Analytical studies of plant proteins based on grain mixtures

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Abstract. Based on the result analysis obtained by grinding the grain mixture on coarse systems
of the break process, a technological scheme of grinding was developed, it includes three break
systems, a scratch system, a grinding system and five reduction systems, which ensured a flour
yield of 70 - 75%. After developing the technology of the grinding process for wheat grain with
the aim to obtain an optimal flour yield of 70-75%, and the preparation of binary mixtures of
composite types of flour with specified technological properties, there was analyzed the samples'
quality in terms of molecular weight (MM) to confirm their nutritional value. The analysis was
carried out to determine the protein molecular mass of wheat grain, flax seeds and wheat flour
on the basis of the developed TSEC technique using polyethylene glycols (PEGs) as standards
and marker proteins to identify the analysis results. Detailed analytical studies of plant proteins
for obtaining qualitative and quantitative characteristics by the TSEC method are presented in
the paper. Analytical characteristics of the method have been studied, which makes it possible
to obtain reproducible results of a kind of qualitative analysis of biopolymers by quantitative
characteristics of molecular weights and quantitative analysis of proteins by optical
densitometry. The developed methods for determining the molecular mass of proteins are
characterized from the metrological point of view.

1. Introduction
Today food quality is considered as a factor of national security, therefore, the determination of the raw
material chemical composition, structure and supramolecular structure is an urgent task for the
commodity assessment of plant proteins in food production technologies. In the diet of the population
of our country, bakery products, most of which are products based on various types of flour and cereals,
occupy a significant share (up to 40%). In this regard, it seems very promising and in demand to form
flour composite mixtures at flourmills with a given content of basic nutrient and biologically active
substances: proteins, carbohydrates, fats, vitamins, micro- and macro-elements from grain processing
products, incl. wheat, triticale, rye, barley and chickpea. Such mixtures will become the basis for specific
manufacturers producing bakery, confectionery, pasta and extruded products, dairy and meat products of functional nutrition: dietary, prophylactic and therapeutic purposes [1].

Determination of the molecular weight distribution (MWD) of native polymers and oligomers is a decisive moment in the science of biological properties of cereals. A special place is occupied by the assessment of the content and complexation of proteins, since proteins are the most important factor in the nutritional value of food products.

Based on the use data obtained with the latest research methods for high-polymer compounds in grain science, the species and varietal specificity of the protein components of plant raw materials and their role in the process of processing into final food products are shown. The nutritional value of grain products depends on the composition and processing technology. According to the chemical composition and properties of the individual components, the grains of cereals of different tribes (tribes) differ from each other; the differences in protein substances are especially noticeable, the properties of which characterize representatives of individual tribes no less than their morphological characters [2].

The main analytical parameters of proteins in food are their molecular weight, amino acid composition, structure and quantitative ratio of various fractions. One of the most powerful methods for the isolation and determination of protein fractions is the method of Thin-layer Size Exclusion Chromatography (TSEC), which is currently the most effective method for studying proteins, since it differs in a number of necessary features: independence from the pH of the medium, temperature, preservation of the native composition, properties and pattern structures.

The purpose of this study is to prepare binary grain mixtures (wheat + flax) to obtain composite types of flour with given technological properties, as well as to develop a technique for analytical work of the TSHC method for analyzing plant proteins in terms of molecular weight (MW) and determining their qualitative characteristics. [3]

2. Materials and methods
Analysis of some physical properties, in particular, geometric dimensions of flax seeds and wheat grains, shows.

To determine the optimal values of the conditioning parameters, the method of contour-graphic analysis was used. The following optimization criteria were used:

- estimated output of premium flour.
- the maximum value of flour whiteness.
- the ratio of the bran yield of break systems to the bran yield of reduction systems, as a characteristic of grinding system efficiency.

The independent variables in the equation are grain moisture and dwell time shown in Table 1.

Based on the results of the study, it can be concluded that the introduction of flax seeds into the mixture does not have a significant effect on the choice of conditioning parameters. The technology for preparing the grain mixture "wheat + flax" is possible immediately before grinding, which is due to the separate preparation of the components and the self-sorting of the mixture while movement [4,5].

