

UDC 637.5 (075.8)

MICROSTRUCTURAL ANALYSIS OF FORCEMEATS OF READY- TO-COOK CHOPPED MEAT WITH FUNCTIONAL INGREDIENTS

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Abstract. Meat products, at different technological stages and as finished articles, retain their morphological features. Microstructure analysis of the raw material, ready-to-cook products, or finished articles allows determining the presence of certain types of tissues, organs, spices – and low-value admixtures the recipe does not provide for, as well as reused raw materials. Microstructural studies of ready-to-cook chopped meat products allows identifying their components, establishing different properties of various tissue and cellular structures, and controlling the articles manufactured. Minced beef as the object of research was modified, with 5 %, 10 %, 15 % of the meat part replaced with lupin flour and 0.5% of elecampane root powder added as aromatic raw material. For microscopic examination, samples of the forcemeats developed were put marks on and fixed in a 10 % neutral formalin solution. The sections, as thick as 0.5–1 cm, were cut on a sledge microtome. They were stained with haematoxylin and eosin, and the PAS reaction. Light microscopy and microphotography of the tissue specimens were performed with a microscope Leica DM 2500 and a camera Leica DFC 450C with the software Leica application suite 4.4. The micrographic investigation of the forcemeats revealed polygonal and round muscle fibres (their dark nuclei were clearly seen under the sarcolemma), concentrations of adipose tissue histologically characterized by a reticulate structure. In the microphotographs, lupin flour looks like groups of round light purple cytoplasm with dark purple nuclei in the centre of polygonal cells; bread looks like loose brown fibres; wavy violet fibres represent ions; and single dark brown spots marked elecampane. It has been shown that histological studies, with the PAS reaction used, are helpful in determining the meat and plant content in the ready-to-cook meat developed, and that haematoxylin and eosin can help determine the functional ingredients content.

Keywords: histology, lupin flour, elecampane, meat, ready-to-cook chopped meat

МІКРОСТРУКТУРНИЙ АНАЛІЗ ФАРШІВ М'ЯСНИХ ПОСІЧЕНИХ НАПІВФАБРИКАТІВ З ФУНКЦІОНАЛЬНИМИ ІНГРЕДІЄНТАМИ

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Анотація. М'ясопродукти на різних стадіях технологічної обробки, а також у готовому вигляді, зберігають свої морфологічні особливості. За допомогою мікроструктурного аналізу сировини, напівфабрикатів чи готової продукції можна визначити наявність тих чи інших видів тканин, органів, спецій, а також малоцінних добавок, непередбачених рецептурою, повторно використану сировину. Проведення гістологічних досліджень м'ясних посічених напівфабрикатів дозволяє виявляти їхні компоненти, диференціювати властивості різних тканинних і клітинних структур, а також здійснювати контроль продукції. Об'єктами досліджень були удосконалені фарші яловичі з заміною 5%, 10%, 15% м'ясної частки на люпинове борошно та додаванням 0,5% порошку кореня дивосилу, як пряно-ароматичної сировини. Для мікроскопічного дослідження матеріал розроблених фаршів маркували і фіксували у 10% нейтральному розчині формаліну. На санному мікроскопі виготовляли зрізи, завтовшки від 0,5–1 мм, які фарбували гематоксином та еозином, ШИК реакцією. Світлову мікроскопію і мікрофотографування гістопрепаратів здійснювали за допомогою мікроскопа Leica DM 2500 та фотокамери Leica DFC 450C програмного забезпечення Leica application suite 4.4. При мікроструктурному дослідженні у фаршах виявили м'язові волокна полігональної і круглої форми з темними ядрами, які добре проглядалися під сарколемою, осередки жирової тканини, яка гістологічно характеризується сітчастою структурою. На мікрофотографіях люпинове борошно зображено у вигляді згрупованих круглих цитоплазм з ядрами темно-фіолетового кольору, розміщеними в центрі клітин полігональної форми, хлібну масу у вигляді – розсіпчастих волокон коричневого кольору; цибулю ріпчасту – у вигляді хвилястих волокон фіолетового кольору, дивосил – у вигляді поодиноких точок темно-коричневого кольору. Результати досліджень показали, що за допомогою гістологічних досліджень з використанням ШИК реакції, доцільно визначати вміст у м'ясних розроблених напівфабрикатах м'ясної та рослинної частин, а з використанням гематоксилину та еозину – вміст функціональних інгредієнтів.

