Subgroup 2

Methods for quality assessment

PROTEIN IDENTIICATION IN MEAT PRODUCTS COMPOSITION

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Background

In recent years the range of meat products into formulations of which the plant and animal materials are included has been extended. It is starch or its derivatives, as well as plant proteins extracted from wheat, corn, soy, green peas, cottonseeds, sunflower, rape, lucerne that are most commonly introduced into meat products. Although meat is a source of protein of high biological value, nowadays there is a trend to creation of combined meat products with the use of plant raw materials. The use of plant proteins allows to partially substitute expensive meat protein and to use emulsions from low grade meat for processed meat products Plant proteins in this case have the function of binders. Soy proteins in the manufacture of meat products have found. the widest application. They are added , mainly, into the product of fine comminution (sausages links, cooked sausages without fat) and of coarse comminution (cutlets, minced meat balls, chopped beef steaks, etc.). canned meats (stewed meat, corned beef, canned ham). Direct introduction of soy proteins isolates in the form of solutions into the muscle tissue is also possible. In recent years the requirements to analysis of foods quality are considerably increased, especially to the evaluation of composition of both the initial raw materials, and the processed meats, and to revealing of different, as a rule, cheaper additives. For the identification of proteins it is useful to apply selective methods, based on electrophoretic or chromatographic mobility of protein fractions, as well as on their specific binding by immune methods.

Objective

The objective of the investigations was to reveal the conditions of electrophoretic identification of protein in polycomponent meat products containing mixed plant and animal proteins.

Materials and methods

The samples of meat (beef and pork) and meat products obtained for certification tests in the system of certification of the testing Center of VNIIMP, containing soy concentrate "Danpro-H" were used in the investigations. The initial composition of the concentrate (%): protein –69.5; carbohydrates – 17.3%; moisture – 4.0; fat – 1.0, cellulose – 4.0; ash – 7.0. For quantitative analysis the method of electrophoresis in 10% polyacrylamide gel in the presence of SDS was used allowing to identify 0.5 – 2 μg of protein fraction with molecular mass from 5 to 400 kD [1,2].

Results and discussion

For practical identification the protein being analyzed should be turned into soluble form in such concentration which provides the possibility of subsequent identification of different fractions. The study of the influence of the ratio of the sample mass to the extraction agent allowed to choose the maximum hydromodule of the system 1: 50 , when it becomes possible to turn the required quantity of fractions of plant and animal protein into solution. The use of extraction solutions has shown that good complex formers, especially Tris, allow to easily turnr the necessary amount of protein into solution (Table).

Table Degree of extraction of beef, pork and soy concentrate proteins by different systems, %

Object	Solvents				
	Water	0.1 M Buffer phosphate, pH 7.4	0.1 M Borate buffer with potassium chloride, pH 9.5	0.1 M Tris-buffer pH 9	4% solution of sodium chloride
Beef	37.9 ±2.2	66.4 ± 3.7	37.0 ±1.8	64.5 ±3.5	29.6±1.6
Pork	44.8±2.5	68.0 ±3.4	53.0±2.7	72.1± 3.1	40.1± 2.0
Soy concentrate	9.4±0.5	11.9±0.6	11.5±0.8	17.8±0.6	3.9±0.2

Study of the influence of temperature on the degree of proteins extraction has shown (phosphate and tris- buffer, degree of dilution 1:50, time length – 30 min.) that with the increase of the temperature from 8 to 75°C the degree of transfer of proteins of beef and pork muscle into the solution, as a rule, is reduced to 7-10%. The degree of extraction of soy proteins in the temperature interval from 8 to 50°C changes little (10-17%) and in the temperature interval from 55 to 75°C increases to 30%. Comparing the data on the degree of extraction of proteins of different origin on can note that animal proteins turned into solution in greater degree at low temperatures of extraction, while the solubility of soy proteins, on the contrary, increased with the increase of the temperature. Such patterns of relationships can be probably explained by denaturation of animal proteins which begins after 45°C and brings to decrease of their solubility, which corresponds to literature data that solubilization of soy proteins is maximum at 70-75°C.

Electrophoresis of the extracts has shown that for the proteins of animal origin the presence of a large number of fractions with molecular mass in the region 12-150kD is typical, and for plant proteins - in the region less than 12kDa. Extracts of soy proteins almost don't contain fractions with high molecular masses. When comparing the densitograms of the extracts obtained in different buffer systems it was found that when tris-buffer was used for the extraction a larger number of protein fractions was determined in the solution in comparison with the use of phosphate buffer. One can suppose that tris-buffer has a disaggregating property which substantially facilitates subsequent identification of proteins by the method of electrophoresis.

