomeration and gel formation. Tenderization of meat by HPP treatment was associated with modification in sarcomere structure, but no rupture of ligaments was observed. In recent years, HPP has also been applied due to the structural changes that the treatment induces in meat products. These structural changes are based on the effect of high pressure on the cellular structure and on the biopolymers present in the food. They can lead to various food applications, such as creating new textures, improving water binding and gelling processes.
- Change in quality characteristics of meat after HPP treatment - The effect of HPP on myoglobin depends on the processing temperature, it was found that the discoloration of high-pressure treated meat is due to the lightening effect caused by the denaturation of myoglobin at about 400 MPa.

  Pressure induces significant changes in the levels of some volatile compounds, possibly coming from microbial activity. Some alcohols and aldehydes are reduced, while other compounds, such as 2,3-butanedione and 2-butanone, are more abundant in pressure-treated meats. Low molecular weight aromatic components are not affected by pressure due to covalent bonds.
- Effect of HPP on lipid oxidation - The increase in lipid oxidation values was observed at higher pressure levels (300, 400 MPa). Some changes in individual fatty acids were observed, while high pressure did not affect the ratio of polyunsaturated/saturated fatty acids.

II. GOAL AND TASKS

GOAL

- Innovative approach in the application of the HPP beef processing technology (alone and in combination with biologically active substances), to improve the physicochemical, biochemical, microbiological and organoleptic qualities of raw beef and its products.

TASKS

FIRST STAGE - Study of the combined influence of HPP treatment and water extracts of thyme and oregano on the quality indicators of raw beef from different types of m.Longissimus dorsi (m.LD) and m.Semimembranosus (m.SM) muscles.

- Determination of the effect of HPP treatment by better parameters (600 MPa, 3 minutes at 4°C) alone and combined with aqueous extracts of thyme and oregano on

the physicochemical parameters: pH, water holding capacity and colorimetric study of raw back muscles: Longissimus dorsi (m.LD) and Semimembranosus (m.Sm);

- Determination of the influence of HPP treatment with set parameters (600 MPa, 3 min at 4C°) alone and combined with aqueous extracts of thyme and oregano on raw beef muscles: Longissimus dorsi (m.LD) and Semimembranosus (m.Sm) on the biochemical indicators: spectrometric study of myoglobin, protein and lipid oxidation, fatty acid composition and electrophoresis;
- Determination of the effect of HPP treatment combined with aqueous extracts of thyme and oregano on the microbiological stability of meat from the two muscle groups.
- Organoleptic analysis.

SECOND STAGE - Study of the influence and effect of HPP technology in two processing modes – standard and extended time and the combined influence of HPP processing with standard time and with fixed concentrations of antioxidants in minced meat from m. Semimembranosus (m.SM). Investigation of the influence of the two regimes of HPP treatment on inoculated pathogens in minced meat from m.SM.

- Study of the influence of single HPP treatment with two modes (580 MPa,/ 4 min./ 4°C - standard and 580 MPa/ 8 min/ 4°C - extended time) and combined HPP standard treatment and antioxidant agents with fixed concentrations, during 40 days of refrigerated storage on the physicochemical parameters: (pH, water holding capacity and colorimetric study) in minced meat from m. *Semimembranosu*s
- The influence of single HPP treatment with two modes (580 MPa/ 4 min./ 4°C - standard and 580 MPa/ 8 min/ 4°C - extended time) and combined HPP standard treatment and antioxidant agents with fixed concentrations, during of 40 days of cold storage the effect on biochemical parameters (myoglobin, lipid oxidation and fatty acid analysis and electrophoresis) on minced meat from m.*Semimembranosus*
- Determination of the optimal HPP treatment regime (standard and extended time) independently of the microbiological status of minced meat from *Semimembranosus* during 40 days of refrigerated storage as an indicator of extended shelf life.
- Study of the combined effect of HPP standard processing and antioxidant agents with fixed concentrations on the microbiological status in minced meat from m.*Semimembranosus*, as an indicator of extended shelf life.
- Determination of the optimal HPP processing regime (standard and extended time) on inoculated mixtures of pathogenic microorganisms in minced beef. The aim to determinate safety of the finished product during 40 days of refrigerated storage.
- Testing the influence of HPP treatment (standard and extended time) alone and appropriately combined with antioxidant agents with fixed concentrations to preserve and improve the organoleptic qualities of minced meat from the Semimembranosus muscle.
- Creating a recipe composition of m.S*emimembranosus* minced meat, treated with HPP and a suitable additive.
- Preparation of an economic analysis for the appropriateness of the results of the development.

III. MATERIALS AND METHODS

3.1. Materials.

3.1.1. For the First stage - a model system of beef from Longissimus Dorsi (m.LD) and Semimembranosus (m.SM) muscles, treated with HPP alone or pre-flavored with aqueous extract of thyme or oregano.

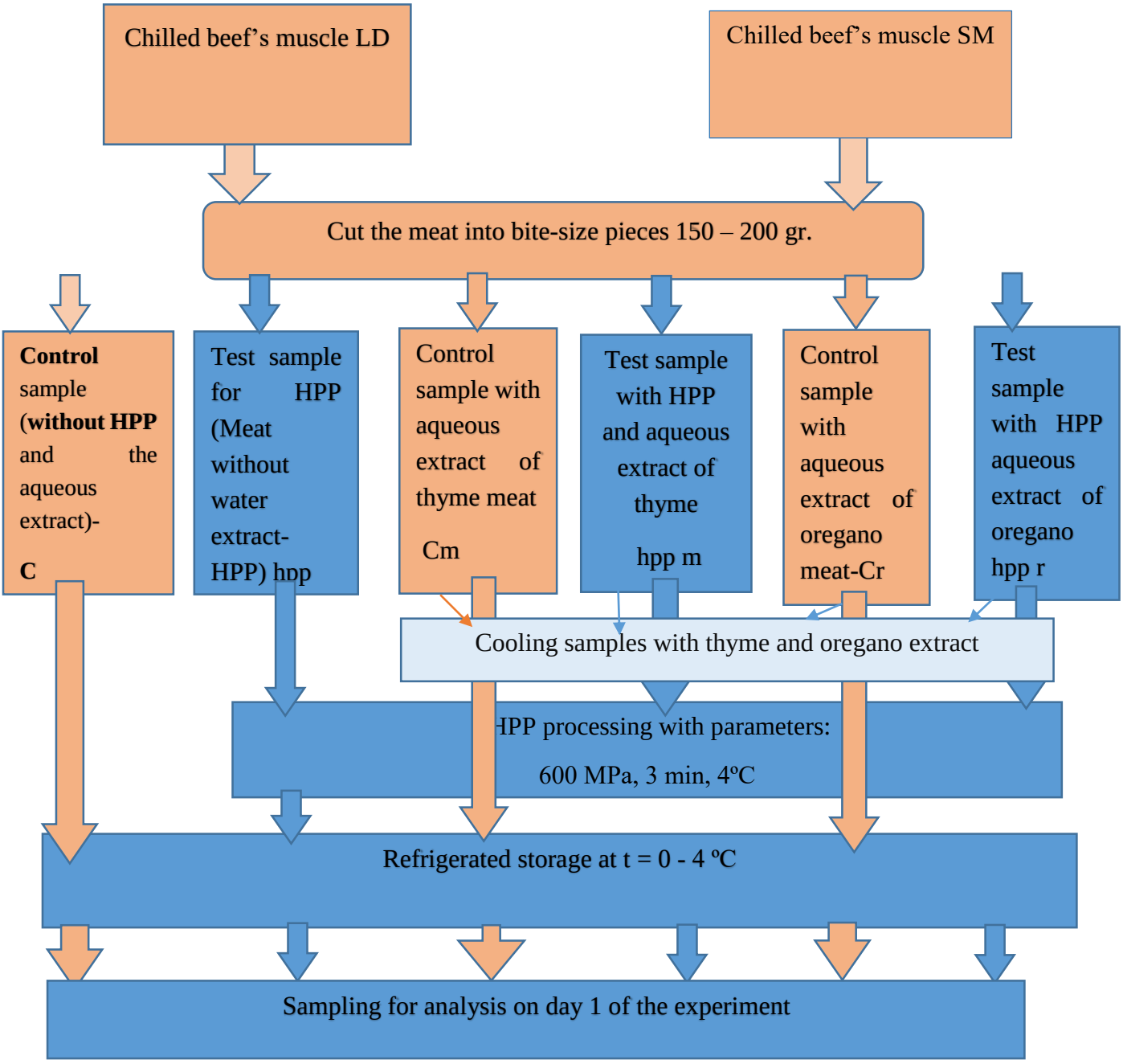


Figure 1 Experimentя with a model system of beef *Longissimus dorsi* (m.LD) and *Semimembranosus* (m.Sm) muscles treated with HPP alone or marinated with an aqueous extract of thyme or oregano.

Legend: cont - control (*untreated with hpp and aqueous extract of thyme or oregano****); hpp*** *– meat processed only* ***hpp; cont-m*** *- meat treated with aqueous extract of thyme* ***hpp-m*** *meat treated with hpp and aqueous extract of thyme;* ***cont – r*** *-meat treated with oregano extract;* ***hpp-r*** *- meat treated with hpp and oregano water extract*

Raw materials:

- The studies were conducted with beef quality, purchased in the commercial network, in compliance with the requirements for slaughtering and obtaining meat from the Large ruminants from the *Longissimus dorsi* (m.LD) and *Semimembranosus* (m.SM) muscle groups. The samples were divided into six rupees – control (untreated with hpp and without additives); hpp – meat processed only hpp; cont-m - meat treated with aqueous extract of thyme hpp-m meat treated with hpp and aqueous extract of thyme; cont – r -meat treated with oregano extract; hpp-r - meat treated with hpp and oregano water extract.
- Experiments with beef from different parts of the carcass with a high commercial value: Cutlet - includes the long muscle of the back (m. *Longissimus dorsi thoracis et lumborum*) Weissbrat, means in practice the outer part of the thigh and includes the semitendinosus muscle (m. *Semimembranosus*).
- Method for flavoring the meat: The meat from the two muscles is cut into pieces of 150 g - 200 g and poured with 250 ml. aqueous extract, for m.LD and 350 ml for m.SM. They stay in the liquid for 24 hours at a temperature of 4C°. The next day, the meats were taken out, drained from the water extract.
- Stalks of thyme (*Thymus vulgaris L*) and oregano (*Origanum vulgare*), purchased dried from a supplier in Sofia. The following were used for flavoring: aqueous extract of thyme and oregano in a ratio of 1:15.
- Method for obtaining the extract: For this purpose, 30 g of dried thyme is mixed with 450 ml. distilled water and 30 g of dried oregano are mixed with 450 ml. distilled water. The dried herbs are averaged to a size of 3 millimeters and placed in boiling water and the mixture is allowed to boil for 3 min. The infusion is left to cool for 60 min. The cooled mixture is filtered and made up to the original amount with distilled water.

Packaging materials: Vacuum envelopes of the company Vakpak Medical OOD, with dimensions - (200x420 mm) of polyamide connecting layer/polyethylene.

HPP processing

• Processing under high hydrostatic pressure (Avure facility, USA) with parameters: pressure 600 MPa, processing time 3 min., carried out in the company "AKVABAR" OOD, Sofia, Bulgaria.

3.1.2. Preliminary survey to establish the need for the use of different additives - additives for a model system of minced meat - from *Semimembranosus* muscles (m.SM), treated with HPP, alone and in combination with antioxidants.

Raw materials.

- Minced meat - from the *Semimembranosus* muscle (m.SM), purchased from the butcher in Karditsa, Greece for preliminary experimental purposes. The animal is aged between 12 - 24 months, slaughtered on 08.01.2020. The meat minced in the butcher's shop was divided into 12 pieces. samples of 50 g, respectively: 1. Control: no additive, 2. 0.05% essential oil of oregano: 0.025 ml= 25 µl in 50 g. Meat; 3. 0.1% essential oil of oregano: 0.050 ml= 50 µl in 50 g of meat; 4. 0.15% essential oil of oregano: 0.075 ml= 75 µl in 50 g of meat; 5. 0.2% essential oil of oregano: 0.100 ml= 50 µl in 50 g of meat; 6. 0.1%

dry oregano: 0.05 g in 50 g of meat; 7. 0.2% dry oregano: 0.10 g in 50 g of meat; 8. 0.3% dry oregano: 0.15 g in 50 g of meat; 9. 0.1% Sodium lactate /80%/ = 0.125 ml = 0.0625 ml in 50 g of meat; 10. 0.1% Sodium Sodium lactate /60%/ = 0.167 ml. . 60%= 0.01% in 50 g of meat; 11. 0.3% Medoliva Plus = 0.15 g in 50 g of meat; 12. 0.5% Medoliva Plus = 0.25 g in 50 g of Meat.

- Dry oregano (*Origanum vulgare*), purchased in dried form from a supplier in the city of Sofia.
- Oregano essential oil purchased from the commercial network in Greece, produced by "Herbstore", Greece.
- Sodium lactate (80% and 60% solution) was purchased from International Corporation, China. Building 9, 2277 Zhuhongzhi Blvd., Shanghai
- Medoliva Plus - is a natural extract of olives and citrus fruits / lemon fruit juice, encapsulated in maltodextrin or another similar food encapsulating carrier, by lyophilization. Only pure water is used for its extraction and drying. Production of the company "PolyHealth", Greece.

3.1.3. For the Second stage - a model system of minced meat from *Semimembranosus muscles* (m.SM), treated with HPP, alone and in combination with antioxidants

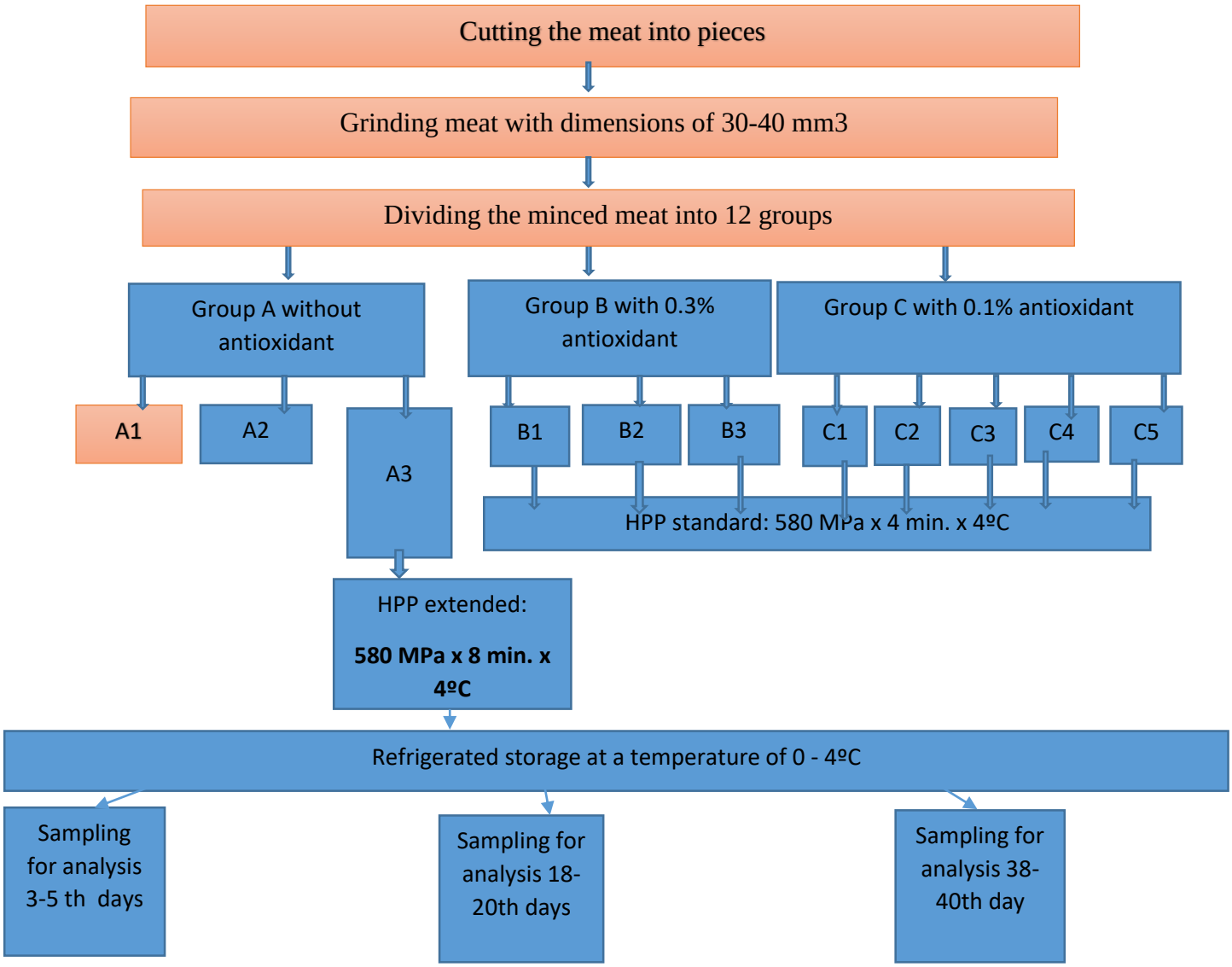


Figure 2. Experimental set-up of a model system of minced meat from *Semimembranosus* muscle (m.Sm) treated with HPP alone and in combination with antioxidants or antimicrobial agents.

Legend: Group A: A1. minced beef, vacuum packaging, without HPP processing; A2. minced beef, vacuum packaging, with HPP treatment+ 580MPa x 4min; A3. minced beef, vacuum packaging + 580MPa x 8min; Group B: B1 minced beef + HPP treatment+ 580MPa x 4min; B2. minced beef + HPP + Medoliva Plus 0.3% B3. minced beef + HPP + sodium lactate 0.3%; Group C: C1. minced beef + HPP treatment+ 580MPa x 4min; C2 minced beef + HPP + Medoliva 0.1%; C3. minced beef + HPP + Beetroot 0.1%; C4. minced beef + HPP + Radish 0.1%; C5. minced beef + HPP + Rosemary 0.1%.

