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Histological Identification of Animal Protein Ingredients

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Abstract: The results of microstructural research of powdery animal protein additives produced from pork collagen-containing connective tissue and blood plasma are presented. The analyzed samples had similar composition of collagen and elastin animal proteins. Generally, a protein agent based on animal connective tissue fibers only or in combination with granular eosinophilic protein of a different biological origin is taken as a sample. The rate of hydrolytic changes of connective tissue intercellular substance during technological processing of agents was found different. Plant protein and carbohydrate components were not found.

Key words: Protein additives, connective tissue, blood plasma, microstructural research

INTRODUCTION

Active implementation of resource-saving technologies in meat-processing enterprises is the most promising way to increase output of meat products and provide high quality level. Tough competitive environment make manufacturers to use traditional meat raw materials along with ingredients of general-purpose which provide balanced nutrition value of finished products and retain traditional organoleptic characteristics.

Animal protein additives (animal proteins) are produced from different raw materials: pork skin, pork vein, beef vein, pork or beef blood plasma, milk whey and eggs. The production of 'animal proteins', as natural products, is based on processing thermally (defatting and dewatering), chemically (partial proteolysis) and mechanically (grinding) in order to transform connective tissue protein into a better digestible form, for unprocessed one is poorly digested in normal conditions (Zhebeleva *et al.*, 2008; Krishtafovich, 2013). Animal proteins based on connective tissue and blood plasma hold a special place among the used protein ingredients. Recently they have become interesting in production area because such agents are natural meat components and they cannot be considered as alien to meat ingredients in meat and meat-containing recipes. In addition, animal protein additives were found to have a high digestibility level *in vitro* (97-98%) (Zhebeleva and Krishtafovich, 2008; Krishtafovich, 2014a).

In the Russian market, the major suppliers of animal protein additives offer those based on pork connective

tissue as well as on a mixture of connective tissue and blood plasma (Krishtafovich, 2014b).

The research which we had conducted earlier enabled us to find high technological properties (water-binding, fat-binding capabilities and ability to emulsify fats) of such protein ingredients, which determined their use in production of meat-containing half-finished products. The aim of that work was to improve properties of minced combined meat based on mechanically separated poultry and properties of weak gluten flour dough (Krishtafovich and Zhebeleva, 2014a,b). Collagen animal proteins can be produced from cooled pork skin and pork trimmings by drying and grinding without any chemicals. They are powders of different dispersion degrees (Khvylya *et al.*, 2011).

Microstructural research (Khvylya *et al.*, 2011) makes it is possible to identify the origin of protein agents (absence of plant components), to differentiate them and to determine the tissular and cellular formation morphology in combined meat products which contain the given protein additives.

Therefore, histologic research of four samples of powdery animal protein additives was conducted. They were a protein additive based on pork connective tissue and pork blood plasma (sample 1), two types of protein additives based on pork connective tissue (sample 2 and sample 3) and a protein additive based on pork blood plasma (sample 4). The animal proteins produced by the firms Scanpro and Vepro were studied. The research was conducted in accordance with the

State Standard P 51604-2000 "Meat and meat products. A method of histologic identification of composition." Dry powder samples of the protein additives were mixed with beef minced meat in the ratio of 5 to 1 and then they were put into a thermostat for 30 min at a temperature of +35°C to make the hydration of components better. After that, we made slices of 15 µm thick in a freezing microtome MIKROM HM-525 and stained them with Ehrlich's hematoxylin and 1% water-alcohol solution of eosin. The agents were enclosed in glycerin-gelatin, studied and photographed in a light microscope Axioimager A1 'Carl Zeiss' (Germany).

Study of the protein additive (sample 1) showed that a powdery agent consisted of a mixture of two protein components. The first one had basophilic aggregations and elastic fibrils fragments, typical for collagen material. The second one was a quite homogeneous eosinophilic lumpy substance with limited sponginess (Fig. 1).

It can be seen from the obtained data that the degree of hydrolytic decomposition of the first protein agent component (sample 1) is quite high. Particles start to bind water well even at room temperature. Intensely colored cell nuclei remnants and separate fragments of elastic fibers of native structure are preserved in the agent formula. However, the nature of a protein eosinophilic component was not clearly identified.

According to the research, a connective tissue protein additive (sample No. 2) was found to consist of a hydrolyzed collagen component (blue color) with basophilic aggregations, inclusions of fibroblast

remnants of nuclear formations (dark blue color) and occasional fragments of elastic fibers (pink color) (Fig. 2). Judging by the morphological characteristics of the visualized components, the sample 2 is considerably similar to the collagen component of the sample 1. The content of elastic fibers that does not bind water is quite high.