Preparation of a grain mixture for mixing and formation of a homogeneous composition during its processing, taking into account significant differences in physical properties of the components, is a rather difficult task. The stability of such a mixture depends on similarity of the component physical properties, first of all, the size and shape of the particles, which are fundamental characteristics of bulky material.

For this reason, experimental experiments were carried out to determine the necessary formation conditions for the binary grain mixture and then there were modeled the main mixing methods:

- active methods - with a high relative speed of component movement, which are based on a convective movement mechanism (paddle mixers),
- passive ones - based on the movement of layers’ sliding relative to each other (drum mixers).

The obtained quality of mixtures was assessed by the coefficient of heterogeneity (coefficient of variation).
\[ V = \frac{100}{\bar{c}} \sqrt{\frac{\sum (c_i - \bar{c})^2}{n-1}} \]  

(1)

where \( \bar{c} \) - arithmetic mean of the key component concentration,  
\( c_i \) - current concentration value,  
n – number of measurements.

The samples’ number and weight were determined in accordance with the recommendations [1] and amounted to: number - 8; weight by calculation -15g, actual weight - 50g.

Comparison of the methods of grain mass mixing showed that the passive method is significantly inferior to the active one; therefore, the active method of mixing was used, which ensured a satisfactory quality level of the product obtained.

3. Results

Analysis of the grinding process and croup formation. Grinding modes. At the first stage of the study, the approximate values of the modes of grain mixture grinding for the 1-3 break systems (I-III br.s.) were determined.

Based on the calibration charts, the values of the roll mill gaps have been established, which provide the extraction range from 25-70% for the I break system and 45-70% for the II break system. The roll gap varied from 0.75mm to 0.20mm on the I break system, from 0.2mm to 0.05mm on the II break system and from 0.05mm to 0.00 on the III break system.

The main part of the intermediate grinding products of the grain mixture lies in the particle size range from 600 to 150 \( \mu \)m, which later determined the set of sieves for the analysis of gross products, the formation of the milling technological scheme and the production of the final product.

Optimum modes were achieved with the following roll mill gaps: I break system - 0.40mm; II break system - 0.20mm; III break system -0.05mm.

Analysis of the grain mixture quality

After developing the technology of the grinding process for wheat grain with the aim to obtain an optimal flour yield of 70-75%, and the preparation of binary mixtures of composite types of flour with specified technological properties, there was analyzed the samples’ quality in terms of molecular weight (MM). The analysis was carried out to determine the protein molecular mass of wheat grain, flax seeds and wheat flour on the basis of the developed TSEC technique using polyethylene glycols (PEGs) as standards and marker proteins to identify the analysis results.

Molecular weight is one of the most important physicochemical constants of protein. It serves to identify each protein, to understand the processes in which its functional activity is manifested. The possibilities for determining the molecular characteristics of biopolymers are significantly expanded in the presence of a linear dependence of the retention volume (\( V_i \)) on the logarithm of the biopolymer MM (lgMi). Unlike nonlinear, this relationship allows considering the chromatogram as a mirror image of the molecular weight distribution (MWD) of the analyzed sample on a logarithmic scale. As a result, the processing of chromatographic data is simplified and accelerated and it becomes possible to calibrate the chromatographic system, which improves the interpretation of the chromatogram in MWD [6,7].

Glass plates with dimensions of 100x200 mm were used for the analysis. The test samples were applied in an amount of 1-5 \( \mu \)l (100-500 \( \mu \)g). The sorbents used are Sephadexes G - 50, G - 75, G - 100, G - 150, G - 200 of the "Superfine" brand. The obtained chromatograms were taken on chromatographic paper Filtrak FN PN 7, Filtrak FN PN 3 (Germany) as a paper replica. Elution systems used: distilled water (pH 7.0), weak saline solutions (8% NaCl); phosphate-buffered solutions (pH 8.5, 8.0, 6.86, 4.5). Optimization of the process was carried out using the angle of inclination (\( \beta \)) varied in the range of 12-18o. The quantitative assessment was carried out on a Den-Scan densitometer (LenChrom Company) and a Shimadzy CS-920 scanner. The criterion for choosing the optimal separation parameters was the process speed (U) and the slope of the calibration curve (\( \beta \)) in conjunction with the sorbent grade.