Technological scheme of minced meat – muscle Semimembranosus (m.Sm), inoculated with a mix of pathogens and treated with HPP

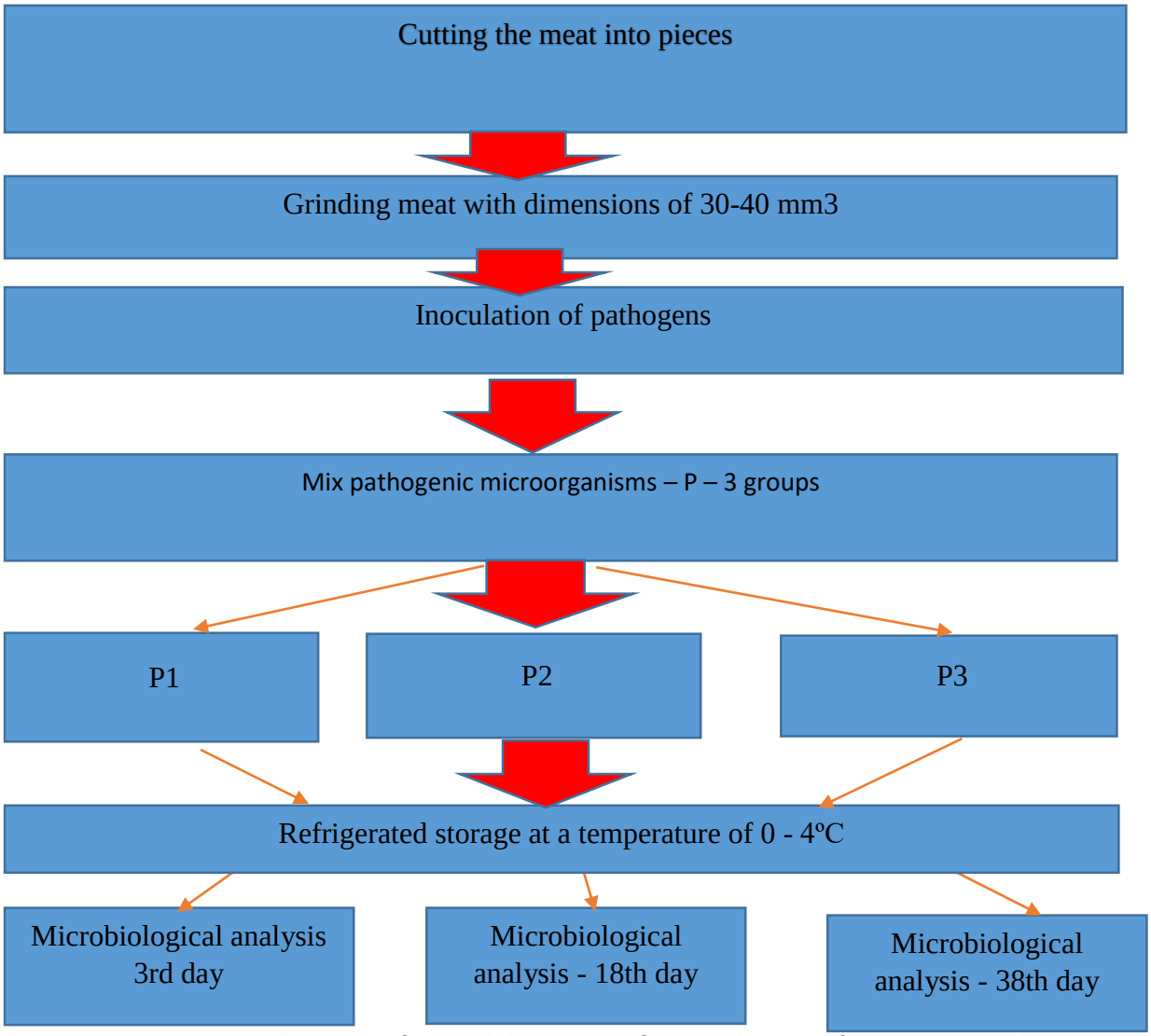


Figure 3 Experimental set-up of a model system of minced meat- from *Semimembranosus* muscle (m.Sm) inoculated with a mix of pathogens and treated with HPP under two regimes.

Legend: P1- mix of pathogens without HPP; P2 - a mix of pathogens with HPP (580 MP/4 min./ 4ºC); P3 - mix of pathogens with HPP (580 MP/8 min./ 4ºC)

Raw materials

• Semimembranosus beef muscle meat (m.Sm) I quality, "Tremas" slaughterhouse, Karditsa, Greece, in compliance with the requirements for slaughter and production of meat from LR. The meat was purchased on 04.02.20 from Tremas farm, the entire amount of 10 kg is from the Semimebranosis muscle. The animal is aged between 12-24 months, slaughtered on 31.01.2020, weighing 255 kg. Ear tag of the animal - EL4100151007. Slaughterhouse code S79, sequence of slaughtered animal - 1, animal category AR3+. Most of the tendons were cleaned and minced on a 30–40 mm3 machine, which formed the test samples of minced beef, which were divided into the following: 11 test samples of groups A, B and C and 3 with pathogens P. The samples were divided into 100 g.

Group A: A1. from beef, vacuum packaging, without HPP processing; A2. from beef, vacuum packaging, with HPP treatment+ 580MPa x 4min; A3. from beef, vacuum packaging + 580MPa x 8min;
Group C: B1. from beef + HPP treatment + 580MPa x 4min; B2. from beef + HPP + Medoliva Plus 0.3%; B3. from beef + HPP + Sodium Sodium lactate 0.3%.
Group C: C1. from beef + HPP treatment + 580MPa x 4min; C2 from beef + HPP + Medoliva 0.1%; C3. from beef + HPP + Beetroot 0.1%; C4. from beef + HPP + Radish 0.1%; C5. from beef + HPP + Rosemary 0.1%.
Antioxidant agents in a ratio of 0.3%: **"Medoliva Plus"** 0.3%, - is a natural extract of olive polyphenols and citrus/lemon fruit juice and extracts, encapsulated in maltodextrin or another similar food carrier.; **Sodium lactate** (60% solution)
Antioxidant substances in a concentration of 0.1%:
"Medoiva" A natural antioxidant produced from the fruit of the olive (aqueous extract of the fruit of the olive), with a high content of hydroxytyrosol and tyrosol, which are known as the most effective natural antioxidants. The product also contains significant amounts of caffeic and coumaric acid, catechins and anthocyanins. The content of the product MEDOLIVA in hydroxytyrosol / tyrosol is 30 times more than in extra virgin olive oil. Chemical composition: Olive extract, maltodextrin. . Production of the company "PolyHealth", Greece.
Red beetroot powder, contains components of iodine, an effect of preventing atherosclerosis has been noticed. Beetroot contains a large amount of magnesium, strengthens blood vessels and prevents the formation of blood clots, has a healing effect on hypertension. Beetroot contains a large amount of cellulose and pectin components, according to research, develops resistance to gastric ulcer. It is obtained by liquid-solid extraction. It has a purplish red color. offered by the company FruitMax- Chr. Hansen – Denmark;
Radish powder - obtained from the roots of edible red radishes, *Radish sativus L*. by extraction and refining. It is a kind of water soluble, natural red color. The color consists mainly of anthocyanin, which belongs to glucose derivatives of Pelargonidin. Appearance: Red dark red powder. Available from the company FruitMax- Chr. Hansen – Denmark;

Rosemary oil. Rosemary 0.1%. is a natural extract of rosemary essential oil. For centuries, one of the most common medicinal uses of the decoction has been to improve memory, not just to flavor food. This spice, and specifically the flower tops, contains antibacterial and antioxidant rosmarinic acid, as well as essential oils such as cineole, camphene, borneol, bornyl acetate, and α-pinene, which are known to have anti-inflammatory, antifungal, and antiseptic properties.

Packaging materials: Vacuum bags - FlexoPack, Greece (200x420 mm) with a polyamide/polyethylene binding layer. This type of packaging limits the negative impact of oxidation on minced beef. The closing of the vacuum bags was done by a Henkelman packaging machine (Netherlands). The indicators of the vacuum bags are: PA-PE based on (oxygen permeability 75 cc /m2 / 24 h/ 1 atm) water permeability 5 gr /m2 / 24 h.

(High Pressure Processing - HPP) processing: HPP processing was carried out at a facility of the American company AVURE, located in Mytilos, Kitros village, Pieria district, Greece. **Standard** processing: **Pressure 580 MPa. Water temperature 4°C, retention time 4 min**. **Extended** processing time: **Pressure 580 MPa, temperature 4°C, holding time 8 min.** Applies to samples without added substances (A3) and samples with inoculated pathogen mix (P3).

3.2. Analysis methods.

3.2.1. Technological: Sampling was carried out according to the requirements of BDS EN ISO 3100-1:2002.

3.2.2. Physicochemical: 1. Determination of the pH value by means of a pH meter: First stage - Hanna, USA (MP, pH sample, glass electrode), Second stage - with pH meter Hanna – Food care, HI 99161, Romania. 2 Determination of water holding capacity (WHC): A classical method of determination of Grau and Ham (1952) was used.

3. Determination of color characteristics - The color characteristics of the samples were determined instrumentally according to the CYELab system (Brewer and Wu, 1993), by measuring the reflectivity and the extraction of pigments with a colorimeter. Colorimeter model Lovinbond GAM – Stem 500.

3.2.3. Biochemical:

1. Protein oxidation is determined by the content of carbonyl substances, as described by Oliver et al. (1987) and modified by Mercier et al., 1998**;**

2. Determination of myoglobin and its derivatives according to the method of Krzywicki (1979);

3.Determination of changes in the protein spectrum of meat products by denaturing protein electrophoresis in polyacrylamide gel SDS-PAG according to Laemmli's method (Laemmli, 1971),, with a slight modification from Ketnawa and Rawdkuen (2011).

A protein electrophoresis system was used - Pharmacia LCB MultiDrive XL equipped with an Omni PAGE WAVE Electrophoresis System (Cleaver Scientifics);

4. Determination of the secondary derivatives of lipid oxidation by means of 2-thiobarbiturate-acid-reactive compounds (lipid oxidation) **For the first stage**, the method of Lynch and Frei (1993) and modified by Mercier al. (1998). **For the Second stage** - the determination of the secondary derivatives of lipid oxidation by the method of Oxford Biomedical, proposed by the laboratory of microbiology, University of Thessaly, Greece Spectrophotometer LANGE, DR 5000, Germany;

5. Determination of fatty acid composition of fat in meat - The extraction of total lipids was carried out according to the method of Bligh & Dyer (AOAC, 1959), and the methyl esters of fatty acids were analyzed on a gas chromatograph

3.2.4. Microbiological: BDS EN ISO 6887-2:2004; EN ISO 4833-1,2; BDS 1035. BDS EN ISO 4833:2004; ISO 4831, ISO 4832; ISO 6579-1:2002; ISO 7954; ISO 6888-1-3, ISO 6888-2:2003; ISO 15213; ISO 15214:1998; ISO 21525-2:2004; ISO16649-2:2001; ISO 11290-2:2017; ISO 10272-2

3.2.5. Organoleptic assessment - 5-point scale developed by University of Applied Sciences Thessaly, Greece - Department of Food Technologies, Karditsa.

3.2.6 Statistical analysis - the software package JMP v7. Two-factor analysis of variance (Fit model procedure). Statistical differences between groups were applied multiple comparisons (Tukey-Kramer HSD) at $P<0.05$. The root mean square error (RMSE) of the model used was used to assess the reliability of differences between groups.

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IV. RESULTS AND DISCUSSION

4. Analysis of results

4.1. FIRST STAGE - Combined effect of HPP treatment and water extracts of thyme and oregano on quality indicators of raw beef from different types of m.*Longissimus dorsi* (m.LD) and m.*Semimembranosus* (m.SM) muscles.

4.1.1. Physicochemical parameters, color characteristics and lipid oxidation

In the present study, high pressure (600 MPa) was applied alone or in combination with an aqueous extract of thyme and oregano, leading to oxidation processes of lipids and proteins. Tables 1 and 2 show the results of the physicochemical tests performed on raw beef for the 2 muscle groups studied - m.*Longissimus dorsi* (m.LD) and m.*Semimembranosus* (m.SM).

Table 1 Physicochemical parameters and lipid oxidation of raw beef treated with high hydrostatic pressure and antioxidants in *Longissimus dorsi*- (LD) muscle

Processing	Antioxidant	pH	WHC(%)	L	a	b	lipid oxidation, mg TBARS kg. meat
Control	Without antioxidant	5.590	36.69	40.71	21.54	10.73	0.315
	thyme	5.627	40.11	47.58	15.21	14.41	0.327
	oregano	5.620	41.00	49.96	15.74	15.49	0.345

HPP	without antioxidant	5.835	41.20	57.89	19.22	12.76	0.296
	thyme	5.862	43.31	60.20	14.51	15.66	0.215
	oregano	5.817	42.79	58.65	15.07	16.62	0.290
Effect							
Processing	contol	5.612^{b}	39.26^{b}	46.08^{b}	17.50	13.55	0.267
	HPP	5.838^{a}	42.43^{a}	58.91^{a}	16.27	15.01	0.319
Antioxidant	without antioxidant	5.713^{b}	38.94^{b}	49.30^{b}	20.38^{a}	11.75^{b}	0.306
	thyme	5.744^{a}	41.71^{ab}	53.89^{a}	14.86^{b}	15.04^{ab}	0.271
	oregano	5.718^{ab}	41.90^{a}	54.30^{a}	15.40^{b}	16.05^{a}	0.317
RMSE (n=24)		**0.020**	**1.87**	**2.86**	**2.05**	**2.71**	**0.079**
Authenticity	processing	P<.0001	P=0.0036	P<.0001	P=0.2274	P=0.2743	P=0.1188
	antioxidant	P=0.0379	P=0.0308	P=0.0186	P=0.0009	P=0.0432	P=0.5867
	processing*antioxidant	P=0.1243	P=0.4714	P=0.0717	P=0.7317	P=0.9525	P=0.5978

Legend: control – raw beef untreated, HPP – raw beef processed by high pressure, BZS – water holding capacity, L – color brightness, a – yellow color component, b – red color component. Values associated with different letter designations are significantly different (P<0.05).

Table 2. Physicochemical parameters and lipid oxidation of raw beef treated with high hydrostatic pressure and antioxidants in m. *Semimembranosus* (SM) muscle

Processing	Antioxidant	pH	WHC(%)	L	a	b	lipid oxidation, mg TBARS kg. meat
Control	without antioxidant	5.738	35.16	36.93^{b}	18.93	10.53	0.320
	thyme	5.739	39.65	46.61^{a}	15.25	14.46	0.324
	oregano	5.784	37.73	47.63^{a}	12.82	15.89	0.277
HPP	without	5.873	40.15	52.23^{a}	23.00	13.93	0.246
	thyme	5.939	40.06	51.72^{a}	17.59	16.06	0.218
	oregano	5.870	43.55	51.00^{a}	20.28	15.94	0.251
Effect							
Processing	contol	5.736^{b}	37.52^{b}	43.72	15.66^{b}	13.63	0.307^{a}
	HPP	5.894^{a}	41.25^{a}	51.65	20.29^{a}	15.31	0.238^{b}
Antioxidant	without antioxidant	5.805	37.66	44.58	20.96^{a}	12.23^{b}	0.283
	thyme	5.839	39.85	49.17	16.42^{b}	15.26^{a}	0.271
	oregano	5.827	40.64	49.31	16.55^{b}	15.92^{a}	0.264
RMSE (n=24)		**0.091**	**3.06**	**3.29**	**2.12**	**2.04**	**0.043**
Significance	Processing	P=0.0014	P=0.0079	P<.0001	P<.0001	P=0.0581	P=0.0010
	antioxidant	P=0.7578	P=0.1598	P=0.0151	P=0.0005	P=0.0043	P=0.6754
	processing*antioxidant	P=0.4723	P=0.1917	P=0.0039	P=0.0742	P=0.2853	P=0.2017

Legend: control – raw beef untreated, HPP – raw beef processed by high pressure, BZS – water holding capacity, L – color brightness, a – yellow color component, b – red color component. Values associated with different letter designations are significantly different (P<0.05).

Exposure to high hydrostatic pressure was found to increase pH values in both muscle groups. The influence of antioxidants was proven in muscle group m.LD, where we found higher values of this indicator in the group treated with thyme compared to the control group without antioxidant. The increase in pH (acidity) after HPP treatment is a consequence of

pressure ≥ 400 MPa. In Mc Ardle's study, high pressure processing at 200 MPa did not change the pH values of beef. (McArdle, 2010)

Impact on this indicator of the applied antioxidant substances, show higher values when combining the action of high pressure and thyme and a slight reduction with oregano for both muscle groups. The combined effect depends, both on the antioxidant and the type of meat, it was found during storage of pork fillets at 4°C (9 days) that the pH of fillets containing sage was quite variable, while the pH of those with panax and rosemary increases, and those with trigonella and mustard decreases. (McCarthy et al., 2001).

The influence of HPP processing in the analysis results in an increase in the water-holding capacity of raw beef in both muscle groups studied from 7 to 10%. The obtained results differ from the Wada study, which showed that the BAC of sardine meat for an untreated sample was 37.1%, and after HPP treatment it decreased to 33.5% and considered this parameter as related to lipid oxidation. (Wada, 1996). We found an influence of added antioxidants only for the group with oregano in muscle group LD, where we found higher values compared to the group without antioxidant. The interaction between the two factors has not been proven.

High hydrostatic pressure treatment was found to increase the brightness (L) values of raw beef in the LD muscle group at a very high level of evidence P<.0001. The increase in L* values or "whitening" as a lightening effect of pressure has been attributed to myoglobin denaturation, heme displacement or release, and ferrous ion oxidation (Cheftel & Culioli, 1997). Other authors have suggested that the lighter appearance of meat may also be due to changes in water content (Mussa, 1999). In the SM muscle group, there is a proven interaction between the treatment with HPP and added antioxidants from oregano and thyme in the direction of increasing the values of the indicator compared to the control group, not treated with HPP and without added antioxidants.