Ключові слова: гістологія, люпинове борошно, дивосил, м'ясо, посічені напівфабрикати.

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DOI: <http://dx.doi.org/10.15673/fst.v12i4.1208>

Introduction. Formulation of the problem

Ready-to-cook chopped meat products with functional ingredients are well-balanced combinations of high sensorial qualities, nutritional value, and healthiness. They are supposed to be regularly consumed by a lot of people as part of everyday diet, without any specific guidelines. The quality control of meat and meat products is nowadays a most topical issue. It is due to the recent global changes in all branches of meat and meat-processing industry [1]. A promising method of quality control is micrographic investigation. It allows identifying certain components, establishing different properties of tissue and cellular structures [2]. Histological study is a direct method of determining the composition of raw materials and manufactured articles.

Analysis of recent research and publications

Enriching ready-to-cook chopped meat with functional ingredients is considered in a number of works. A lot of researchers have studied the histology of meat raw materials in which plant raw materials are used (A. Semenova, T. Kuznetsova, Ye. Tuniyeva, S. Khvylyya, V. Pchelkina, M. Paska, M. Pospiech et al., J. Vanha, F. Kvasnicka). But this paper is the first to study the microstructure of ready-to-cook chopped meat products with lupin flour and elecampane included in the composition of their forcemeat, to identify these elements.

In Japan, for more than twenty years, various methods of cooking meat with an elevated level of calcium. To make cutlets, schnitzels, ready-to-serve foods, small bones of animals are added to the forcemeat [5]. In the USA, the production of a protein-mineral food additive from bones and bone residues are extensively studied [6]. In the UK, food bones are processed by Johnson-Fowdler's method to obtain edible fat, soluble protein, and food phosphate [7].

The studies [8,9] are devoted to introducing protein components (mainly soybean concentrates, wheys, etc.) into chopped meat.

It is now becoming especially important to manufacture ready-to-cook meat products that combine meat raw material and plant proteins and contain complete proteins. As a protein-containing raw material of plant origin, lupin takes pride of the place. In a World Congress in the USA, lupin was recognized as an important, so to say, reserve fund of high quality protein substances. Besides protein, a lupin grain contains 25–40% of nitrogen-free extractive substances, 9% or more of fat, 3–4% of ash. The average protein content of lupin flour is 38.6% in relation to the dry substances (DS) – this parameter is 3 times as high as that of first grade wheat flour, and by 2.2% higher, in relation to the DS, for soybean flour. About 90% of the total protein substances content in a lupin seed is represented by easy-to-assimilate fractions – albumins and globulins, whereas for soybean wheat, it is only 67% [10,11].

A promising functional ingredient of ready-to-cook chopped meat is the elecampane root containing up to 44% of inulin. It is proved that elecampane contributes to metabolic rebalancing, invigorates, helps stay young and healthy [12,13].

The timeliness of the topic of the research is due to the importance of the task of controlling the formulations of ready-to-cook chopped meat products according to the normative documents. That is why it is essential to identify raw components basing on a histological study of compound forcemeats.

A microstructure analysis allows determining the structural changes in model forcemeats and finished ready-to-cook chopped meat products, studying how heat treatment forms differences in the structure of the layers near the surface and deep ones, how it effects on the coherence of the structural elements of forcemeat, on the density of its consistency, on reducing the sineresis in forcemeat.

At different stages of their technological processing, and when finished, too, meat products retain their morphologic features. So, microstructure analysis of the raw material, ready-to-cook products, or finished articles allows determining the presence of certain types of tissues, organs, spices – and low-value admixtures the recipe does not provide for, as well as reused raw materials [14].