Thus, the results of electrophoresis of the extracts, obtained at low temperatures (8°C) allow to suppose the presence of the admixture proteins of animal origin in food products of plant origin. It is not possible to fix the presence of admixture plant proteins, therefore, to discover the admixture of soy proteins in meat products, such conditions of protein isolation are necessary under which plant proteins are extracted to maximum degree, and the degree of extraction of proteins of animal origin is minimum. The extraction temperature 75°C meets these conditions. With the use of such approach the analysis has shown that at the electrophoretograms of low-temperature extracts of proteins of muscle tissue of pork there were a large number of bands corresponding to protein fractions with molecular masses from 15 to 150 kD. On the contrary, on the phoretograms of pork extracts, obtained at 75°C , only three bands are visible: the pronounced binary bands in the region of molecular masses less than 12 kD and the band , corresponding to proteins with molecular masses 320-350 kD. A sharp decrease in the amount of soluble protein fractions can be explained by their denaturation which agrees with earlier obtained data about

decrease in the degree of extraction of proteins along with the increase of the temperature. In contrast to animal proteins during extraction of soy concentrate there was a reverse relationship. While on the phoretograms of the extracts obtained at 8°C one could distinguish only proteins with molecular mass less than 12 kD , as the temperature of extraction increased, bigger protein fractions began to solve. Thus, in the extract of the soy concentrate, obtained at 75°C, three main groups of proteins were well distinguishable: with molecular masses 15-3 kD, 50-55 kD and 65-75 kD, which can be considered as the characteristic ones for subsequent identification.

The evaluation of the contents of soy protein in meat semi-foods (ground pork added with soy), not subjected to thermal treatment,, can be determined by the formula: $X + C \cdot P /100$, where X – content of soy proteins in the meat product, %, C – content of soy proteins in the model ground meat, found according to electrophoretic calibration, %; P contents of total protein in the ground meat, determined by the method of Kjeldahl, %.

Calibration of the evaluation content of soy protein in the mixture with meat protein in the semi-foods with the use of characteristic bands on electrophoretograms, corresponding to the sum of bands taken in the region 65-75 kD can be taken from the following row:

Sum of the areas of peaks in the range of molecular masses 65-75 kD	25	50	75	100	150	200	250	300
Contents of soy proteins, C, %	10	17	20	30	40	60	70	90

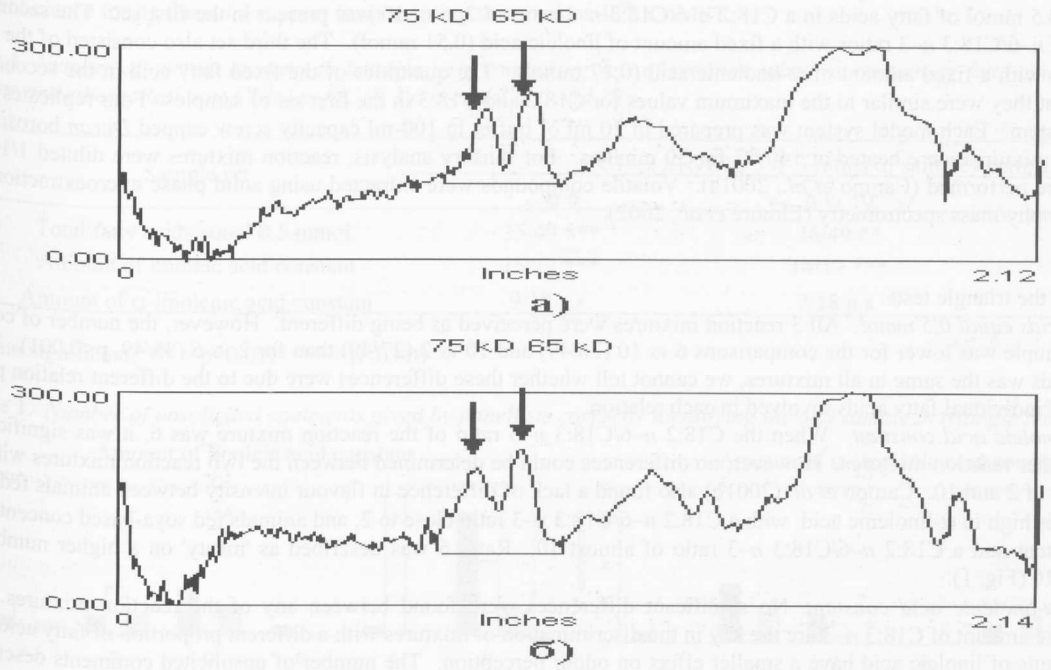

Fig. Densitograms of extracts of ground pork containing soy protein in the amount 30% а
50% .....б -

Conclusions

Thus, the use of tris-buffer solution with pH 9 with the extra amount of the extraction agent 1: 50. temperature 75°C and the time of extraction 0.5 hr allows with the help of electrophoresis in 10% polyacrylamide gel to evaluate the amount of plant and animal proteins in meat products having 10-75% of mass admixtures of soy proteins, using characteristic protein bands 15-30 kD, 50-55 kD, 65-75 kD.

Pertinent literature

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