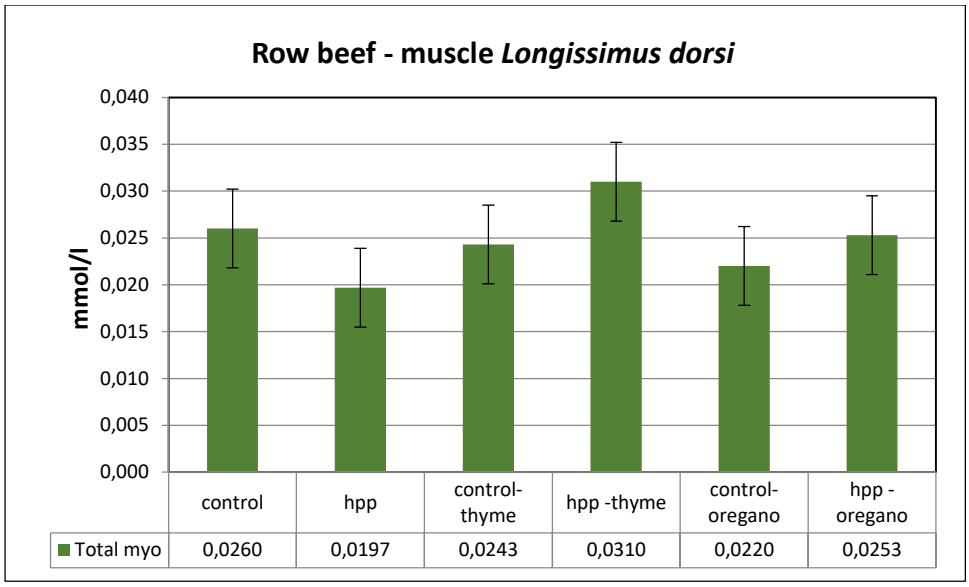
According to the results obtained for the red component of the meat, the impact of HPP works differently in the two types of muscle groups. Treatment with HPP leads to an increase of this indicator in the SM muscle group, while in the LD group we obtain a statistically unproven decrease in the values. Adding thyme and oregano to meat leads to lower values of this indicator in both muscle groups. A higher value of the red component of the color is found in SM, compared to LD. It can be assumed that the better results in SM are due to the different composition of the muscle and the smaller amount of myoglobin. The impact of the applied high hydrostatic pressure is weakly expressed and statistically insignificant in the direction of increasing the values of the yellow component of the meat. The influence of added antioxidants leads to a reliable increase in the values of this indicator in both muscle groups, more pronounced in the group treated with oregano. The results for LD, regarding the red and yellow color components, correspond to those obtained by McArdle, 2010 for beef from *m. Pectoralis profundus*. Lipid oxidation also causes changes in meat color, being one of the most important quality parameters. (Jung et al., 2013).

The obtained results show that the HPP treatment affects the **lipid oxidation** of the raw beef meat only in the SM muscle group in the direction of decreasing the index values for both muscle groups, more significantly in the SM group. Treatment with antioxidants resulted in insignificant changes in the lipid fraction. The influence of the treatment with HPP alone on the index of lipid oxidation showed a reliable decrease in the values of the MDA index in the

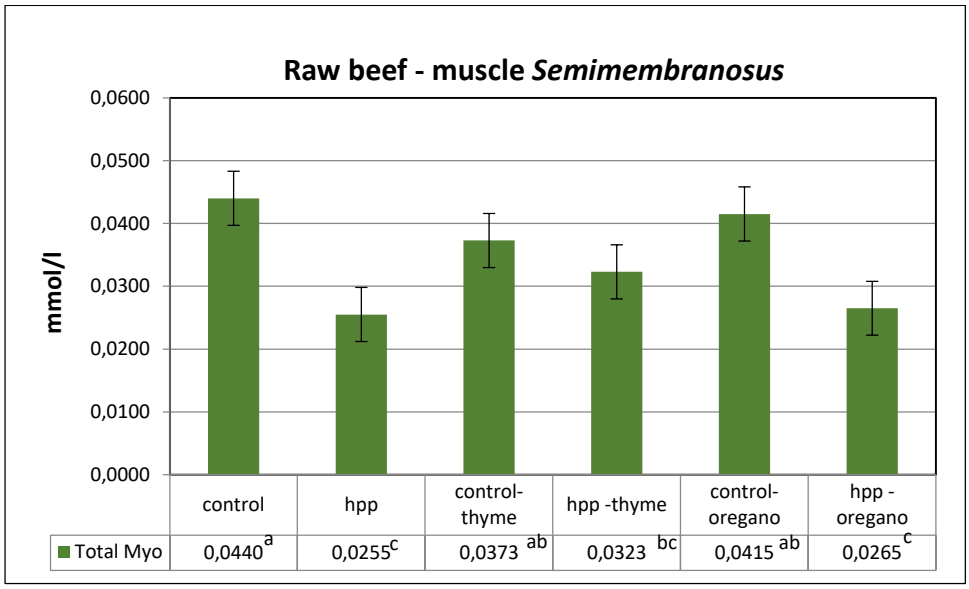
SM group. It can be assumed that the good results are also due to the high quality of the vacuum packaging

4.1.2. Myoglobin and its forms, changes in meat protein

Figures 4 and 5 show the results of the tests on the total myoglobin index in the two beef muscles examined.



Фиг.4 Total myoglobin content of raw beef in muscle *Longissimus dorsi*

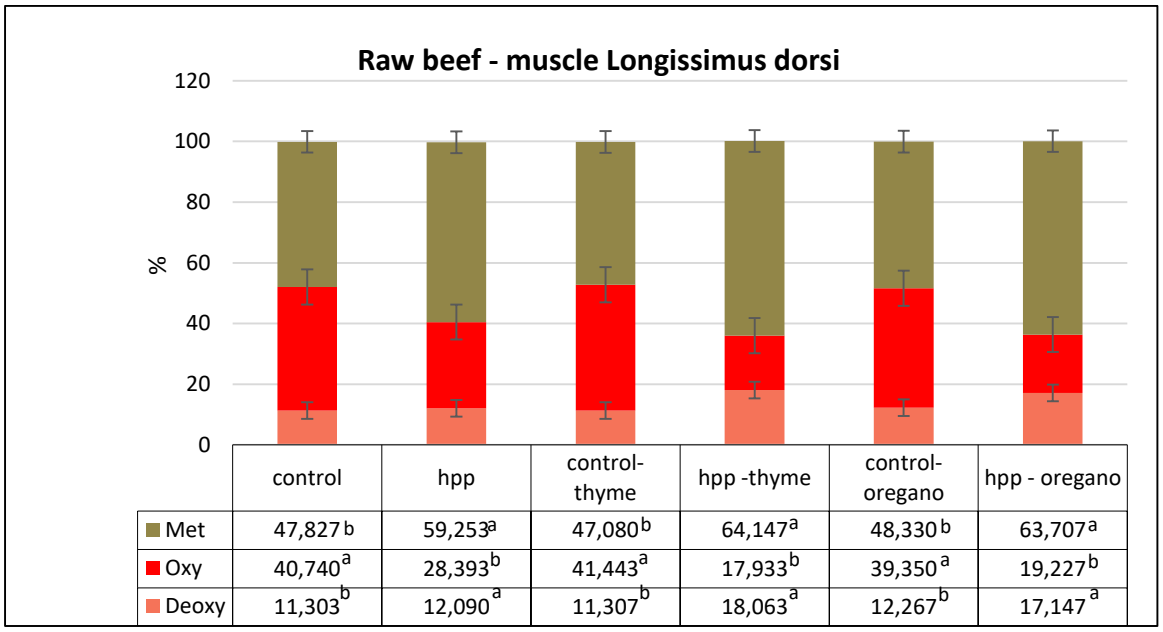


Фиг.5 Total myoglobin content of raw beef in muscle *Semimembranosus*,

* *Values associated with different letter designations are significantly different (P<0.05).*

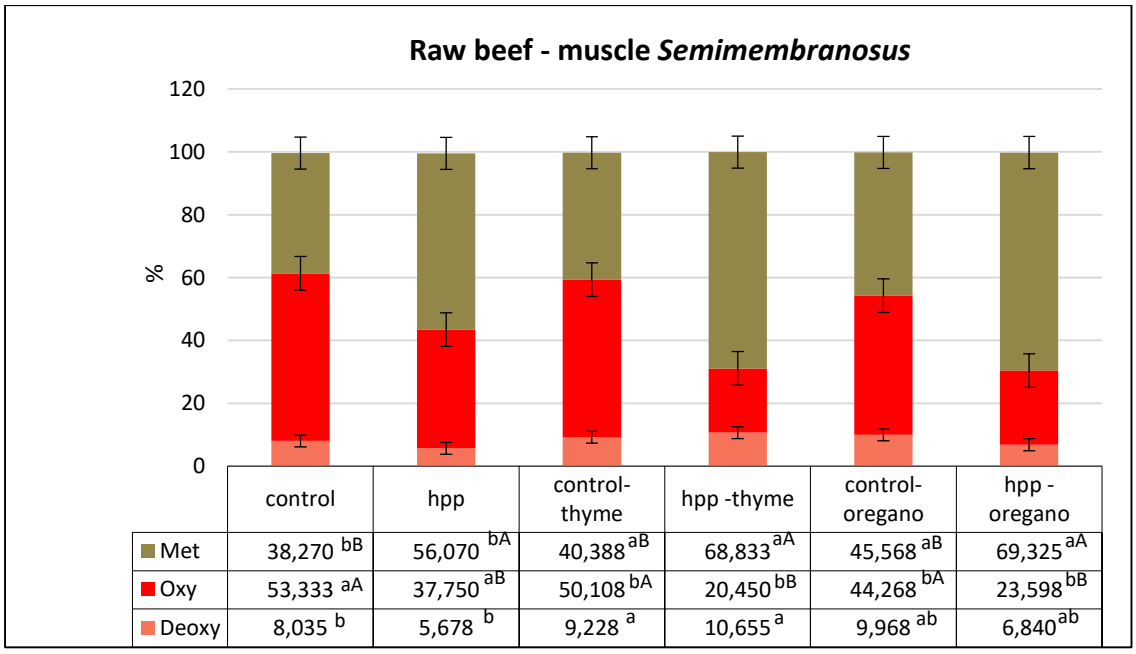
In the LD muscle group, no significant differences were found following the administration of HPP and the treatment with thyme and oregano. In the SM muscle group, we found an interaction between the factors HPP treatment and added supplements. The highest value was obtained in the untreated control, which significantly differed from all groups treated with HPP, where we found a decrease in the values of the indicator within 27 to 42%. Reduction of

myoglobin content by high pressure treatment is considered to be dependent on the degree of high pressure along with the denaturation of the globular protein. (Wada1996.)



	control	hpp	control-thyme	hpp -thyme	control-oregano	hpp - oregano
Met	47,827 b	59,253 a	47,080 b	64,147 a	48,330 b	63,707 a
Oxy	40,740 a	28,393 b	41,443 a	17,933 b	39,350 a	19,227 b
Deoxy	11,303 b	12,090 a	11,307 b	18,063 a	12,267 b	17,147 a

Fig.6 Forms of myoglobin of raw beef in muscle group LD *Values associated with different letter designations differ significantly (P<0.05)



	control	hpp	control-thyme	hpp -thyme	control-oregano	hpp - oregano
Met	38,270 bB	56,070 bA	40,388 aB	68,833 aA	45,568 aB	69,325 aA
Oxy	53,333 aA	37,750 aB	50,108 bA	20,450 bB	44,268 bA	23,598 bB
Deoxy	8,035 b	5,678 b	9,228 a	10,655 a	9,968 ab	6,840 ab

Fig.7 Myoglobin forms of raw beef in muscle group LD,

** Values associated with different letter designations are significantly different (P<0.05), with lowercase letters associated with the influence of applied additives and uppercase letters associated with technological processing (HPP)*

In fig. 6 and fig.7 show the results of the changes in the forms of myoglobin in the two studied muscle groups.

In LD muscle, we found that under the influence of HPP, the content of deoxymyoglobin and metmyoglobin increases at the expense of a decrease in the content of oxymyoglobin. Metmyoglobin levels were too high and treatment with the added supplements did not significantly affect the values of this indicator.

In the SM muscle group, we found a significant increase in deoxymyoglobin values in the thyme-supplemented group compared to the unsupplemented control group. Treatment with high hydrostatic pressure does not significantly affect this indicator. For oxymyoglobin and metmyoglobin, the same trends are present as for the first muscle group. Higher methemyoglobin and lower oxymyoglobin values were found after HPP administration. In these indicators, unlike muscle group LD we also obtained a reliable influence of the incorporated antioxidants.

A significant decrease in oxymeiglobin values and an increase in metmyoglobin were found in the oregano and thyme groups compared to the unsupplemented groups. The different forms of myoglobin deoxymyoglobin, oxymyoglobin and metmyoglobin have different sensitivities to high pressure and thus the proportions of myoglobin forms present in fresh meat before and after HPP are a determinant of the degree of myoglobin oxidation. (Bak et al., 2012). The denaturation process confirms the CIE values that beef under pressure changes from bright cherry red to dark red or brown. This suggests that high-pressure processing can induce the same type of discoloration and, respectively, myoglobin, as heat processing. Denaturation of metmyoglobin is associated with the shift of hematin iron to the ferric state

In fig. 8 and fig.9 show the results of the change of carbonyls under the influence of the inserted additives and the technological treatment with HPP

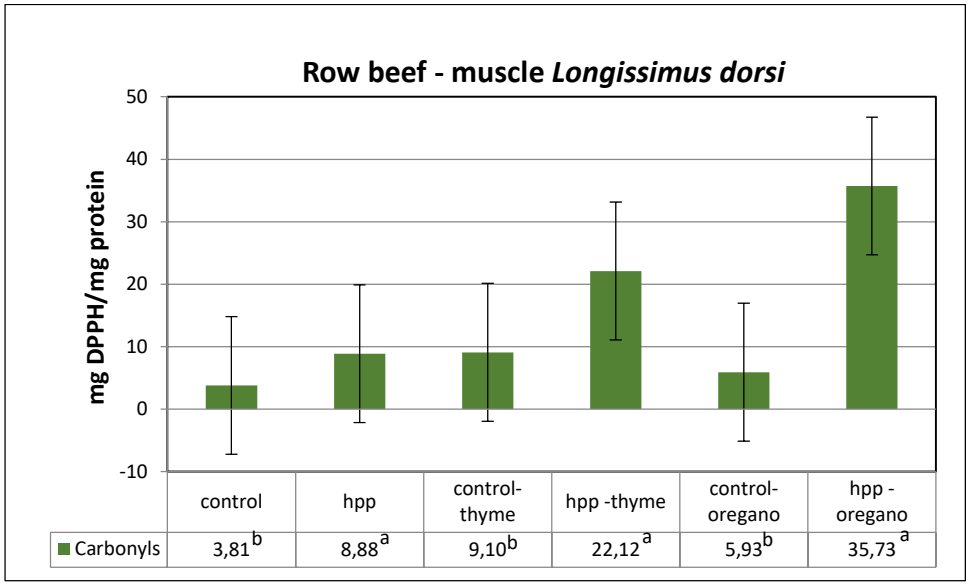


Fig. 8 Content of carbonyls in raw beef in muscle group LD, * Values associated with different letter designations differ significantly ($P<0.05$)

In the LD muscle group, we found that carbonyl content reliably increased after HPP treatment, with values ranging from 133% in the unsupplemented control group to 502% in the oregano-supplemented group.

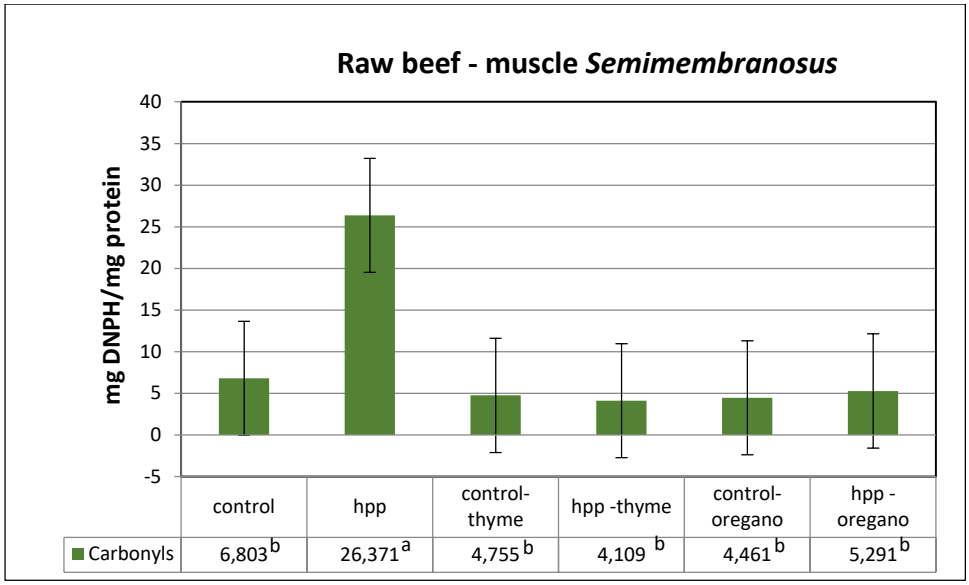


Fig.9 Content of carbonyls in raw beef in muscle group SM,

** Values associated with different letter designations differ significantly (P<0.05)*

In the SM muscle group, we found an interaction between the factors treatment and, distinguishing the group treated with high hydrostatic pressure without supplements, where the values were 4 to 6 times higher than the other studied groups. Muscle proteins are susceptible to oxidative reactions caused by various oxidation initiators, such as oxidizing lipids, metal ions, and other prooxidants generated during meat processing, which corresponds to the results obtained for carbonyls in both muscle groups. (Estévez et al., 2011).

In Fig. 10, electrophoresis in a polyacrylamide gel SDS-PAGE of *Longissimus dorsi* and *Semimembranosus* muscle groups of beef gives information about changes in the protein spectrum of meat products, before and after HPP treatment and addition of biologically active components for the purpose of stabilization in the storage process. The extracts did not change the number and intensity of the protein fractions. No differences were reported for samples subjected to high pressure processing (HPP).

Technological processing under high pressure does not change the primary structure and molecular mass of the main proteins in meat from both muscle groups. These results are in agreement with the data obtained by Csehi et al. (2015), according to which beef proteins (sarcoplasmic and myofibrillar) do not change during high pressure treatment of 600 MPa, do not denature and are more resistant to high pressure than pork.