Microstructure analysis allows not only identifying counterfeits, but also controlling the compliance of meat products with the approved recipe. The method makes it possible to quantify individual constituents of a product as well [15].

The purpose of the study is determining the microstructure of ready-to-cook chopped meat products, with lupin flour and elecampane used in their composition, to identify these ingredients.

For this purpose, the following **objectives** should be achieved:

1. Prove the feasibility of using lupin flour and elecampane in the technology of ready-to-cook chopped meat.
2. Develop recipes and a technology, develop forcemeats and take samples to study.
3. Determine the microstructure of ready-to-cook chopped meat products, with lupin flour and elecampane used in their composition.
4. Identify the raw components, and make conclusions.

Research materials and methods

The research was carried out by the Normal Morphology, Pathomorphology, and Judicial Veterinary Medicine Department at Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv.

The objects of the study were functional beef forcemeats (with 5%, 10%, 15% of the meat part replaced with lupin flour and 0.5% of elecampane root powder added as aromatic raw material), and a control sample of

minced beef developed according to GOST (State Standard) 52675-2006. The flour used was of lupin of the "Kharchovyy" variety grown in the Institute of Agriculture of the National Academy of Agrarian Sciences of

Ukraine. Lupin flour is a light-yellow homogeneous fine powder, smelling and tasting neutral.

4 forcemeat samples were selected for the study (Table 1).

Table 1 – Formulations of ready-to-cook chopped meat products

Raw material	Raw material consumption per 100 kg of finished products, kg			
	Net weight			
	Control	Sample 1	Sample 2	Sample 3
Beef (minced meat)	54.0	51.3	48.6	45.9
Lupin flour	–	2.7	5.4	8.1
Slaughter fat	5.0	5.0	5.0	5.0
Wheat bread	13.0	13.0	13.0	13.0
Breadcrumbs	2.0	2.0	2.0	2.0
Bulb onions	3.0	3.0	3.0	3.0
Ground black pepper	0.1	0.05	0.05	0.05
Powdered elecampane	–	0.05	0.05	0.05
Salt	1.2	1.2	1.2	1.2
Water	21.7	21.7	21.7	21.7

For microscopic examination, samples of the forcemeats developed were put marks on and fixed in a 10 % neutral formalin solution. Then, the fixed material was dehydrated in a number of alcohol solutions with increasing concentrations (70, 80, 90, 96%), densified in two portions of chloroform, and embedded in paraffin wax. On a sledge microtome, sections, as thick as 0.5–1 cm, were cut. They were stained with haematoxylin and eosin, and the PAS reaction (a test used to detect glycoproteins, polysaccharides, some mucopolysaccharides, glycolipids, and some fatty acids in tissues. The tissue under study, on being acted upon by the Schiff reagent, is treated with hydroiodic acid. If the reaction is positive, the tested tissue is stained red). Light microscopy and microphotography of the tissue specimens were performed with a microscope Leica DM 2500 and a camera Leica DFC 450C with the software Leica aplitation suite 4.4 [1].

Results of the research and their discussion

The micrographic investigation of the forcemeats has revealed polygonal and round muscle fibres, with the dark nuclei clearly seen under the sarcolemma. It indicates that for the forcemeat, fresh, refrigerated meat was used. Besides, among the muscle fibres, concentrations of adipose tissue have been observed that are histologically characterized by a reticulate structure. In the locations of fat inclusions, vacuoles have been detected, different in shape and size, which explains the net-like look of the section. Lupin flour looks like compact groups of round light purple cytoplasm with dark purple nuclei in the centre of polygonal cells; bread looks like loose brown fibres; wavy violet fibres represent bulb onions; and single dark brown spots marked elecampane.

In Fig. 1, there are lupin flour vacuoles stained with haematoxylin and eosin (a), with eyepieces 10 and camera lens 30, and those stained with the PAS reaction (b), with eyepieces 10 and camera lens 20. They are spherical

polygonal violet-coloured vacuoles with the nuclei in the centre of the cells, and are good absorbents.