The sample of the protein additive No. 3 was found to be a homogeneous powder with the main component of basophilic color, which presents a highly hydrolyzed collagen (blue color) with inclusions of remnants of fibroblast fragments and occasional pieces of elastic fibers (Fig. 3).

The comparison of microstructural characteristics of collagen protein components showed that the sample 3 is considerably similar to the samples 1 and 2. According to the research findings, the sample 3 is assumed to have a higher degree of hydrolytic changes in collagen than the sample 2 has. At the same time, there is another protein in limited quantities in the agent, which is in a globular form and has affinity to oxyphilic strainers.

The additive based on pork blood plasma (sample 4) was found to be a homogeneous powder which has eosinophilic aggregations of fine-lumpy and partially hydrolyzed protein material (pink color) with inclusions of basophilic structures remnants of probably cell nuclei (Fig. 4)

Judging by the morphological characteristics, the sample looks like a small-grained protein mass and is

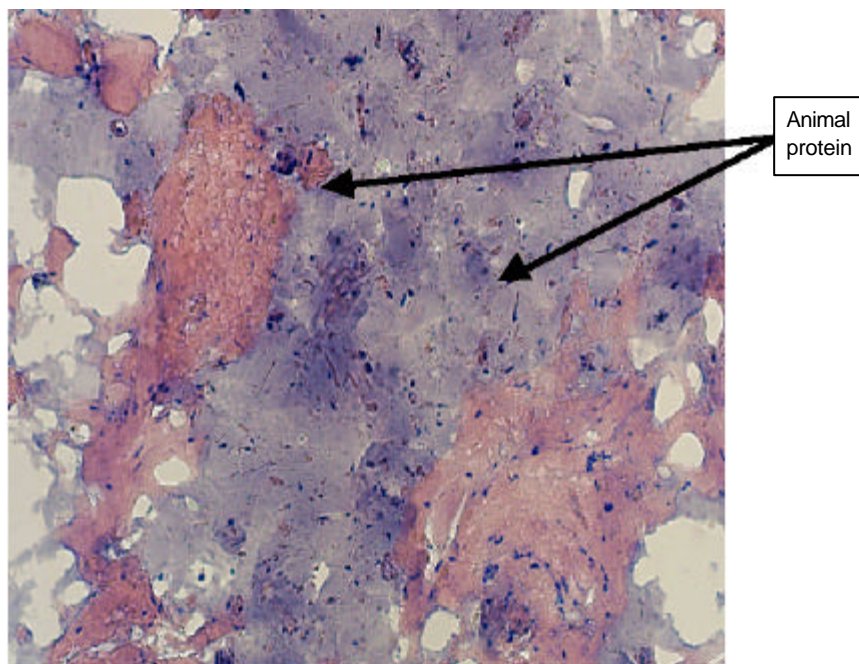


Fig. 1: Microstructure of a protein additive (sample 1)

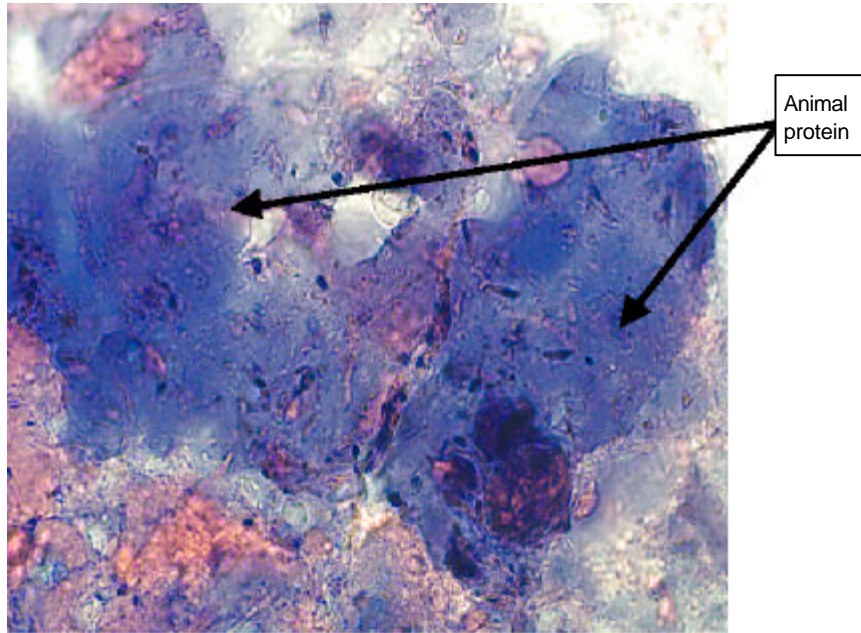


Fig. 2: Microstructure of a protein additive (sample 2)