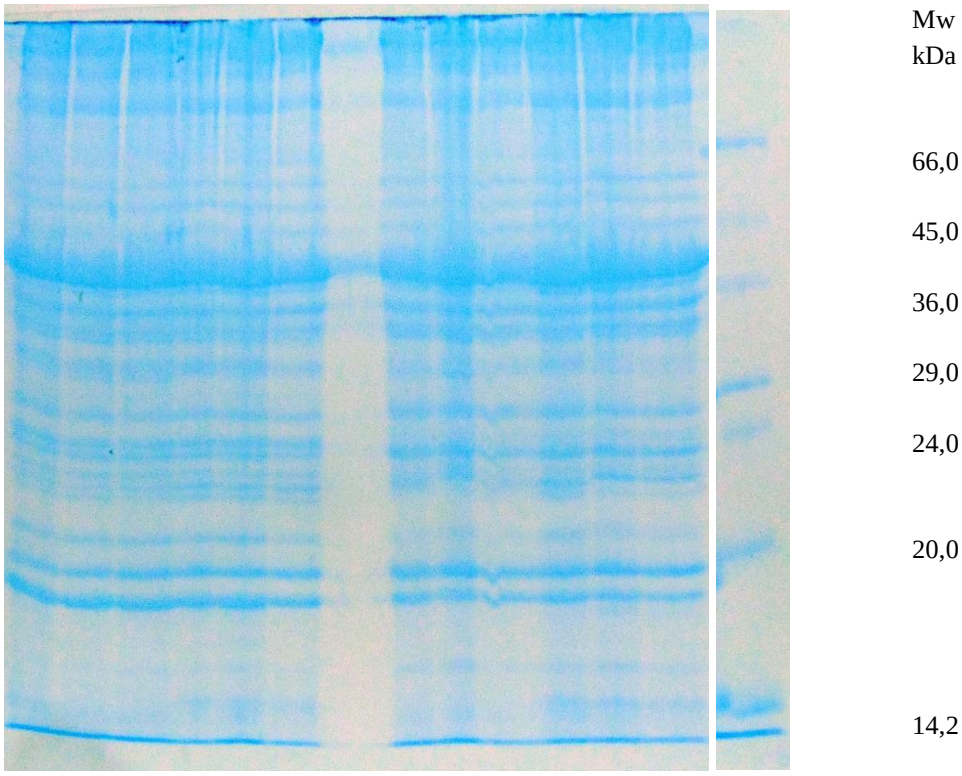


Figure 10. SDS PAGE electrophoresis of beef proteins at different processing options.

Legend: 1-Musculus *semimembranosus* (MSm) control; 2- Musculus *semimembranosus* (MSm) marinated with oregano; 3- Musculus *semimembranosus* (MSm) marinated with thyme; 4- control Musculus semimembranosus (MSm) treated with HPP; 5- Musculus semimembranosus (MSm) treated with HPP and marinated with oregano; 6- Musculus semimembranosus (MSm) treated with HPP and marinated with thyme; 7- Control Musculus *longissimus dorsi* (MLD); 8- Musculus *longissimus dorsi* (MLD) with oregano; 9- *Musculus longissimus dorsi* (MLD) with thyme; 10- control *Musculus longissimus dorsi* (MLD) treated with HPP; 11- *Musculus longissimus dorsi* (MLD) treated with HPP and oregano; 12- *Musculus longissimus dorsi* (MLD) treated with HPP and thyme; M- protein marker.

4.1.3. Changes in fatty acid composition of a model system of *Longissimus dorsi* and *Semimembranosus* muscle groups

Table 3. Fatty acid groups (g/100g fat) of raw beef in *Longissimus dorsi and Semimembranosus* muscles.

processing	antioxidant	Σ SFA		Σ MUFA		Σ PUFA		Σ CLA		Σ Branched FA		Σ ω3		Σ ω6		Σ ω6/Σ ω3	
		mLD	m SM	m LD	m.SM	m. LD	m SM	m. LD	m. SM	m.LD	m.SM	m. LD	m. SM	m. LD	m. SM	m. LD	мускул SM
control	without additives	44.26	48.83	35.62	40.99^{ab}	17.72	8.17	0.459	0.315	1.949	1.698	7.081	2.675	10.635	5.497	1.535	2.248
	thyme	43.02	53.44	34.44	36.96^{b}	21.00	7.26	0.250	0.355	1.270	1.899	7.142	2.315	13.854	4.942	1.900	2.239
	oregano	39.97	47.08	33.82	41.62^{ab}	24.31	9.53	0.246	0.283	1.571	1.493	8.747	3.231	15.564	6.296	1.772	1.868
HPP	without additives	49.01	43.08	39.02	52.54^{a}	10.20	12.85	0.244	0.261	1.530	1.221	3.242	4.332	6.958	8.522	2.214	1.983
	thyme	47.20	45.32	42.81	44.47^{a}	8.02	8.30	0.220	0.349	1.740	1.552	2.390	2.800	5.632	5.502	2.384	2.190
	oregano	45.00	42.10	42.79	42.35^{a}	10.24	13.92	0.224	0.262	1.752	1.368	3.801	4.872	6.435	9.051	1.856	1.868
processing	control	42.41^{b}	49.78^{a}	34.63^{b}	39.85	21.01^{a}	8.32^{b}	0.318	0.317	1.597	1.697^{a}	7.657^{a}	2.740^{b}	13.351^{a}	5.578^{b}	1.736^{b}	2.150
	HPP	47.07^{a}	43.50^{b}	41.54^{a}	43.12	9.49^{b}	11.69^{a}	0.230	0.290	1.674	1.380^{b}	3.144^{b}	4.001^{a}	6.342^{b}	7.692^{a}	2.152^{a}	2.014
antioxidant	without additives	46.63	45.95^{ab}	37.32	41.76	13.96	10.51	0.351	0.288	1.740	1.460	5.161	3.504	8.797	7.010	1.875	2.115
	thyme	45.11	49.38^{a}	38.63	40.71	14.51	7.78	0.235	0.352	1.505	1.726	4.766	2.558	9.743	5.222	2.142	2.215
	oregano	42.48	44.59^{b}	38.30	41.98	17.27	11.72	0.235	0.272	1.661	1.430	6.274	4.051	11.000	7.673	1.814	1.916
RMSE (n=24)		**3.27**	**3.81**	**4.85**	**2.28**	**5.52**	**3.61**	**0.113**	**0.083**	**0.302**	**0.312**	**1.960**	**1.374**	**3.865**	**0.076**	**0.371**	**0.380**
significance (P)	processing	0.011	0.001	0.011	0.002	0.001	0.031	0.123	0.432	0.596	0.021	0.001	0.034	0.002	0.031	0.039	0.383
	supplem.	0.126	0.048	0.890	0.482	0.554	0.095	0.164	0.141	0.417	0.125	0.412	0.103	0.624	0.093	0.301	0.285
	proc.*additives	0.974	0.681	0.568	0.012	0.561	0.522	0.285	0.834	0.068	0.534	0.874	0.607	0.449	0.478	0.395	0.833

Values associated with different letter designations are significantly different (P<0.05).

Table 4. Essential fatty acids (g/100g fat) of raw beef in *Longissimus dorsi* and *Semimembranosus muscles*.

processing	antioxidant	lauric C-12:0		palmiticC-16:0		stearic C-18:0		palmitoleic C-16:1n7		heptadecenoic C-17:1n7		linoleic C-18:2c9,12/19:0		eicosatrienoic C-20:3n3		oleic C-18:1cis-FA	
		m. LD	m.SM	m. LD	m. SM	m. LD	m. SM	m. LD	m. SM	m. LD	m. SM	m. LD	m. SM	m. LD	m. SM	m. LD	m. SM
control	without additives	4.73	4.52	19.96	24.59	15.23	16.13	2.04	2.34	4.88	1.61	10.42	5.14	5.51	1.67	25.53	33.23^{ab}
	thyme	0.64	7.83	20.65	25.07	19.30	15.94	1.34	2.00	2.51	1.69	13.46	4.53	5.27	1.27	27.33	28.96^{b}
	oregano	2.76	3.84	19.14	24.48	14.90	15.54	1.64	2.36	6.45	2.20	15.06	6.02	6.84	1.98	22.25	33.34^{ab}
HPP	without additives	0.23	0.54	22.54	24.75	23.41	14.60	1.50	2.59	0.48	2.00	6.59	8.25	2.40	2.89	34.20	34.52^{a}
	thyme	0.44	0.60	22.57	25.87	19.04	15.15	2.13	2.80	0.89	1.40	5.38	5.26	1.85	1.68	36.84	36.56^{a}
	oregano	1.02	1.59	23.03	23.17	17.66	14.18	2.23	2.65	1.42	2.57	6.07	8.75	2.84	3.22	35.97	33.35^{ab}
processing	control	2.71^{a}	5.40^{a}	19.92^{b}	24.71	16.47^{b}	15.87	1.67	2.23^{b}	4.61^{a}	1.83	12.98^{a}	5.23^{b}	5.87^{a}	1.64^{b}	25.04^{b}	31.84
	HPP	0.57^{b}	0.91^{b}	23.38^{a}	24.60	20.04^{a}	14.65	1.95	2.68^{a}	0.93^{b}	1.99	6.01^{b}	7.42^{a}	2.37^{b}	2.60^{a}	35.67^{a}	34.81
antioxidant	without additives	2.48	2.53	21.25	24.67	19.32	15.37	1.77	2.46	2.68	1.80	8.50	6.69	3.95	2.28	29.87	33.87
	thyme	0.54	4.22	20.65	25.47	19.17	15.55	1.74	2.40	1.70	1.54	9.42	4.90	3.56	1.48	32.09	32.76
	oregano	1.89	2.71	19.14	23.82	16.28	14.86	1.93	2.51	3.94	2.38	10.57	7.39	4.84	2.60	29.11	33.35
RMSE (n=24)		**1.44**	**2.66**	**1.77**	**2.11**	**3.23**	**1.72**	**0.52**	**0.51**	**1.47**	**0.93**	**3.77**	**2.24**	**1.38**	**1.00**	**5.30**	**2.41**
significance (P)	processing	0.009	0.001	0.001	0.895	0.037	0.093	0.266	0.041	0.001	0.677	0.002	0.025	0.001	0.027	0.001	0.006
	Supplem.	0.097	0.374	0.300	0.300	0.226	0.706	0.786	0.914	0.069	0.194	0.647	0.085	0.296	0.081	0.612	0.643
	proc.*additives	0.067	0.174	0.759	0.588	0.113	0.897	0.093	0.474	0.144	0.693	0.471	0.509	0.857	0.621	0.685	0.009

Values associated with different letter designations are significantly different (P<0.05).

Table 3 shows the groups of saturated, polyunsaturated, monounsaturated, conjugated, conjugated linoleic acids, the sum of ω3, ω6 and their ratio in raw beef in *Longissimus dorsi* and *Semimembranosus* muscle groups. On the table 5 shows some of the main fatty acids, the share of which constitutes about 80% of their total sum.

In the LD muscle group, we found that the application of HPP treatment leads to a reliable increase in saturated and monounsaturated fatty acids by 5 and 7%, at the expense of a decrease in polyunsaturated fatty acids by about 12%. As a logical consequence of this, we register a decrease in Σ ω3 and Σ ω6 fatty acids, as well as an increase in the ratio between them. In this muscle group, no reliable influence of the applied antioxidants was found. We register a minimal increase in the content of poly- and monounsaturated fatty acids at the expense of the decrease in saturated fatty acids.

In the SM muscle group, however, we obtained an opposite effect of HPP treatment with respect to some groups of fatty acids. It reliably increases by about 3% the content of polyunsaturated fatty acids at the expense of reducing the content of saturated fatty acids in the same order. We also record an increase in Σω3 and Σω6 fatty acids, while their ratio is preserved. In this muscle group, we also found a reliable influence of the interaction between the two factors - HPP treatment and the influence of the added essential oils of thyme and oregano on the content of monounsaturated fatty acids, where we obtained 4.6 to 15.6% lower values of the non-HPP-treated thyme-supplemented group versus the HPP-treated groups. When examining the results of the influence of processing factors and antioxidants on some of the main fatty acids in the LD muscle group, we found a reliable decrease in the content of lauric, heptadecene, linoleic and eicosacenoic acids, as well as an increase in palmitic, stearic and cis isomers of oleic acid under the influence of high hydrostatic pressure treatment. No influence of added antioxidants was found.

In the SM muscle group, we found an increase in stearic, palmitoleic, linoleic and eicosacenoic acids, as well as a decrease in lauric acid content after HPP treatment. In this muscle group, there is also a proven interaction between the two factors in the group of fatty acids with the largest share in beef – the cis isomers of oleic acid, where in the groups without supplement and with thyme supplement we recorded significantly higher values compared to the untreated HPP group with thyme. An increase in saturated fatty acids was found, which is an indicator of lipid oxidation in the technological treatment of *Longissimus dorsi* muscles, while in *Semimembranosus* muscles it leads to an improvement of the fatty acid composition in terms of saturated and trans fatty acids. The reasons for this probably lie in the different content of intermuscular lipids in the two types of muscles.

4.1.4. Changes in the Microbial Status of a Beef *Longissimus Dorsi* and *Semimembranosus* Muscle Group Model System

The results of the microbiological studies of the two muscles when treated with HPP and treated with aqueous extract of thyme and oregano, show that the total number of aerobic (ABM) mesophilic and psychrotrophic microorganisms, as well as the content of molds and yeasts in the examined beef samples are within the permissible limits norms, according to the requirements of BDS and ISO. For OBM (m.Sm/CFU/g - for control -1.5. 106, after HPP - 2.10^2, HPP with thyme and oregano - $<1x10^2$), for (m.Ld/CFU/g - control, after HPP - 1.6. 10^2, HRR with thyme - $<1x\ 10^1$, and oregano - $<1x\ 10^1$). Molds and yeasts (m.Sm/CFU/g -$4x10^2$ for control, after HPP - 1.10^2, HPP with thyme and oregano $<1x\ 10^2$), for (m.Ld/CFU/g - control $2x10^2$, after HPP - $1.6x10^2$, HPP with thyme $<1.1x\ 10^2$, samples with HPP with oregano - 1.1. 10^2). The results show and confirm the scientifically

proven properties of thyme as a strong antiseptic. The absence of Coliforms, Salmonella, Listeria, Coagulase-positive staphylococci, Anaerobic sulphite-reducing clostridia proves the purity and suitability for use of the examined beef.

In this regard, the application of HPP treatment at 600 MPa was shown to be a very effective method to reduce the bacterial load and lead to an extension of the safe shelf life of chilled meat products. The ability of psychrotrophic microorganisms to recover in meat at low temperatures appears to be a critical factor in evaluating the results of HPP processing.

4.1.5. Organoleptic analysis

The organoleptic analysis carried out at the Institute of Cryobiology and Food Technologies (ICHT) - Sofia, 53 Cherni Vrah Blvd., involved 5 experienced persons from the institute. All were women over the age of 50. The analysis performed included: The meat samples of (m. *Longissimus dorsi*) (m.*Semimembranosus*) The results showed that the roasted meat samples of (m. *Longissimus dorsi*) (m.*Semimembranosus*) showed that HRR-treated pure meat was slightly dry, more so for SM. Thyme imparts a pleasant aroma, but an unusual taste. Oregano is unacceptable as an extract because of the bitter taste.

4.2 Preliminary attempts to establish the need for the use of various additives - additives to improve the color and organoleptic characteristics of minced meat - from *Semimembranosus* muscles (m.SM), as the second part of the analysis. Organoleptic analysis.

* The sensory analysis performed does not include cold pasteurization and does not lead to additional changes in the organoleptic sensations.

Table 5 Sensory analysis of ground beef from the Semimembranosus muscle (m.SM) with different additives

Analyzed sample	Time	Sensory evaluation
0.05% essential oil of oregano: 0.025 ml= 25 μl in 50 g of meat;	3 d	A faint aroma of oregano essential oil and flavor in the meat.
0.1% essential oil of oregano: 0.050 ml= 50 μl in 50 g of meat;	3 d	the taste of a sample with 0.1% essential oil of oregano is pleasant and the aroma of spice is acceptable.
0,15 % етерично масло от риган: 0,075 ml= 75 μl в 50 гр. Месо;	3 d	Still the astringent aroma of oregano oil, but also a slightly bitter taste of the meat.
0.2% essential oil of oregano: 0.100 ml= 50 μl in 50 g of meat;	3 d	Strong aroma of oregano essential oil and unpleasant bitter taste of the meat.
0.1% dry oregano: 0.05 g in 50 g of meat;	3 d	The dry spices did not change the taste and aroma, as is the result of the action of the essential oil. When chewing them, an unpleasant sensation remains from the dry parts of the oregano stems.
0.2% dry oregano: 0.10 g in 50 g of meat ;	3 d	The dry spices did not change the taste and aroma, as is the result of the action of the essential oil. When chewing them, an unpleasant sensation remains from the dry parts of the oregano stems.
0.3% dry oregano: 0.15 g in 50 g of meat.	3 d	The dry spices did not change the taste and aroma, as is the result of the action of the essential oil. When chewing them, an unpleasant sensation remains from the dry parts of the oregano stems.

0.1 % Sodium lactate /80%/ = 0.125 ml = 0.0625 ml in 50 g of meat	3 d	Sodium lactate imparts moisture to the meat.
0.1% Sodium lactate /60%/ = 0.167 ml. . 60 %= 0.01% in 50 g of meat	3 d	Sodium lactate imparts juiciness and moisture to the meat, with a lower percentage giving a more pleasant sensory sensation.
0.3% Medoliva Plus = 0.15 g in 50 g of meat	3 d	"Medoliva Plus" conveys a pleasant buttery taste and aroma of olive oil, helps to give the minced meat a more saturated dark red color.
0.5% Medoliva Plus = 0.25 g in 50 g of meat	3 d	The higher concentration does not lead to acceptable organoleptic characteristics, such as taste, aroma and color.

4.3 Second stage - minced meat from the *Semimembranosus* muscles (m.SM), treated with HPP, alone and in combination with antioxidants or antimicrobial agents.

4.3.1. Physicochemical parameters and color characteristics, lipid oxidation.

The experimental design is divided into 3 groups. In group A, the influence on the investigated indicators was investigated in different modes of treatment with HPP. In groups B and C, the influence of different antioxidants in combination with HPP was studied.

Group A: A1. Minced beef, vacuum packaging, without HPP processing; A2. Minced beef, vacuum packaging, with HPP treatment+ 580MPa x 4min.; A3. Minced beef, vacuum packaging + 580MPa x 8min.;
Group B: B1. minced beef + HPP t + 580MPa x 4min; B2. minced beef + HPP + Medoliva Plus 0.3%; B3. minced beef + HPP + Sodium lactate 0.3%;
Group C: C1. minced beef + HPP treatment + 580MPa x 4min; C2 minced beef + HPP + Medoliva 0.1%; C3. minced beef + HPP + Beetroot 0.1%; C4. minced beef + HPP + Radish 0.1%; C5. minced + HPP + Rosemary 0.1%.