In Fig. 2, there is a powdered elecampane root stained with haematoxylin and eosin (a) and with the PAS reaction (b), with eyepieces 10 and camera lens 20. The fibres are elongated, clear-shaped, clumped, not loose.

In the control sample sections (Fig. 3a) stained with haematoxylin and eosin (a) and by the PAS reaction (b), with eyepieces 10 and camera lens 20, the muscle fibres are polygonal and sharp-outlined (1). Besides, large reticulate and oval cells of adipose tissue are observed (2). Loose violet fibres represent onions (3), because they have been ground. Bread is shown as single loose brown elements (4). The forcemeat structure is even and homogeneous, some loosening of the fibres is observed, as the forcemeat has been stirred.

After the PAS reaction, in the control sample section (with eyepieces 10 and camera lens 20), the plant (1) and meat (2) parts are clearly distinguishable (Fig. 3b). They make it possible to determine whether the forcemeat components ratio is right, and to conclude that the meat part of the forcemeat is quite considerable.

In Fig. 4a, there is a forcemeat section containing 5% of lupin flour and 0.5% of elecampane root. With haematoxylin and eosin, with eyepieces 10 and camera lens 10, the presence has been established of polygonal, sharp-outlined muscle fibres (1), large reticulate and oval cells of adipose tissue (2), loose violet fibres of onions (3), single loose brown fibres of bread (4), spherical polygonal violet-coloured vacuoles with the nuclei in the centre of the cells – lupin flour (5), and individual clear-shaped, dark-brown fibres of elecampane (6). The forcemeat structure is even and homogeneous.

In Fig. 4b, by the PAS reaction, with eyepieces 10 and camera lens 20, the plant (1) and meat (2) parts have been clearly identified. The plant components content is supposed to increase by 5%, according to the formulation developed, but the meat content is close to that in the control sample and remains quite considerable.

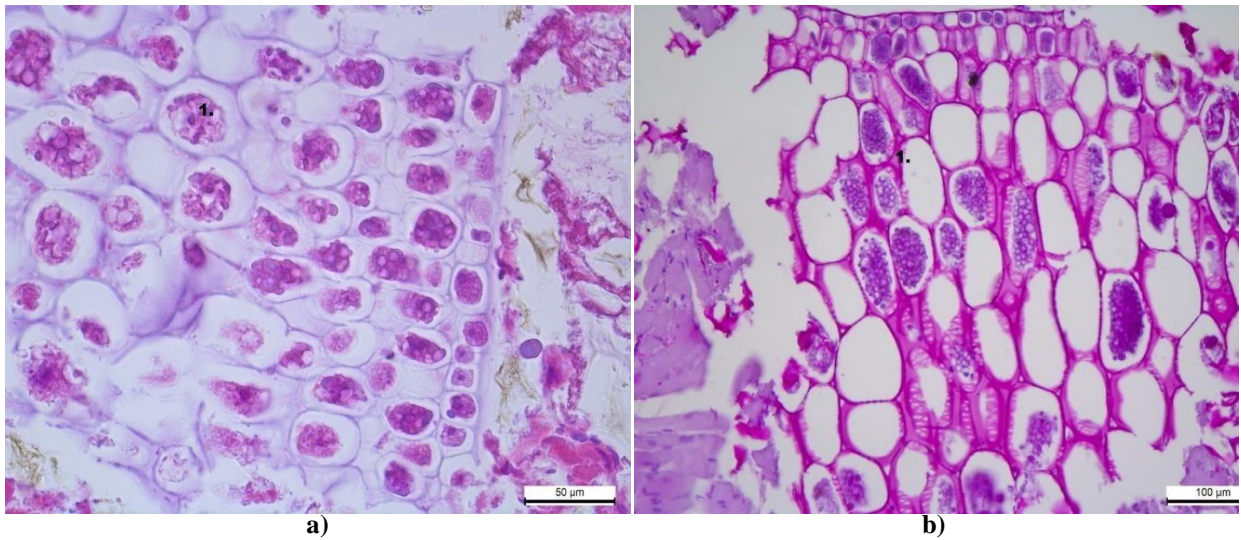


Fig. 1. Microphotographs of lupin flour
a) Haematoxylin and eosin (eyepieces 10, camera lens 30); b) PAS reaction (eyepieces 10, camera lens 20)