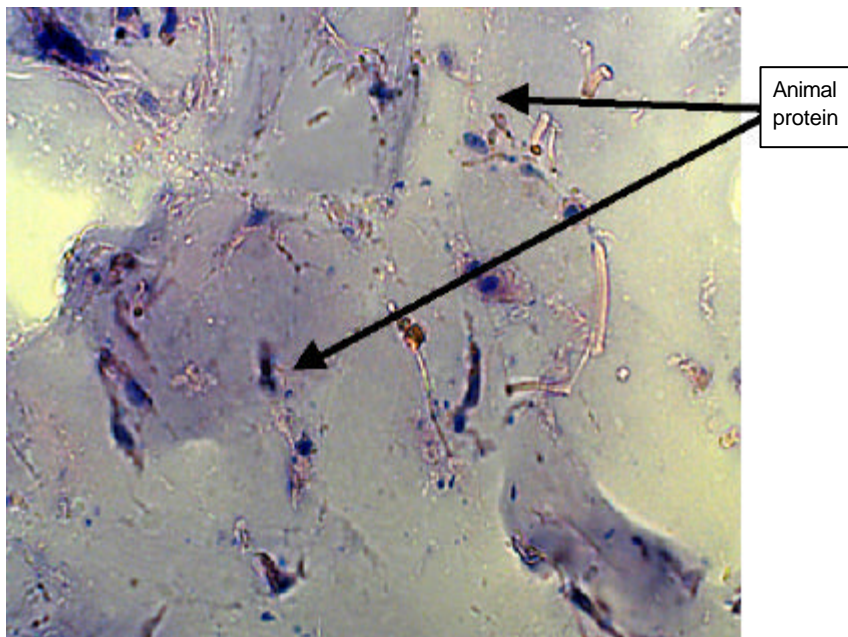


Fig. 3: Microstructure of a protein additive (sample 3)

considerably similar to decomposition product of muscle fibers, which is formed when meat is tendering or being processed technologically. According to the obtained data, a protein additive based on pork blood plasma does not contain such fibrous components of animal origin as collagen and elastin.

Thus, the analyzed samples of different powder protein additives have a similar composition of fibrillary collagen and elastin animal proteins or globular proteins of blood plasma. In general, they present a protein agent based on only fibers of animal connective tissue and its fibrous intercellular substance of different degrees of hydrolytic

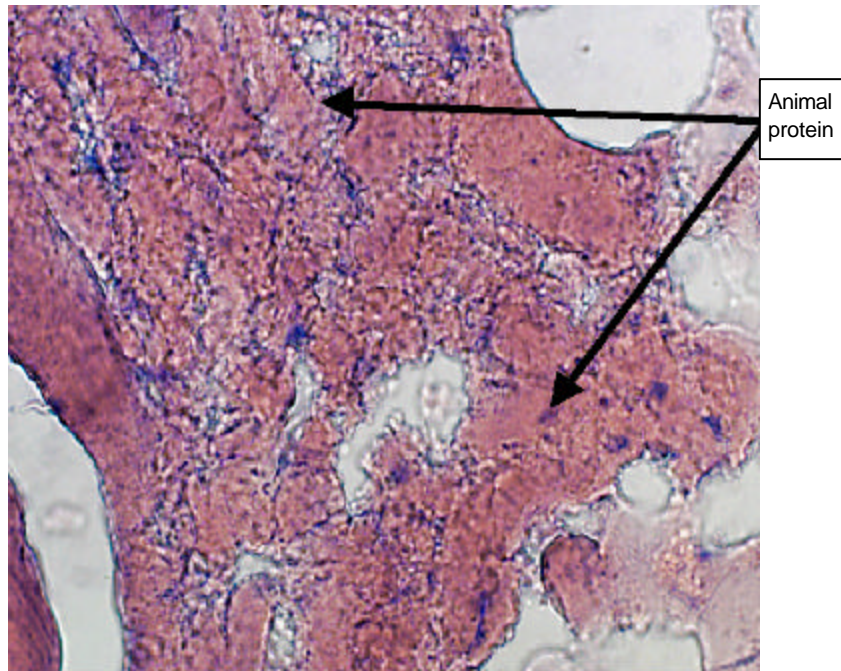


Fig. 4: Microstructure of animal protein (sample 4)

decomposition or in combination with granular eosinophilic protein of a different biological origin. The degree of hydrolytic changes in the connective tissue intercellular substance after technological processing is a little different. Plant components of protein and carbohydrate origin were not found in the analyzed protein additives.

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