On the table 6 shows the results for physicochemical parameters, color characteristics and lipid oxidation of different HPP treatment regimes

Table 6 Physicochemical indicators and lipid oxidation of raw beef processed with high hydrostatic pressure

Processing	Storage	pH	WHC(%)	L	a	b	lipid oxidation, mg MDA / kg meat
A1	5 d	$5,68^{d}$	$34,71^{ab}$	$46,17^{e}$	$25,50^{a}$	$10,60^{bc}$	0,069
	20 d	$5,47^{e}$	$18,44^{c}$	$47,07^{e}$	$26,03^{a}$	$9,80^{c}$	0,160
	40 d	$5,38^{e}$	$23,43^{bc}$	$51,40^{d}$	$18,97^{b}$	$7,23^{d}$	0,225
A2	5 d	$5,83^{abc}$	$39,04^{a}$	$61,83^{ab}$	$12,20^{c}$	$11,93^{bc}$	0,115
	20 d	$5,86^{abc}$	$40,11^{a}$	$58,43^{bc}$	$18,67^{b}$	$15,03^{a}$	0,172
	40 d	$5,79^{bcd}$	$34,59^{ab}$	$62,50^{a}$	$11,90^{c}$	$10,33^{bc}$	0,261
A3	5 d	$5,87^{ab}$	$38,05^{a}$	$58,27^{c}$	$11,40^{c}$	$12,40^{b}$	0,146
	20 d	$5,73^{cd}$	$23,73^{bc}$	$54,23^{d}$	$17,13^{b}$	$15,03^{a}$	0,179
	40 d	$5,94^{a}$	$35,12^{ab}$	$64,70^{a}$	$9,80^{c}$	$10,60^{bc}$	0,261
Effect							
Processing	A1	5,51	25,53	48,21	23,50	9,21	$0,151^{b}$
	A2	5,83	37,91	60,92	14,26	12,43	$0,182^{a}$
	A3	5,85	32,30	59,06	12.78	12,68	$0,195^{a}$

Storage	5 d	5,79	37,26	55,42	16,37	11,64	0,110^{c}
	20 d	5,70	27,43	53,24	20,61	13,29	0,170^{b}
	40 d	5,79	31,05	59,53	13,56	9,39	0,249^{a}
RMSE (n=27)		**0.05**	**4.53**	**1.23**	**1.00**	**0.75**	**0.021**
significance	Processing	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P=0.0011
	Storage	P=0.0003	P=0.0008	P<0.0001	P<0.0001	P<0.0001	P<0.0001
	proc.*storag.	P<0.0001	P=0.0112	P=0.0002	P=0.0001	P=0.0027	P=0.2265

Values associated with different letter designations are significantly different (P<0.05).

According to the results obtained in the table. 8, we establish a proven effect of the interaction of the factors: processing and storage for all the studied indicators, except for lipid oxidation, where both factors influence independently. The highest values in terms of **pH** were the group treated with higher duration of exposure to HPP at a storage period of 40 days, and the lowest in the non-HPP treated control for the same storage period. The tendency is towards an increase in the values of the indicator after treatment with HPP, with the longer treatment we obtain higher values. The **water-holding capacity** increases after the impact of HPP, while as time increases, the storage value of the indicator decreases. A similar trend is observed in the brightness of the meat. The **red** component reliably decreases after the applied treatment, and the **yellow** component increases. The results during storage remained stable, as the results of the analysis of McArdle, 2010 who reported that **L*** values under pressure (35, 45, 55 ◦C, 400, 600 MPa) beef samples remained stable during storage (for 30 days at +4 ◦C) and showed that the L* values of the control samples were lower ($p < 0.001$) than those of the pressurized samples during storage. The lighter appearance is due to denaturation of myglobin and/or displacement or release of a heme group by Carlez et al. 1995 In lipid oxidation, we found that after treatment with HPP the values of the indicator increased, as found by Sert, 2022, that the MDA values of all samples were higher than expected for the beginning of storage. This can be attributed to the fact that the meat passed through a grinding machine twice before being treated with HPP. For other indicators, the most noticeable is the influence of the storage period on the **water-holding capacity** in the direction of decrease and on the brightness of the meat in the direction of increase with increasing duration of storage. Overall, we report a positive effect of HPP treatment, with no significant differences between the two treatment durations. For this reason, we chose the option with a duration of 4 min. processing for further research as economically more expedient.

On the table 7 shows the results of physicochemical parameters, color characteristics and lipid oxidation in groups of minced beef treated with HPP and added antioxidants + Medoliva Plus 0.3% and sodium lactate 0.3%.

Table 7 Physicochemical indicators and lipid oxidation of minced beef treated with high hydrostatic pressure and antioxidants

Processing	Storage	pH	WHC (%)	L	a	b	lipid oxidation, mg MDA / kg meat
B1	5 d	5.83	39.04	61.83^{cd}	12.20^{b}	11.93	0.115
	20 d	5.86	40.11	58.43^{e}	18.67^{a}	15.03	0.172
	40 d	5.79	34.59	62.50^{bc}	11.90^{b}	10.33	0.261
B2	5 d	5.99	42.93	59.87^{de}	12.43^{b}	11,.67	0.100
	20 d	5.93	41.42	58.30^{e}	18.73^{a}	15.23	0.151
	40 d	5.86	36.15	60.53^{cde}	12.17^{b}	10.87	0.222
B3	5 d	5.85	40.21	64.57^{ab}	9.80^{b}	11.67	0.198

	20 d	5.67	30.30	66.17^{a}	11.40^{b}	15.30	0.206
	40 d	5.67	37.00	64.97^{ab}	11.63^{b}	10.33	0.308
Effect							
Processing	B1	5.83^{ab}	37.91	60.92	14.26	12.43	0.182^{ab}
	B2	5.93^{a}	40.17	59.57	14.44	12.59	0.158^{b}
	B3	5.73^{b}	35.84	65.23	10.94	12.43	0.237^{a}
Storage	5 d	5.89^{a}	40.73	62.09	11.48	11.76^{b}	0.138^{b}
	20 d	5.82^{ab}	37.28	60.97	16.27	15.19^{a}	0.176^{b}
	40 d	5.77^{b}	35.91	62.67	11.90	10.51^{c}	0.263^{a}
RMSE (n=27)		**0.09**	**6.12**	**0.90**	**0.97**	**0.63**	**0.053**
significance	Processing	P=0.0005	P=0.3463	P<0.0001	P<0.0001	P=0.8350	P=0.0154
	Storage	P=0.0316	P=0.2545	P=0.0029	P<0.0001	P<0.0001	P=0.0003
	Processing .* Storage	P=0.3308	P=0.3714	P=0.0006	P<0.0001	P=0.7868	P=0.9194

Values associated with different letter designations are significantly different (P<0.05).

The acidity index reliably increases in the group with the antioxidant "Medoliva". For the same indicator, we found a reliable decrease in values after 40 days of storage.

We did not find any significant differences in the **water-holding capacity** between the different studied groups. Marcos et al. (2010) found a negative correlation between sarcoplasmic protein solubility and expressed moisture in beef from m. *Longissimus dorsi* muscle, suggesting that pressure-induced denaturation of sarcoplasmic proteins could negatively affect water binding in pressurized meats to some extent.

We found a proven interaction between the processing and storage factors in the **color** characteristics brightness and red component of the meat. The addition of sodium lactate increases the brightness of the meat. In the case of the **red** component, groups B1 and B2 stand out when stored for 20 days, where we found significantly higher values for this indicator compared to all other groups. The influence of added antioxidants on **lipid oxidation** is insignificant. We found no significant differences from the untreated group in either. With this indicator, we expectedly register a reliable increase after storage.

On the table 8 shows the results for physicochemical indicators, color characteristics and lipid oxidation in groups of minced beef treated with HPP and added different antioxidants at a concentration of 0.1%.

Table 8 Physicochemical indicators and lipid oxidation of minced beef treated with high hydrostatic pressure and antioxidants

Processing	Storage	pH	WHC (%)	L	a	b	lipid oxidation, mg MDA / kg meat
C1	5 d	5.86^{bc}	36.02	61.07^{bcde}	11.90	11.93	0.114^{d}
	20 d	5.71^{d}	34.37	59.47^{efg}	12.40	13.97	0.181^{cd}
	40 d	5.86^{bc}	34.59	62.50^{abcd}	11.90	10.33	0.261^{ab}
C2	5 d	5.82^{bcd}	43.77	59.20^{efg}	12.93	11.93	0.161^{cd}
	20 d	5.80^{cd}	29.22	60.67^{cdef}	12.67	14.50	0.165^{cd}
	40 d	5.85^{bc}	39.24	61.03^{bcdef}	12.67	10.80	0.219^{bc}
C3	5 d	5.84^{bc}	41.90	59.87^{defg}	15.30	11.40	0.125^{d}
	20 d	5.80^{cd}	37.45	57.13^{g}	15.83	13.23	0.274^{ab}

	40 d	5.87^{abc}	38.53	61.43^{bcde}	14.77	10.60	0.303^{a}
C4	5 d	5.87^{abc}	41.89	62.63^{abc}	13.20	10.60	0.172^{cd}
	20 d	5.93^{ab}	29.93	58.30^{fg}	16.10	13.17	0.216^{bc}
	40 d	5.98^{a}	36.26	63.07^{abc}	12.40	9.80	0.284^{ab}
C5	5 d	5.87^{abc}	36.26	63.50^{ab}	13.19	10.97	0.119^{d}
	20 d	5.76^{cd}	30.62	61.47^{bcde}	13.70	13.70	0.170^{cd}
	40 d	5.92^{ab}	33.79	64.30^{a}	13.17	10.80	0.213^{bc}
Effect							
Processing	C1	5.81	34.99^{ab}	61.01	12.07^{c}	12.08^{a}	0.185
	C2	5.82	37.41^{ab}	60.30	12.76^{bc}	12.41^{a}	0.181
	C3	5.84	39.30^{a}	59.48	15.30^{a}	12,.08^{a}	0.234
	C4	5.92	36.03^{ab}	61.33	13.90^{b}	11.19^{b}	0.224
	C5	5.85	33.55^{b}	63.09	13.35^{b}	11.82^{ab}	0,.167
Storage	5 d	5.85	39.97^{a}	61.25	13.30^{b}	11.37^{b}	0.138
	20 d	5.80	32.32^{b}	59.41	14.14^{a}	13.91^{a}	0.201
	40 d	5.90	36.48^{a}	62.47	12.98^{b}	10.47^{c}	0.256
RMSE (n=45)		**0.04**	**3.27**	**0.91**	**0.91**	**0.51**	**0.024**
significance	Processing	P<0.0001	P=0.0091	P<0.0001	P<0.0001	P=0.0003	P<0.0001
	Storage	P<0.0001	P<0.0001	P<0.0001	P=0.0047	P<0.0001	P<0.0001
	Proc.*stor.	P=0.0044	P=0.0521	P=0.0009	P=0.0533	P=0.5007	P=0.0009

Values associated with different letter designations are significantly different (P<0.05).

We found a proven interaction between the treatment with different antioxidants and the shelf life on the parameters pH, brightness of the meat and lipid oxidation. However, regarding the first 2 indicators, the differences between the different options are too small, as an absolute value, and do not allow us to draw more definite conclusions. In terms of acidity, the lowest value was obtained in group C1 not treated with an antioxidant after 20 days of storage, and the highest in the group of radish treated with an antioxidant after 40 days of storage. The increase in pH after HPP, according to (Mandava et al., 1994), is due to a reduction of available acid groups in the meat as a result of conformational changes associated with protein denaturation.

Regarding the **brightness** of the meat, we recorded the lowest value in group C3 with red beet antioxidant after 20 days of storage, and the highest value in rosemary - group C5 after 40 days of storage. Regarding the **lipid oxidation** indicator, the differences between the individual variants are high enough to allow us to draw some more definitive conclusions.

An increasing trend with increasing storage time is evident. The influence of the added antioxidants from red beet has a negative effect, expressed in an increase in the values of the indicator compared to the untreated group after 20 days of storage.

For the rest of the indicators, we found an independent action of the processing and storage factors. The influence of the different antioxidants on the **water-holding capacity** is most noticeable in the group with added beets, where we register the highest value, with this antioxidant we also get the highest value of the red component. While for the yellow component, the variant with antioxidant red radish stands out, where we find lower values compared to the other studied groups.

The influence of storage is expressed in a decrease in the water holding capacity and an increase in the **red** and **yellow** components of the meat in the groups stored for 20 days compared to the groups stored for 5 and 40 days.

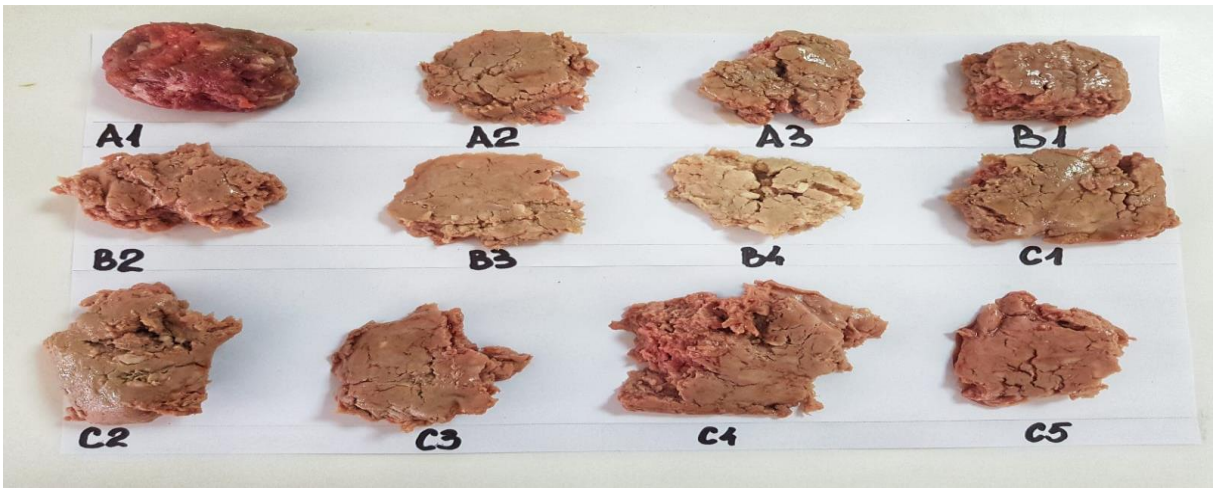


Figure 11. Photograph of the samples on the 5th day of the study - the changes in the instrumentally recorded color characteristics.

Legend: A1. minced beef without HPP processing; A2. minced beef + HPP (580MPa x 4min); A3 minced beef + HPP (580MPa x 8min); B1. minced beef + HPP(580MPa x 4min); B2 minced beef + HPP(580MPa x 4min)+ Medoliva Plus 0.3%; B3. minced beef + HPP(580MPa x 4min)+ sodium lactate 0.3%; C1 minced beef + HPP(580MPa x 4min); C2 minced beef + HPP(580MPa x 4min)+ Medoliva 0.1%; C3. minced beef + HPP(580MPa x 4min)+ + Beetroot 0.1%; C4. minced beef + HPP(580MPa x 4min)+ Radish 0.1%; C5. minced beef + HPP(580MPa x 4min)+ + Rosemary 0.1%

4.3.2. Myoglobin and its forms, determination of changes in meat protein

Fig. 12 and Fig. 13 show the results of the studies on the total myoglobin index of minced beef at different durations of HPP treatment.

According to the obtained results in fig. 12 we found no change in total myoglobin content after HPP treatment. Significant differences were observed only after storage for 20 days compared to the groups stored for 5 and 40 days.

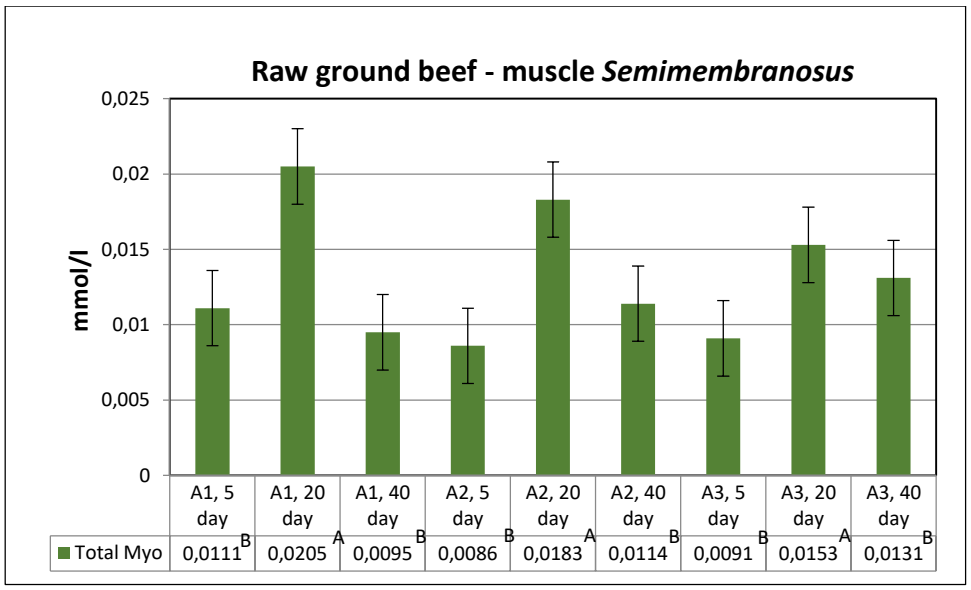
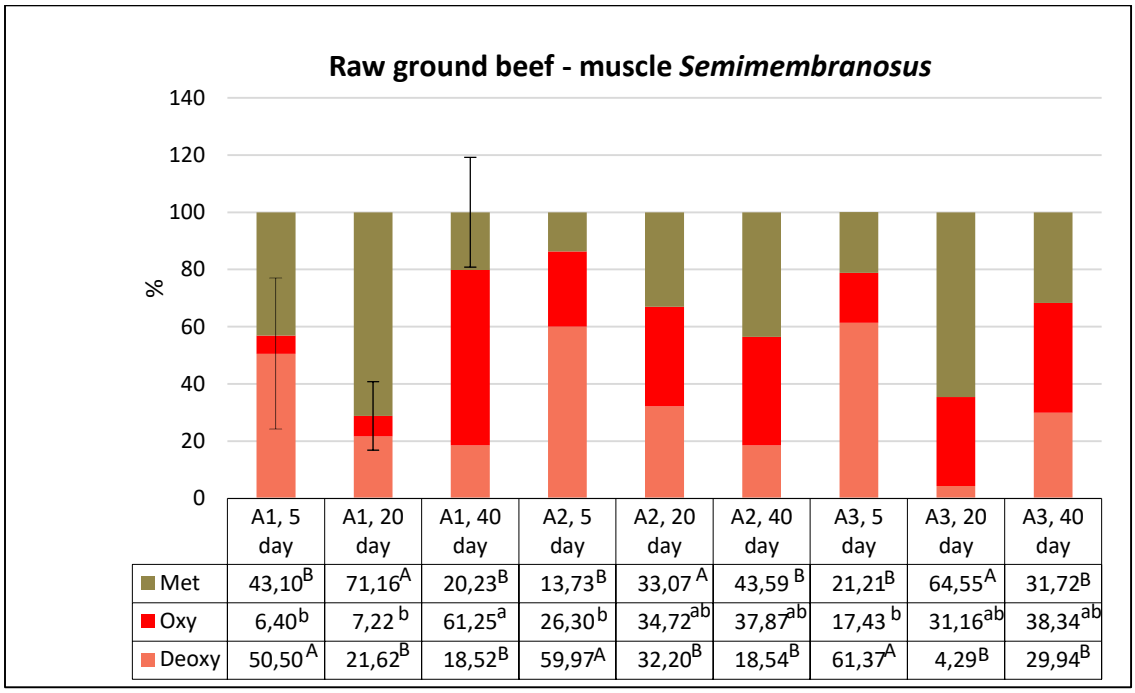


Fig.12 Content of total myoglobin in minced beef - *Semimembranosus* muscle,
* Values associated with different letter designations are significantly different (P<0.05).