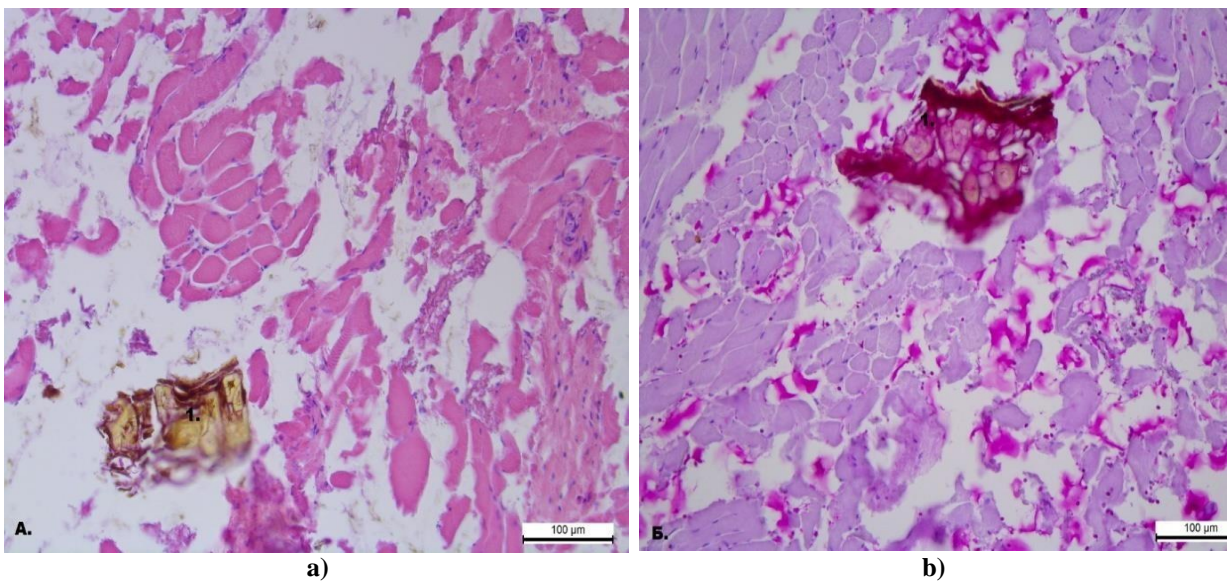


Fig. 2. Microphotographs of elecampane root inclusions
a) Haematoxylin and eosin (eyepieces 10, camera lens 20); b) PAS reaction (eyepieces 10, camera lens 20)