	A1, 5 day	A1, 20 day	A1, 40 day	A2, 5 day	A2, 20 day	A2, 40 day	A3, 5 day	A3, 20 day	A3, 40 day
Met	43,10^{B}	71,16^{A}	20,23^{B}	13,73^{B}	33,07^{A}	43,59^{B}	21,21^{B}	64,55^{A}	31,72^{B}
Oxy	6,40^{b}	7,22^{b}	61,25^{a}	26,30^{b}	34,72^{ab}	37,87^{ab}	17,43^{b}	31,16^{ab}	38,34^{ab}
Deoxy	50,50^{A}	21,62^{B}	18,52^{B}	59,97^{A}	32,20^{B}	18,54^{B}	61,37^{A}	4,29^{B}	29,94^{B}

Fig.13 Myoglobin forms of minced beef

** Values associated with different letters are significantly different (P<0.05), with lowercase letters for oxymyoglobin indicating the presence of an interaction between the two factors, and uppercase letters being related to shelf life*

In fig. 13 shows the effect of 580 MPa pressure on minced beef SM, with the exception of control A1 (not treated with HPP) degradation was observed in almost all analyzed samples. In confirmation of Cheah and Ledwar 1996, reported that HPP causes significant color changes by affecting myoglobin in a protein structure at a pressure of 400 MPa causing irreversible denaturation of myoglobin, and the meat acquires a gray-brown color. According to the results obtained for the forms of myoglobin, we found a decrease in the content of deoxymyoglobin for the entire storage period and an increase in the content of metmyoglobin after 20 days of storage. For oxymyoglobin, we obtained disproportionately high concentration values in the untreated group for a period of 40 days of storage compared to the groups stored for 5 and 20 days.

When examining the content of total myoglobin in fig. 15 minced beef treated with "Medoliva" 0.3% and sodium lactate, no significant differences were found between the individual groups. The large value of the standard error of the model is striking, which is probably due on the one hand to the small number of samples and on the other hand to the different initial states of the meat, possibly related to a different period after slaughter or a different way of storage before purchase of the samples.

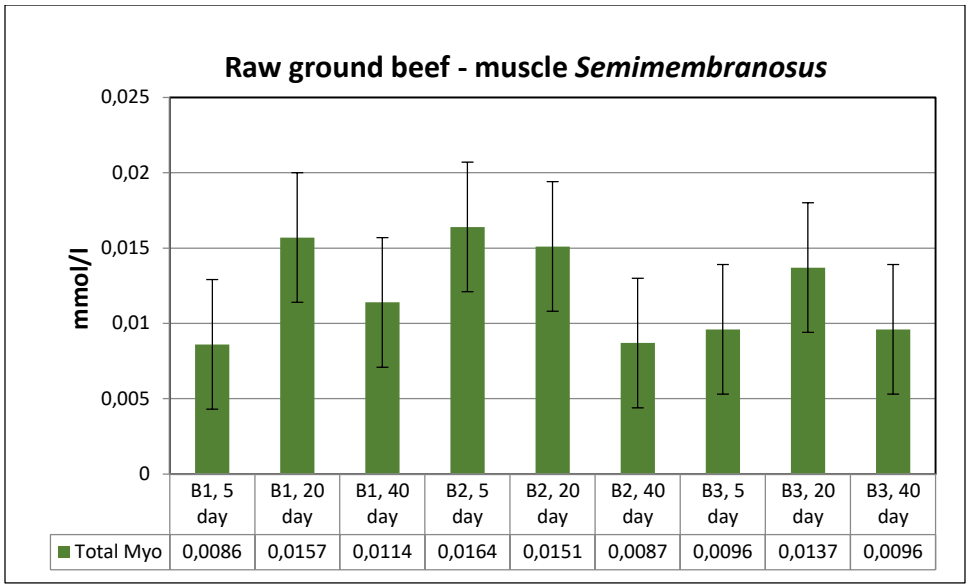


Fig.14 Content of total myoglobin in minced beef treated with group B antioxidants

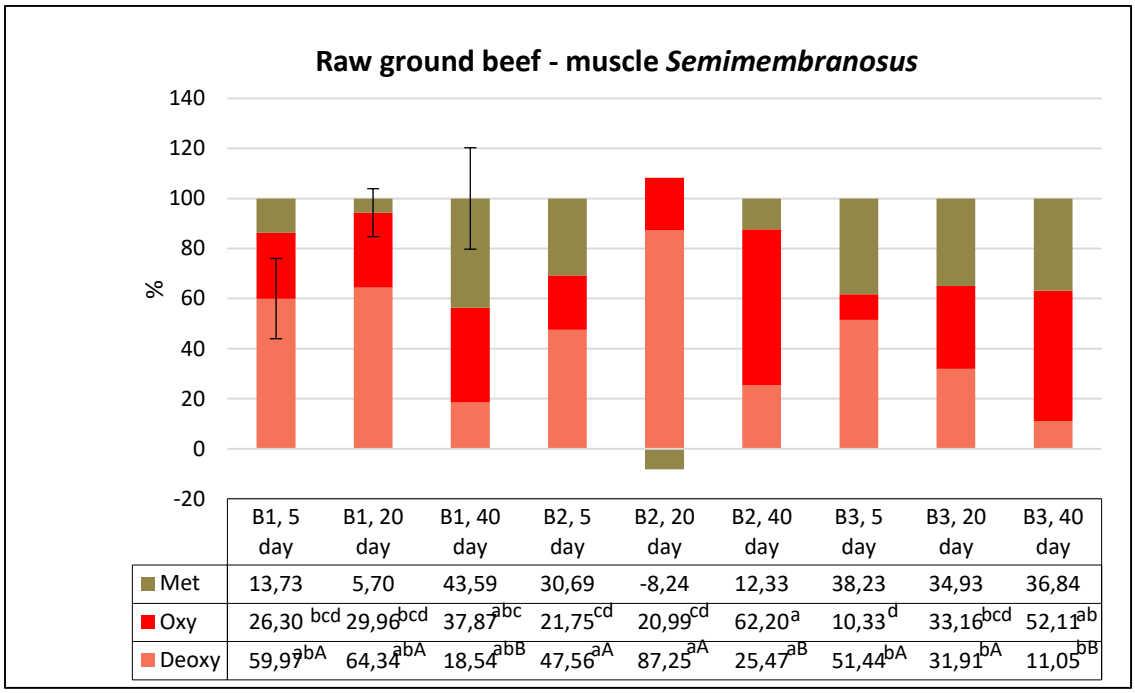


Fig 15. Myoglobin forms of minced beef in groups B. * Values associated with different letter designations are significantly different ($P<0.05$), with lowercase letters for oxymyoglobin indicating the presence of an interaction between the two factors, lowercase letters for deoxymyoglobin indicating the presence of significant differences between groups treated with a different antioxidant, and uppercase letters are related to the storage period

With respect to the myoglobin products in Fig. 16 we found reliable effects of added antioxidants and shelf life for the deoxymyoglobin indicator and an interaction between the two factors for the oxymyoglobin indicator, while we did not find reliable differences for metmyoglobin. However, due to the above considerations for total myoglobin, we believe that we cannot draw any firm conclusions when interpreting the results for these indicators. The combined effect of HPP and 0.3% "Medoliva Plus" (B2) shows the lowest values of metmyoglobin, we assume that it is due to the high level of polyphenols.

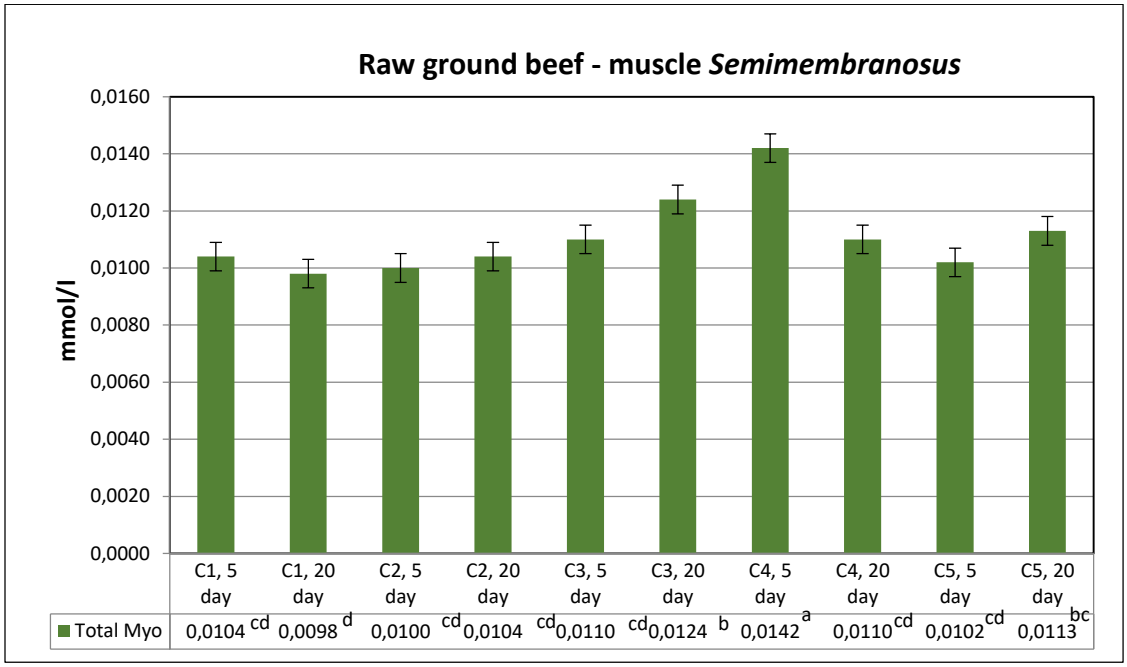
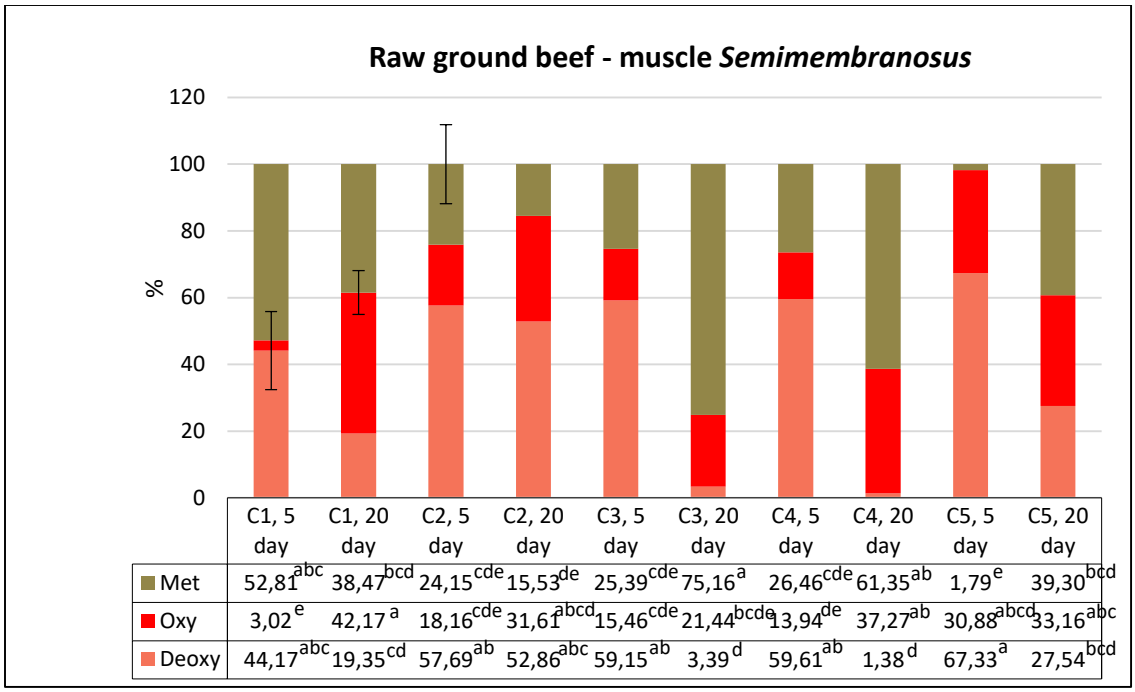


Fig.16 Total myoglobin content in minced beef treated with group C antioxidants, * Values associated with different letter designations are significantly different (P<0.05).

When examining the influence of added group C antioxidants and storage time for minced beef, we obtained a reliable interaction between the two factors. We obtained the highest values for this indicator in the groups with antioxidant red beet when stored for 20 days and red radish stored for 5 days, where we found higher values compared to all other groups. Myoglobin values ranged widely from 0.0098 to 0.0142. The higher values are due to the combined effect of antioxidants. The most positive effect, both from the colorimetric test and from the spectrometric test, is 0.1% Beetroot powder (C3), 0.1% Red radish (C4)



	C1, 5 day	C1, 20 day	C2, 5 day	C2, 20 day	C3, 5 day	C3, 20 day	C4, 5 day	C4, 20 day	C5, 5 day	C5, 20 day
Met	52,81^{abc}	38,47^{bcd}	24,15^{cde}	15,53^{de}	25,39^{cde}	75,16^{a}	26,46^{cde}	61,35^{ab}	1,79^{e}	39,30^{bcd}
Oxy	3,02^{e}	42,17^{a}	18,16^{cde}	31,61^{abcd}	15,46^{cde}	21,44^{bcde}	13,94^{de}	37,27^{ab}	30,88^{abcd}	33,16^{abc}
Deoxy	44,17^{abc}	19,35^{cd}	57,69^{ab}	52,86^{abc}	59,15^{ab}	3,39^{d}	59,61^{ab}	1,38^{d}	67,33^{a}	27,54^{bcd}

Fig. 17. Forms of myoglobin of minced beef in groups C. *Values associated with different letter designations are significantly different (P<0.05), with different letter designations being associated with significant differences with proven interaction between the two factors for all indicators

The effect of storage time and added group C antioxidants on myoglobin forms are shown in Fig. 17. A proven interaction of the two factors was established for all 3 investigated indicators. An interaction between 2 factors is difficult to comment on in principle, especially in the presence of such large values of the standard error, as we obtain in the model we used. However, we can note the reliable decrease in the values of deoxymyoglobin and increase in oxymyoglobin during a longer period of storage, as well as the low levels of metmyoglobin in the group with the addition of "Medoliva"- C2 compared to the group without antioxidant - C1. These results in view of the high pressure processing parameters are consistent with McArdle, who found that the ratio of oxymyoglobin decreased in the range of 400–500 MPa, at the expense of the increased values of metmyoglobin, but from the table it is clear that the combination with antioxidants significantly improves the amount of oxymyoglobin in the tested samples. However, as other studies have found with regard to color, myoglobin denaturation under pressure is similar to heat, acid and urea denaturation, and this is partly due to the disruption of hydrophobic interactions (McArdle et al. 2010).

In fig. 18 shows the protein profiles of the samples determined by SDS-PAGE under reducing conditions according to Laemmli (1970). Multiple bands consistent with the protein profile of beef were observed in the non-HPP minced meat (1 and 2). The intense fractions corresponding to the molecular mass of contractile proteins - actin and myosin - stand out.

The picture is similar for meat treated with HPP and various different antioxidants (from 3 to 12). The different antioxidants and HPP treatment did not show a change in the intensity of the

protein fractions. The solubility of myosin light chain increases with increasing pressure, as demonstrated in a study by Hanjun Ma.

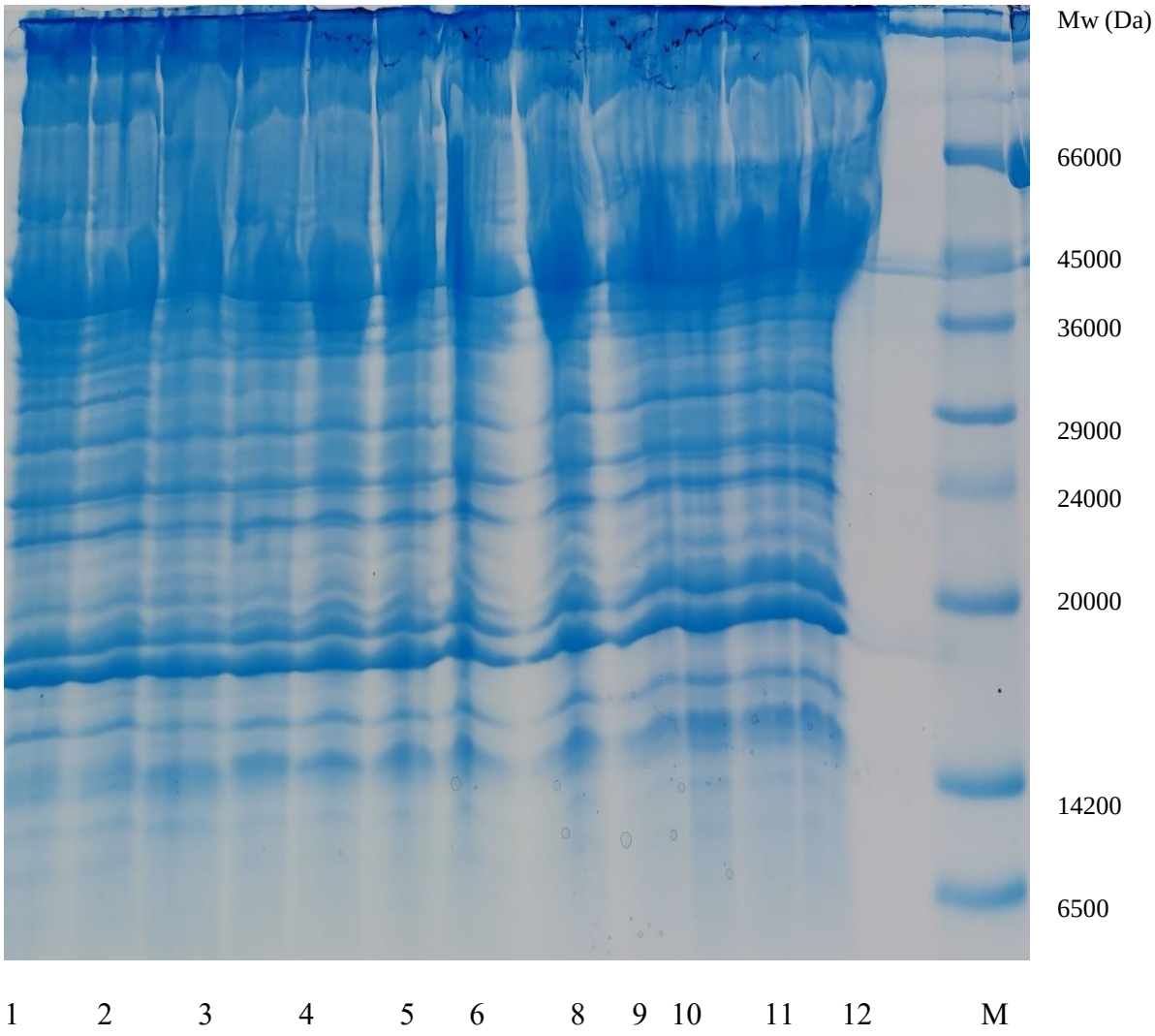


Figure 18. SDS-PAGE of SM minced beef samples

1. A1- control, without HPP, day 3; 2. A1 – control, without HPP, 40 days; 3. A3 – HPP 580 MPa/ 8 min.; 4. A2 /B1 - HPP 580 MPa/ 4 min.; 5. B2 – (HPP 580 MPa/ 4 min) + "Medoliva Plus" 0.3%; 6. B3 – (HPP 580 MPa/ 4 min) + sodium lactate 0.3%; 8. C1 - HPP 580 MPa/ 4 min.; 9. C2 - HPP 580 MPa/ 4 min + "Honey" 0.1%; 10. C3 - HPP 580 MPa/ 4 min + beetroot 0.1%; 11. C4 - HPP 580 MPa/ 4 min + red radish 0.1%; 12. C5 - HPP 580 MPa/ 4 min + rosemary 0.1%*

From a literature review on SDS-PAGE electrophoresis of HPP-treated beef, differences were also reported for samples subjected to high-pressure processing (HPP) when the supernatant and pellet were examined in parallel. The present results show no significant changes in the protein

profile of beef treated with HPP, similar to the sludge study. It can be concluded that further research is needed to clarify the effect of high pressure on the protein profile of meat.

4.3.3. Effect of HPP treatment on model group composition and input additives on fatty acid composition of minced beef

On the table 9 to 14 show the results for the fatty acid profile of minced beef with different durations of exposure to HPP (group A) and a combination of HPP treatment and added different antioxidants in groups B and C.

Table 9 Groups of fatty acids (g/100g of fat) of minced beef in groups A

Processing	Σ SFA	Σ MUFA	Σ PUFA	Σ CLA	Σ Branched FA	Σ ω3	Σ ω6	Σ ω6/Σ ω3
A1	45.24	42,91	9.77	0.337	1.757	2.69	7.07	4.10
A2	43.60	42.52	12.58	0.259	1.201	4.27	8.31	1.97
A3	44.29	42,91	11.21	0.275	1.281	3.76	7.44	1.98
RMSE (n=9)	**3.09**	**1,17**	**3.59**	**0,051**	**0.310**	**1.54**	**2.18**	**2.07**
significance	P=0.8125	P=0.8939	P=0.6515	P=0.2234	P=0.1369	P=0.4872	P=0.7817	P=0.4057

Values associated with different letter designations are significantly different (P<0.05).

Table 10 Main fatty acids (g/100g fat) of minced beef in groups A

Processing	lauric C-12:0	palmitic C-16:0	Stearic C-18:0	palmitoleic C-16:1n7	heptadecenoic C-17:1n7	linoleic C-18:2c9, 12/19:0	eicosatrienoic C-20:3n3	oleic C-18:1cis-FA
A1	0.908	25.16	15.33	2.56	1.06	6.72	1.73	35.44
A2	0.636	24.83	14.73	2.57	2.06	8.03	2.84	34.43
A3	0.608	25.73	14.57	2.77	1.86	7.19	2.45	34.84
RMSE (n=9)	**0.221**	**2.06**	**1.55**	**0.58**	**0.49**	**2.13**	**1.19**	**0.70**

Values associated with different letter designations are significantly different (P<0.05).

The results obtained for the fatty acid profile in group A show that HPP treatment did not significantly affect its composition. No significant differences were found between groups. There was a trend towards a minimal increase in polyunsaturated fatty acids and a decrease in the ω6/ω3 ratio, but within the standard error of the model. Of the main fatty acids, we register a slight decrease in lauric and stearic acids and an increase in heptadecenoic and eicosacenoic acids, also within the standard error.

Table 11 Groups of fatty acids (g/100g of fat) of minced beef in groups B

Processing	Σ SFA	Σ MUFA	Σ PUFA	Σ CLA	Σ Branched FA	Σ ω3	Σ ω6	Σ ω6/Σ ω3
B1	42.02	42.35	14.51	0.248	1.165	4.79^{b}	9.60	2.01^{a}
B2	38.75	42.64	16.64	0.330	1.632	7.14^{a}	9.50	1.34^{b}
B3	43.18	42.54	12.76	0.260	1.228	4.31^{b}	8.45	1.96^{ab}
RMSE (n=9)	**2.43**	**1.54**	**1.83**	**0.070**	**0.331**	**0.85**	**1.33**	**0.25**
significance	P=0.1479	P=0.9724	P=0.1048	P=0.3631	P=0.2504	P=0.0148	P=0.5395	P=0.0291

Values associated with different letter designations are significantly different (P<0.05).

Table 12 Main fatty acids (g/100g fat) of minced beef in groups B

Processing	lauric C-12:0	palmitic C-16:0	Stearic C-18:0	palmitoleic C-16:1n7	heptadecenoic C-17:1n7	linoleic C-18:2c9, 12/19:0	eicosatrienoic C-20:3n3	oleic C-18:1cis-FA
B1	0.463	23.77	14.64	2.408	2.143	9.30	3.33	34.20

B2	0.635	20.74	12.82	2.476	1.822	8.87	4.33	32.47
B3	0.569	24.78	14.64	2.583	2.019	8.18	2.88	34.50
RMSE (n=9)	**0.211**	**1.77**	**1.27**	**0.191**	**0.259**	**1.36**	**0.63**	**1.22**
significance	P=0.6239	P=0.0715	P=0.2057	P=0.5631	P=0.3708	P=0.6208	P=0.0716	P=0.1723

Values associated with different letter designations are significantly different (P<0.05).

According to the results obtained for the fatty acid composition of the groups treated with different antioxidants from group B, we found a reliable increase in ω3 and a decrease in the ω6/ω3 ratio in the group with the antioxidant "Medoliva plus" (B2) compared to the group without antioxidant addition (B1). There was also a trend of an increase in polyunsaturated, conjugated linoleic and branched-chain fatty acids at the expense of a decrease in saturated fatty acids in the group with "Medoliva plus" compared to the group without antioxidant, but within the statistical error of the model. Regarding the main fatty acids, we report a minimal decrease in palmitic, stearic and oleic acids and a minimal increase in eicosacenoic acid in group B2 compared to group B1.

Table 13 Groups of fatty acids (g/100g of fat) of minced beef for groups C

Processing	Σ SFA	Σ MUFA	Σ PUFA	Σ CLA	Σ Branched FA	Σ ω3	Σ ω6	Σ ω6/Σ ω3
C1	43.99	42.59	11.88	0.270	1.223^{ab}	3.98	7.90	1.99
C2	42.70	42.22	13.77	0.226	1.092^{ab}	4.71	9.05	1.93
C3	41.48	41.65	15.72	0.197	0.948^{b}	5.52	10.20	1.85
C4	43.56	42.49	12.67	0.225	1.054^{ab}	4.35	8.31	1.91
C5	46.04	40.59	11.31	0.298	1.759^{a}	3.02	8.29	2.75
RMSE (n=15)	**2.31**	**1.69**	**2.04**	**0.041**	**0.263**	**1.10**	**1.08**	**1.92**
significance	P=0.2509	P=0.6029	P=0.1423	P=0.0771	P=0.0259	P=0.1533	P=0.1496	P=0.3947

Values associated with different letter designations are significantly different (P<0.05).

Табл.14 Main fatty acids (g/100g fat) of minced beef in groups B

Processing	lauric C-12:0	palmitic C-16:0	Stearic C-18:0	palmitoleic C-16:1n7	heptadecenoic C-17:1n7	linoleic C-18:2c9, 12/19:0	eicosatrienoic C-20:3n3	oleic C-18:1cis-FA
C1	0.685	25.24^{a}	14.77	2.59	2.031	7.64	2.61	34.50
C2	0.436	22.07^{b}	13.61	2.24	1.909	8.75	3.09	31.90
C3	0.366	20.54^{b}	13.44	2.07	2.130	9.84	3.72	31.07
C4	0.574	21.84^{b}	13.44	2.17	2.005	8.01	2.79	30.91
C5	0.542	25.03^{a}	16.85	2.15	2.027	7.92	1.96	32.79
RMSE (n=15)	**0.172**	**1.08**	**1.86**	**0.28**	**0.586**	**1.06**	**0.83**	**1.69**
significance	P=0.2610	P=0.0011	P=0.1931	P=0.2411	P=0.9984	P=0.1564	P=0.2020	P=0.1329

Values associated with different letter designations are significantly different (P<0.05).

When examining the fatty acid composition of minced beef treated with antioxidants from group C, we obtained reliable differences between the groups only in branched fatty acids (higher values in group C5 with antioxidant rosemary compared to group C3 with antioxidant red beet) and in palmitic acid, where we found significantly lower values in the Medoliva, Red Beet and Red Radish groups compared to the antioxidant untreated control. There were trends of decreases in saturated fatty acids, increases in polyunsaturated, ω3 and ω6 acids in all groups except for rosemary compared to the untreated antioxidant group, but within standard error. It is likely that

the used concentration of 0.1% of added antioxidants was insufficient to affect the changes in the fatty acid composition of the meat.

4.3.4. Effect of HPP treatment and added antioxidant substances on the microbial status of minced beef

Effect of HPP treatment and embedded antioxidants with antimicrobial activity on the microbial status of a minced beef model system

The effect of applying pressure with a force of 580 MPa and time (4 min.– 8 min.) at a temperature of +4C° was analyzed against the listed microbiota of minced beef:

1. Total number of microorganisms - Total Plate Count; **2. Lactic acid bacteria -** (Lactobacillus) (LAB); **3. Enterobacteria** - Enterobacteriaceae

Assessment of the concentration of microorganisms related to the shelf life of the meat product (Total number of microorganisms, lactic acid bacteria, enterobacteria) is considered against the factors:

1. different holding time (4 min.– 8 min.);

2. combined effect of hydrostatic pressure (580 MPa, 240 s) and added means – "Medoliva Plus"0.3 %, Sodium lactate 0.3%.

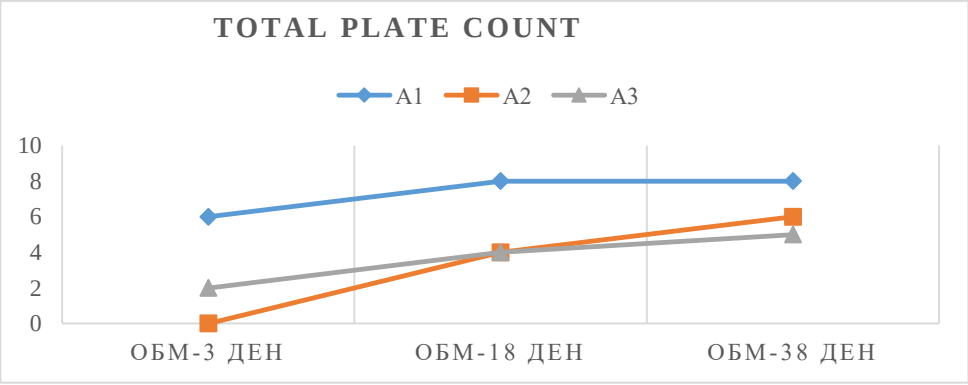


Figure 19. Influence of the time factor on the total number of microorganisms during HPP treatment

Legend: A1. from beef, vacuum packaging, without HPP processing A2. of beef, vacuum packaging, with HPP treatment+ 580MPa x 4min. A3. of beef, vacuum packaging + 580MPa x 8min.

In the microbiological analyzes on total number of microorganisms, the following results were observed: Controls had the high initial number of microorganisms ($1.5.10^6$ cfu/g), which increased by 2 log CU/g on the 18th day of cold storage ($1.3.10^8$) and is maintained at approximately the same level until the 38th day of the study ($5.\ 10^8$).

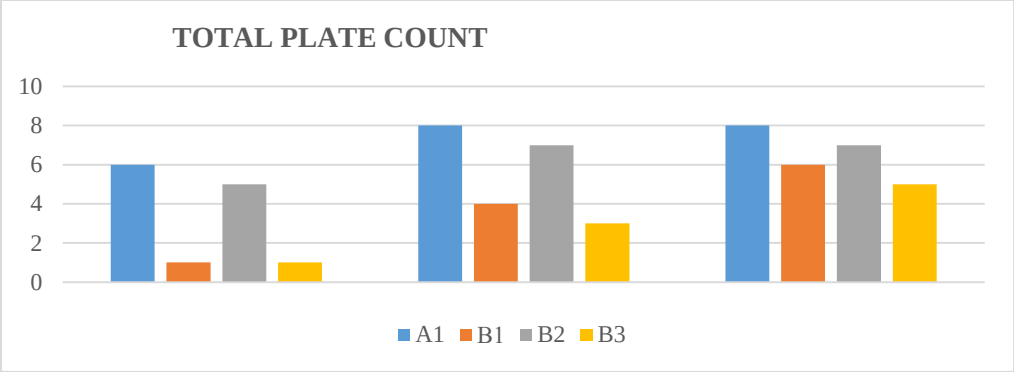


Figure 20. Combined influence of antioxidants with antimicrobial action and HPP treatment

A1. from beef, vacuum packaging, without HPP processing; B1. from beef + HPP + 600MPa x 4min; B2. from beef + HPP + Medoliva Plus 0.3%; B3. from beef + HPP + sodium lactate 0.3%

Figure 20 shows the combined effect of a pressure factor - antioxidants with antimicrobial action. The influence of sodium lactate 0.3% gives a better combined result, both at the beginning of the observation (B3 - 3rd day, <1x 10^2) and during cold storage (B3 - 18th day, 1.1. 10^3 ; B3 – 38th day, 4.2. 10^5), due to the strong preservative effect of lactate and in confirmation of Doores, 2005. Medoliva Plus in a concentration of 0.3%, plays a baroprotective role and eliminates the influence of hydrostatic pressure, probably due to the amounts of oleopropylene: 0.5 μg kg−1 and 1.7 μg kg−1 , lutein-7-O-glucose 1.3 μg kg−1 and 4.3 μg kg−1 , found by (Moudache, at al. 2016). Microbiological studies show a reduction of 1 log CU/g on the 3rd day (2.10^5); 18th day of cold storage (3.2. 10^7) and up to 38th day of research (7.10^7).

In the microbiological analyzes on the lactic acid bacteria, the following results are observed. Controls had a high initial microorganism count (1.1.10^6 cfu/g), which increased by 1 log CU/g on the 18th day of cold storage and by 1.7 log CU/g by the 38th day of the study.

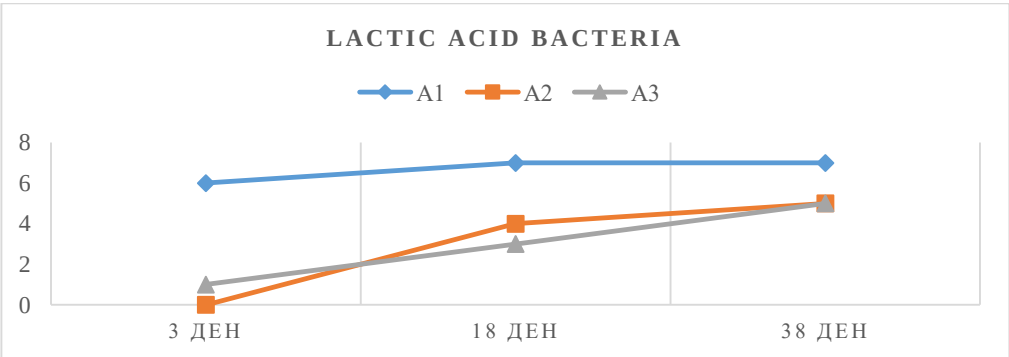


Figure 21. Influence of the factor duration of HPP treatment on the lactic acid bacteria

Legend: A1. from beef, vacuum packaging, without HPP processing; A2. from beef, vacuum packaging, with HPP treatment+ 580MPa x 4 min.; A3. of beef, vacuum packaging + 580MPa x 8 min

.

In fig. 21 shows the influence of HPP treatment with parameters: pressure 580 MPa and retention time (4-8 min) shows a reduction of 6 log CU/g in the number of microorganisms on the 3rd day of treatment (<1 × 10^1) compared to the untreated control . In the process of cold storage on day 18, the reduction of lactic acid bacteria decreased to 3 log CU/g (3 × 10^4), and on day 38 to 2 log CU/g (3.8 × 10^5). Extended processing time does not significantly change the number of lactic acid bacteria compared to those processed for 4 min.