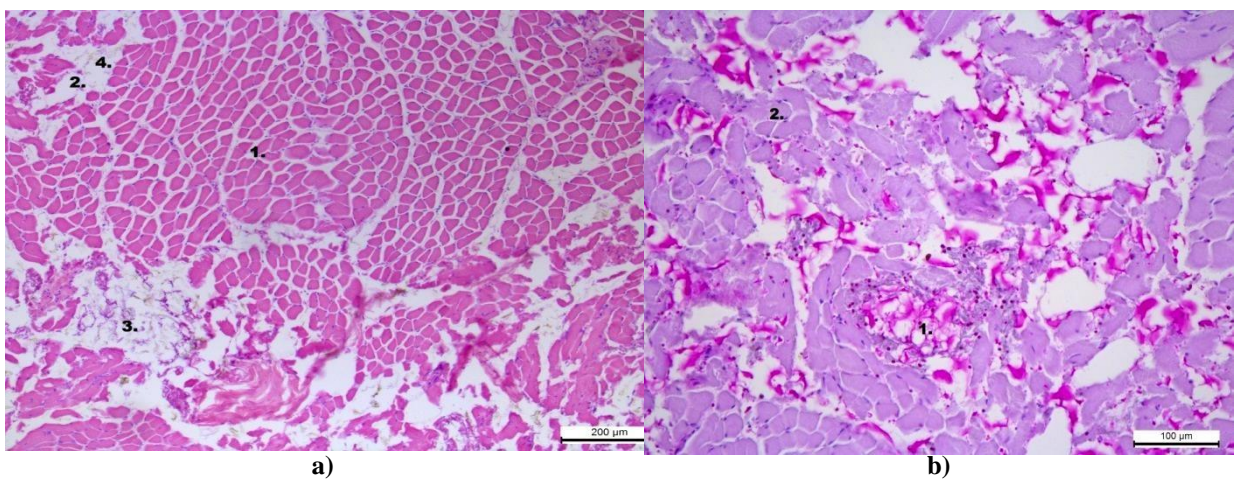


Fig. 3. Microphotographs of sample 1 "Control"
a) Haematoxylin and eosin (eyepieces 10, camera lens 10): 1 – muscle fibres, 2 – adipose tissue, 3 – onions, 4 – bread
b) PAS reaction (eyepieces 10, camera lens 20): 1 – plant part, 2 – meat part

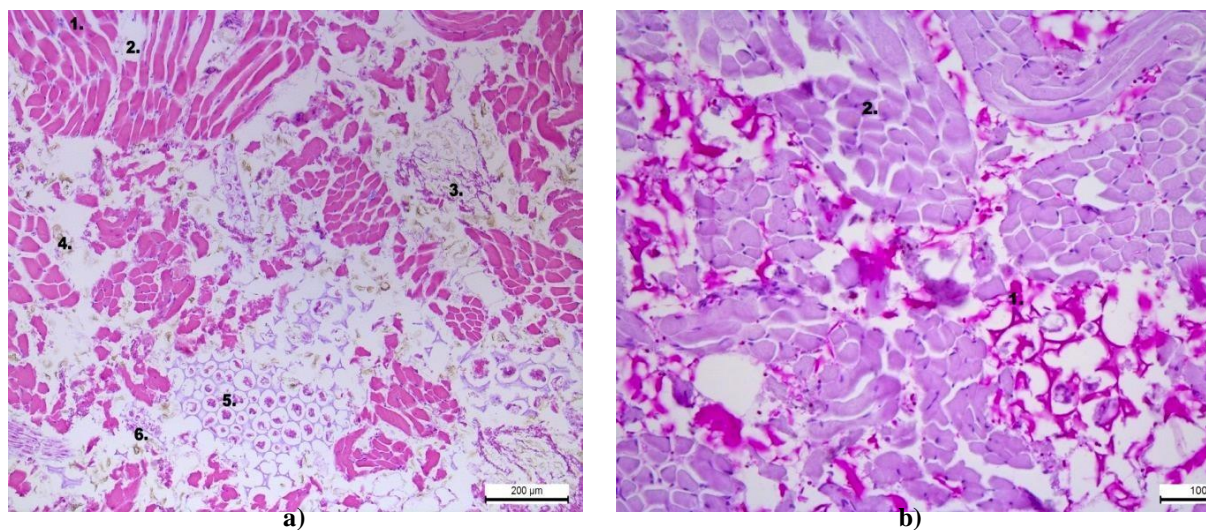


Fig. 4. Microphotographs of sample 2 “5 %”

- a) Haematoxylin and eosin (eyepieces 10, camera lens 10): 1 – muscle fibres, 2 – adipose tissue, 3 – onions, 4 – bread, 5 – lupin flour, 6 – elecampane
 b) PAS reaction (eyepieces 10, camera lens 20): 1 – plant part, 2 – meat part

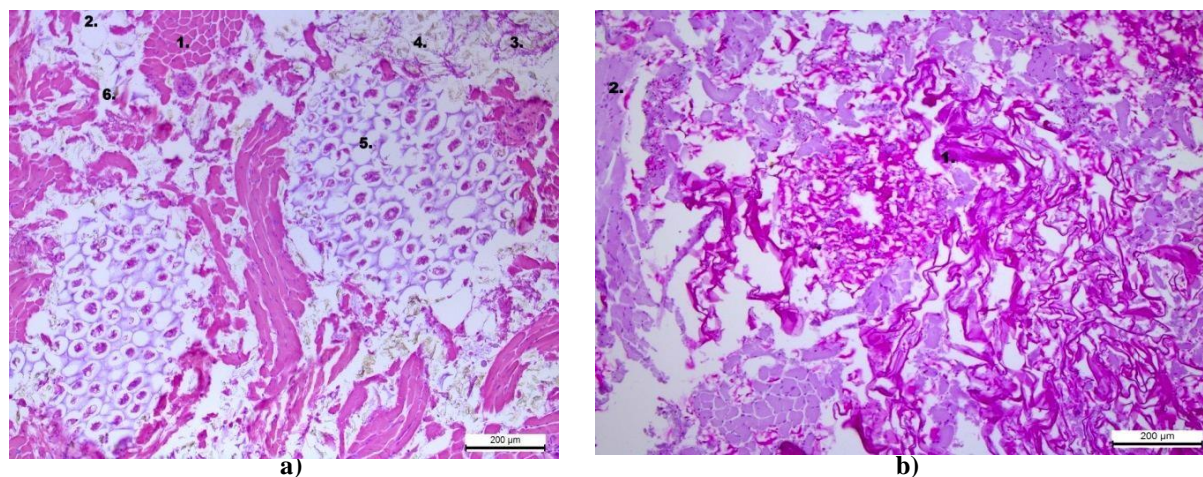


Fig. 5. Microphotographs of sample 3 “10 %”

- a) Haematoxylin and eosin (eyepieces 10, camera lens 10): 1 – muscle fibres, 2 – adipose tissue, 3 – onions, 4 – bread, 5 – lupin flour, 6 – elecampane
 b) PAS reaction (eyepieces 10, camera lens 10): 1 – plant part, 2 – meat part

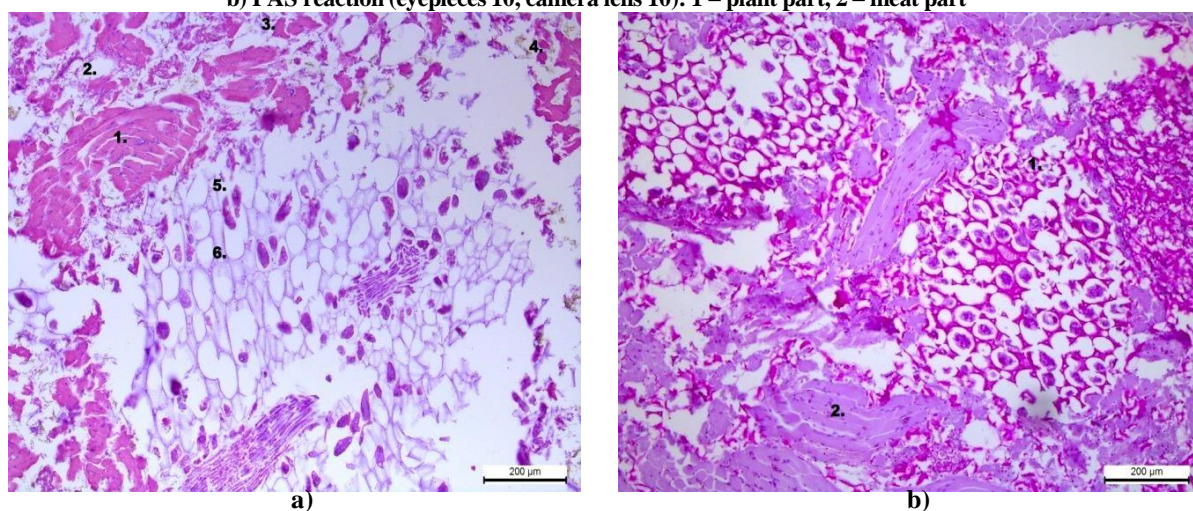


Fig. 6. Microphotographs of sample 4 “15 %”