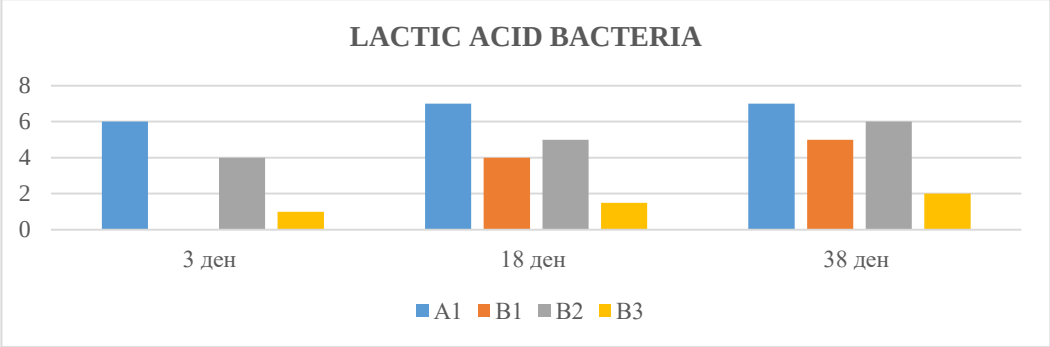


Figure 22. Combined effect of antioxidants with antimicrobial action and HPP treatment

Legend: A1. Minced beef, without HPP processing; B1. minced beef + HPP (580MPa x 4 min); B2. Minced beef + HPP + Medoliva Plus 0.3%; B3. minced beef + HPP + sodium lactate 0.3%

Fig. 22 shows the combined action of HPP and antioxidants with antimicrobial action. Sodium lactate in a concentration of 0.3%, maintained a reduction (B3 –3rd day 2 × 10^1) of 5 log CU/g from 18 (B3 –18th day 4 × 10^1) to 38 days of storage (B3 –38th day 3.× 10^2). The combined effect shows an advantage over the high pressure treatment considered against lactic acid bacteria. A difference of 3 log CU/g at 38 days of storage is significant. Medoliva Plus in a concentration of 0.3% plays a baroprotective role, both in relation to the total number of bacteria and eliminates the influence of hydrostatic pressure. Enterobacteriaceae contamination in the controls was not as high as in the other two studies.

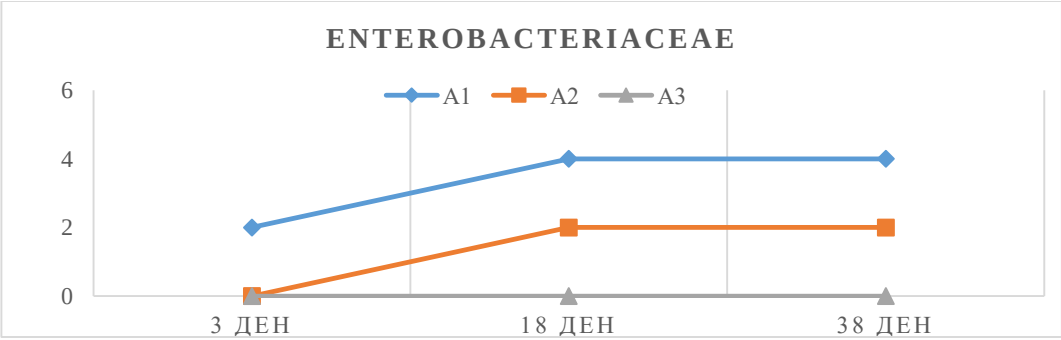


Figure 23. Influence of the time factor on enterobacteria during HPP treatment

Legend: A1. from beef, vacuum packaging, without HRP processing; A2. from beef, vacuum packaging, with HPP treatment+ 580MPa x 4min; A3. of beef, vacuum packaging + 580MPa x 8min

From fig. 23 it is evident that the application of hydrostatic pressure with a force of 580 MPa and retention time (4 min./240s.) are effective parameters for reducing the number of enterobacteria for the entire observation period from the 3rd (<1x 101), 18-(8.6. 102) to the 38th day (<1x 102), compared to controls: 3rd day (5.9. 102), 18th day (1.2. 104) and 38th (3.8. 104). HPP administered over time (8 min./480 s.) inhibited the development of enterobacteria for the entire study period under refrigerated storage conditions.

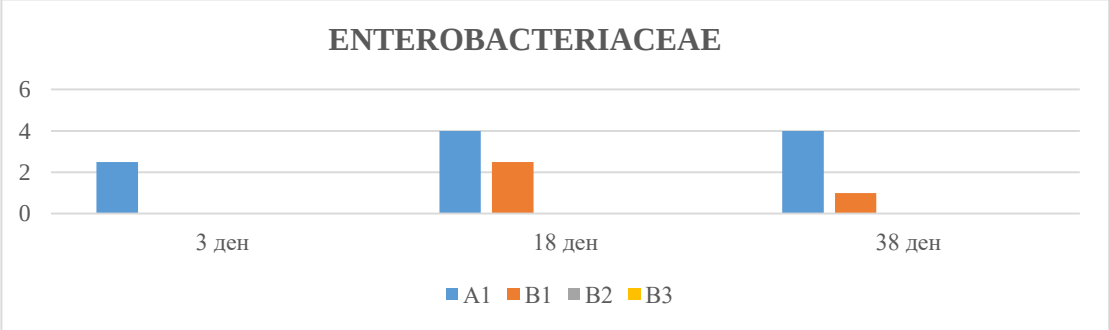


Figure 24. Combined effect of antioxidants with antimicrobial action and HPP treatment on enterobacteria during HPP treatment

Legend: A1. Minced beef, without HPP processing; B1. minced beef + HPP (580MPa x 4 min); B2. Minced beef + HPP + Medoliva Plus 0.3%; B3. minced beef + HPP + sodium lactate 0.3%

In fig. 24 show that the combined action of both antioxidants with antimicrobial agents – Medoliva Plus 0.3%, Sodium lactate 0.3%, do not allow the development of enterobacteria from the 3rd to the 38th day (<1x 101). The effectiveness of both pressure treatment and combined action is an effective process of improving microbial safety and quality during storage.

Minced beef has high moisture and mixed microflora, including pathogenic from the slaughterhouse to packaging. This evaluation from the experiments carried out confirms the literature data that at a pressure of 450 to 600 MPa it reduces the total number of bacteria by 6-8 log (CFU/g) over a period of 7-14 days, depending on the microorganisms present in the meat. This means that pasteurization is possible at pressures above 500 MPa. (Kruk, 2011). The type of food product and the microbial species in it, together with the ability of psychrotrophic microorganisms to recover at low temperatures, are the critical factors in evaluating the effectiveness of HHP processing.

Effect of HPP treatment and embedded antioxidants with antimicrobial activity on the number of inoculated pathogens of minced beef

The inoculated pathogens in the mix (P) include: *Escherichia coli; Salmonella; Listeria monocytogenes; Staphylococcus/sp; Campylobacter jejuni.* The behavior of the pathogens is considered against a factor of different retention time (240–480s) of HPP treatment.

The influence of the high hydrostatic pressure factor on *Salmonella* is undisputed even with standard processing. At the initial value of the control in log (CFU/g) (P1 without HPP treatment) on the 3rd day of the study – **$9.7.10^3$**, it leads to absence after both modes of treatment. (P2- HPP treatment 580 MPa x 4min.) **<1x 10^1** (P3 - HPP treatment 580MPa x 8min.) **<1x 10^1**. On the 38th day of the study, the control values were **9.10^4**, and for P2 and P3 (< **1x 10^1**) the same results were obtained, which is consistent with the studies of (Smelt, 1998).

The influence of HPP on the pathogen *Escherichia Coli* was analyzed on the 3rd day of treatment with HPP, for the control P1 without HPP a result of **$4.7.10^4$** was obtained, which leads to a reduction at P2 - **2x 10^1** (CFU/g) at and absence at P3. **A 4 log** reduction indicates good efficacy, this pathogen belongs to the Enterobacter family.

The inoculated *Listeria Monocytogenes* in the minced beef showed a reduction on the 3rd day by 3 log (CFU/g) P2 (**5x10^1**), compared to the control samples at P1 (**1.6x10^3**) and to absence at P3. During storage compared to the control 18th P1(7.10^4) 38th P1(**3.10^4**) there was a reduction of 38th P2 (**8x 10^2**) by 2 log (CFU/g), and at P3 it continued to no *Listeria* develops. The more difficult elimination of this pathogen is due to its belonging to Gram (+), which are more difficult to process even at a high pressure of 580 MPa, according to (Smelt, 1998).

On Staphylococcus Spp. a significant influence of HPP was observed from control P1 on the 3rd day (**7.2. 10^3**), and their number decreased 3 times, P2 (4x 10^1) and P3 (**<1x 10^1**). This type of microorganisms are Gram (+) due to the structure of the cell wall (Smelt, 1998) and are spherical in shape (Diels and Michiels 2006), which determines their more difficult treatment with HPP.

In Campylobacter Jejuni, as Gram (-), a decrease of 1 log (CFU/g) was observed compared to the controls (P1 - **1.1. 10^3**), and in (P2 - **4. 10^2**), and absence in P3. Experiments with pathogens show a promising result for the application of HPP technology in the used mode of operation to deal with zoonotic problems, both for the control authorities and for the producers of meat products.

4.3.5. Effect of HPP treatment and added antimicrobial and antioxidant substances on the organoleptic characteristics of minced beef.



Figure 25. Photo of samples during organoleptic evaluation

Organoleptic analysis

The organoleptic evaluation of minced meat from musclus Semembranosus was carried out in the microbiology laboratory of the University of Thessaly in Greece. Participants are not professional tasters, but future or current food technologists. The microbiology teacher and the students present in the laboratory participated in the analysis.

The majority of them are women aged 21 to 33, and men aged 20 to 40. Each participant had an evaluation sheet, on the basis of which an evaluation was given on: General Appearance, Color, Structure, Aroma, Taste. The rating scale is from 1 to 5. (5 - Excellent, 4 - Very good, 3 - Good, 2 - Acceptable, 1 - Not acceptable). The organoleptic analysis of minced beef with different additives and processed with HPP technology showed the best overall score for the samples with added red radish and corresponds to the research of other authors as well (Schindle, 2010).

V. CONCLUSIONS

1. Based on the results obtained from the study of beef from different parts of the carcass with high commercial value - ***m. Longissimus dorsi thoracis et lumborum*** and ***m. Semimembranosus*** applied high hydrostatic pressure in combination with treatment with biologically active substances of the selected muscle groups, the following more important conclusions can be drawn:

- The increase in pH values in experiments with marinated veal with thyme and oregano from muscle groups of ***Longissimus dorsi (LD)*** and ***Semimembranosus (SM)***, after HPP treatment) prove that the marinade has a minimal effect and is not of interest for wider studies with the aim decrease in the values of this indicator. In the experiments with the addition of antimicrobial substances to a meat product, a significant increase in the indicator was observed in the group treated with "Medoliva", which is a natural olive extract, as a powder, but this ability of the product does not lead to spoilage of the meat

product.The increasing trend is also observed in the group with added substances with antioxidant action, of which 0.1% added radish powder is the most important.

- It was found that the water-holding capacity increases significantly after treatment with HRR technology. The addition of the thyme and oregano marinade also has an effect on both muscle groups. When comparing the two muscle groups ***Longissimus dorsi (LD)*** and ***Semimembranosus (SM)***, an increase in the indicator was found, more pronounced for muscle group LD. When studying the influence on the water-holding capacity, the influence of antioxidants during the storage period was proven. The highest value was measured for the combination of HPP and the antioxidant effect of beetroot powder, and the lowest for the added rosemary essential oil.
- Attempts made to improve the negative effect of HPP treatment on meat on color on both LD and SM muscles showed a higher redness in the SM muscle.
- The influence of high hydrostatic pressure on the oxidation of beef lipids, expressed as TBARS reflectance values (MDA), was studied. An increase in saturated fatty acids was found, which is an indicator of lipid oxidation in the technological processing of ***Longissimus dorsi muscles***, in contrast to ***Semimembranosus muscle.***
- The results obtained for protein oxidation, which leads to various modifications of amino acids leading to carbonyl formation, were analyzed. Data on carbonyls in meat after high hydrostatic pressure treatment. show an increase in the values of this indicator as a result of the HPP treatment in the meat of the LD group in all its variants, and in the meat of the SM group the influence of HPP treatment is low on the HPP factor and oregano additive factor.

- Microbiological studies on the general microflora show the effectiveness of the HPP technology. The indicators: the total number of aerobic mesophilic, psychrotrophic microorganisms, molds and yeasts in the examined samples in muscle groups LD and SM are within the permissible norms, according to the requirements of BGS and ISO. The antibacterial properties of thyme in combination with HPP technology lead to an extension of the shelf life of raw meat from both muscles.

2. Based on the results obtained from the research of minced meat - minced meat from the ***Semimembranosus muscle (m.Sm)***, the applied high hydrostatic pressure HPP treatment, alone and in combination with antioxidants or antimicrobial agents, the following more important conclusions can be made:

- The most positive result on ground muscle SM is the addition of beetroot powder. The obtained results showed that of the added antioxidants, a reliable reduction of the yellow component of the meat product was found only in the red radish, and the highest value was obtained in the group stored for a period of 40 days.
- In the case of the yellow component of the meat, the version with ozonized water for 15 min, stored for 40 days, significantly differs, in which we obtained a value significantly

lower than all other groups. The 20th day storage time showed higher values compared to the 5 and 40 days stored groups.

- Immediately after NPP with parameters (pressure 580 MPa and time 4 min), the highest levels of MDA showed the samples with 15 min treatment in ozonized water, and during the refrigerated storage the results were stable and slowly rising until the 40th day of observation . The highest values were obtained for the group of added antioxidants - radish powder - 0.1% and the lowest for Medoliva Plus 0.3%, (contains olive + citrus polyphenol + citric acid), as well as with added antioxidant Rosemary 0.1%.
- The SM muscle minced meat study showed a significant effect of high hydrostatic pressure (HPP) processing on total micro-organisms, lactic acid bacteria and effectively inhibited Enterobacteriaceae from day 5 to day 40. Combined action with antimicrobial agents has no additional effect.
- The performed SDS-PAGE electrophoresis of HPP, concluded that the change in protein structure did not confirm the result in other scientific publications, which means that further studies are needed to clarify the effect of high pressure on the protein profile of the meat.

VII. CONTRIBUTIONS

1. Scientific contributions

• It was experimentally established that samples treated with HPP lead to an increase in the acidity index, which is not a sign of a spoilage process.

• The statistical analyzes performed show a proven effect of the HPP treatment factor on the water-holding capacity in the meat variants with thyme and oregano inserted for m.LD meat, as well as in the oregano variant for m.SM. The increased water holding capacity (WHC) of beef as a result of the applied high hydrostatic pressure, when using it as a raw material for a processed meat product The structure and sensory perception (fragility, juiciness, taste) are improved.

• It has been established that the added "Medoliva Plus" in a concentration of 0.3% plays a baroprotective role and eliminates the influence of hydrostatic pressure on the total number of microorganisms, but with regard to the development of enterobacteria (Enterobacteriaceae) it and the other three antimicrobial agents used have an equally positive effect and do not allow microorganisms to develop until the 38th day.

2. Scientific and applied contributions

- From the analyzes it was concluded that the treatment with parameters: 580 MPa, temperature 4°C, holding time 4 minutes is the optimal mode of operation to achieve highly effective elimination of pathogenic (*Escherichia Coli, Salmonella, Listeria Monocytogenes, Staphylococcus/ Sp , Campylobacter Jejuni*) and reduction of total microflora, to extend the safe shelf life of chilled minced beef and improve colorimetric parameters when using appropriate natural antioxidants. Applying the same pressure, but

with an extended retention time of 8 min., is not economically justified, as no significant differences were found between the effect of treatment with a retention time of 4 min.

- The combined treatment with HPP and sodium lactate was found to suppress the growth of both total microorganisms and lactic acid bacteria, maintaining its strong antimicrobial abilities throughout the 38-day storage period.
- As a consequence of the microbiological results, it can be proposed to replace the synthetic preservatives with a single or combined application of high hydrostatic pressure, which is ecologically and economically beneficial. It makes it possible to process both organic foods and foods that have entered the market widely under the name "clean label".
- An innovative product has been developed - minced beef, processed with HRR technology and added red radish powder, which has a significantly improved taste, color and texture. The wide range of results obtained with the use of different antimicrobial and antioxidant substances makes it possible to develop new taste preferences in meat production.
  The impact of the supplement "Medoliva Plus" has a baroprotective effect, but the baroresistance in relation to lactic acid bacteria can be reported as positive or negative depending on the characteristics of the specific strains. The use of fermented foods (with antimicrobial metabolic organisms) is a new trend in the food industry. The use of "Medoliva Plus" acts protectively against these strains of microorganisms, when HPP treatment is applied to an already fermented meat product.

VI. LIST
DISSERTATION RELATED PUBLICATIONS

1. **Pepa Nacaridou**, Daniela Miteva, Silvyia Ivanova, 2021. Alternative technology for beef meat shelf life. *Bulgarian Journal of acriculture,* 27(4), 792-797; ISSN 1310-0351 – print, ISSN 2534-983X – online; (*Web of Science*, Scopus).
2. **Pepa Natsaridu**, 2021. Influence of high pressure processing at 580 MPa and other antimicrobials on minced beef. *Agricultural Academy; Animal Sciences*, LVIII, 4/26-57; ISSN 0514-7441 (Print), ISSN 2534-9856 (On-line); (*Web of Science, CABI)*
3. **Pepa Natsaridu,** 2019. Application of HPP technology in meat-processing production. *Food Processing Magazine,* 9/31-35, ISSN 1311-0179; (*Web of Science, CABI)*

X. PARTICIPATION IN TRAINING:

- ✓ Participation in training on the topic: Principles and application of HPP technology in the food industry. HACCP control and labelling. The training was held at the tolling center "Pascal Processing B.V", Helmond, Netherlands, 19-22.02.2018 Attached training protocol Jasper van Altena Food Technolist EMEA

- ✓ Participation in training by Errol V. Raghubeer, Avure Technologies, Erlanger, KY, USA, training in AquaBar LTD, Sofia www.avure-hpp-foods.com,
- ✓ Training under the Erasmus + program, module "Training agreement Mobility of students and doctoral students for internships" at the University of Thessaly, Kardisa, Greece - 25.11.2019 - 25.04.2020, which includes practice and training in modern research methods and techniques on meat and meat products processed with HPP technology, entrance exam in English and online training during the program. During the training, the doctoral student learned methods of statistical processing of the obtained data in order to determine the linearity of the analysis methods used. She has mastered analytical methods and techniques for determining changes in meat and meat products after HPP processing.

XI. PARTICIPATION IN PROJECTS:

- ✓ **SSA- HTAI PROJECT** - No. 139 "Development of high-protein meat-based foods with increased antioxidant effect".
- ✓ Supervisor: Assoc. Dr. Daniela Miteva

SSA - HTAI PROJECT - No. TN 6: "Modern methods for extending the shelf life and monitoring the freshness of food products"

Head: Assoc. Dr. Daniela Miteva, Assoc. Dr. Svetla Dyankova