- a) Haematoxylin and eosin (eyepieces 10, camera lens 10): 1 – muscle fibres, 2 – adipose tissue, 3 – onions, 4 – bread,

5 – lupin flour, 6 – elecampane**b) PAS reaction (eyepieces 10, camera lens 10): 1 – plant part, 2 – meat part**

In Fig. 5a, there is a forcemeat section containing 10% of lupin flour and 0.5% of elecampane root. With haematoxylin and eosin, with eyepieces 10 and camera lens 10, the presence has been established of polygonal, sharp-outlined muscle fibres (1), large reticulate and oval cells of adipose tissue (2), loose violet fibres of onions (3), single loose brown fibres of bread (4), spherical polygonal violet-coloured vacuoles with the nuclei in the centre of the cells, forming a large clump, – lupin flour (5), and individual clear-shaped fibres of elecampane (6). The forcemeat structure is slightly stratified, the components are not observed to be evenly stirred up, as lupin flour absorbs moisture.

In Fig. 5b, by the PAS reaction, with eyepieces 10 and camera lens 10, the plant (1) and meat (2) parts have been clearly identified. The plant components content increases by 10%, according to the formulation developed, but the sensorial qualities remain as high.

In Fig. 6a, there is a forcemeat section containing 15% of lupin flour and 0.5% of elecampane root. With haematoxylin and eosin, with eyepieces 10 and camera lens 10, the presence has been established of polygonal, sharp-outlined muscle fibres (1), large reticulate and oval cells of adipose tissue (2), loose violet fibres of onions (3), single loose brown fibres of bread (4), spherical polygonal vacuoles with the nuclei in the centre of the cells, forming a large clump, moisture-absorbent, – lupin flour (5), and individual clear-shaped, dark-brown fibres of elecampane (6). The forcemeat structure is slightly stratified, the components are not observed to be evenly stirred up, as lupin flour absorbs moisture. The forcemeat

structure is defragmented, inhomogeneous, loose, the meat and plant parts are distributed unevenly.

In Fig. 6b, by the PAS reaction, with eyepieces 10 and camera lens 10, the plant (1) and meat (2) parts have been identified. The plant components content increases by 10%, according to the formulation developed. It increases its content in forcemeats, but affects the sensorial qualities.

Conclusion

Histologic (microstructure) research has revealed, by the PAS reaction, the content of the meat and the plant parts in the ready-to-cook products developed. By means of haematoxylin and eosin, it has been determined how the forcemeat components under study look like. The research has shown that with 5% of lupin flour introduced, the forcemeat is even and homogeneous; 10% – slight stratification in the forcemeat is possible; 15% – the meat and the plant parts are unevenly distributed, the forcemeat is non-uniform, inhomogeneous, loose. A microstructure analysis of the forcemeats prepared by the formulations modelled indicates that more than 15% of lupin flour introduced into the forcemeat composition results in a loosened structure of the product. Thus, the optimum lupin flour amount is 10 %.

Thus, the microstructure study has allowed establishing a quality model of the useful product, without affecting its sensorial qualities. Further work is to be aimed at starting its serial production.

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Отримано в редакцію 04.05.2018
Прийнято до друку 06.11.2018

Received 04.05.2018
Approved 06.11.2018

Цитування згідно ДСТУ 8302:2015

Paska M., Bal-Prylypko L., Masliichuk O., Lychuk M. Microstructural analysis of forcemeats of ready-to-cook chopped meat with functional ingredients // Food science and technology. 2018. Vol. 12, Issue 4. P. 110-116. DOI: <http://dx.doi.org/10.15673/fst.v12i4.1208>

Cite as Vancouver style citation

Paska M., Bal-Prylypko L., Masliichuk O., Lychuk M. Microstructural analysis of forcemeats of ready-to-cook chopped meat with functional ingredients . Food science and technology. 2018; 12(4): 110-116. DOI: <http://dx.doi.org/10.15673/fst.v12i4.1208>