The study of the grinding process was carried out on a laboratory mill unit PCA with grooved rollers for break systems (P-10 l/cm) and rollers with a micro-rough surface for grinding.
Figure 1. General view of the process of molecular weight distribution (MWD) of biopolymers and the formation of chromatographic zones in TSEC.

Sample preparation of marker proteins was carried out by dissolving in distilled water (0.2 mg of protein in 0.05 ml of water) with the addition of a few drops of 0.1 HCl until a clear solution was obtained. Proteins with known molecular weights were used: chain B insulin (MW 6000), ribonuclease (MW 13 700), chymotrypsin (MW 25 000), egg albumin (MW 43 000), and bovine serum albumin (MW 68 000).

The results of experimental studies of standard proteins are presented in Figures 2 and 3. The molecular weights of the studied proteins were determined by the ratio dependence of the analyte path length on the molecular weight logarithm (logMM).

Graphs of the dependence of the path length of proteins on the logarithm of MW are shown in Figures 4 and 7.

Figure 2. Chromatogram of standard proteins on G-100Sf. Parameters: pH=8.0, β=15, t=150 min, 1- insulin, 2 - ribonuclease, 3 - chymotrypsin, 4 - ovalbumin, 5 - bovine serum albumin.

Figure 3. Chromatogram of standard proteins on G-200Sf. Parameters:

- pH=8, β=15, t=150 min, 1 - insulin, 2 - ribonuclease, 3 - ovalbumin, 4 - bovine serum albumin.
Whole proteins. It is known that dilute alkali solutions dissolve gluten, as well as extract gluten proteins directly from wheat meal and flour. The whole proteins of wheat grain, flax seeds and wheat flour were extracted with 0, 2% sodium hydroxide (NaOH) solution in 50% ethanol. As a result of extraction for an hour with a 0, 2% sodium hydroxide (NaOH) solution in 50% ethanol, it is possible to transfer 94-98% of all grain protein into a solution [8].

To determine the molecular mass of whole proteins of wheat grain, the obtained extract was subjected to separation on Sephadexes G-50 (Fig. 8), G-75, G-100, G-200. When analyzing the samples, eight fractions with a molecular weight of 6 300 to 84 000 were found when eluted with a phosphate buffer solution (pH 6,86). Elution time 1 hour 10 minutes Slope angle - 12°. Elution with distilled water for the same time yielded fractions with molecular weights ranging from 5 600 to 80 000.

Separation of proteins on Sephadex G-75 and elution in phosphate buffer solution (pH 4,5) yielded 6 fractions with molecular weights from 7 000 to 35 000. Elution time was 1 hour and 30 minutes. Elution with distilled water yielded 7 fractions with molecular weights ranging from 9 000 to 51 000. Elution time was 2 hours 10 minutes. Tilt angle 15°.
The study of total proteins showed: wheat grain on Sephadex G-100 included 10 fractions with \( MW \) from 5,500 to 81,000; flour - 9 fractions with \( MW \) from 5,200 to 75,000; flax seeds - 7 fractions with \( MW \) from 6,000 to 98,000 when eluted with phosphate buffered saline (pH 8.0). Elution time - 2 hours 30 minutes, tilt angle 18°. When eluted with distilled water, fractions with \( MW \) from 5,000 to 48,000 were obtained. Elution time was 2 hours 15 minutes. Tilt angle 15° [9,10].

Separation of wheat grain proteins on Sephadex G-200 obtained 8 fractions with a molecular weight of 10,500 to 76,000 in phosphate buffered saline (pH 6.86). The separation time was 5 hours at an angle of inclination of 18°, and under the same conditions of chromatographic separation in distilled water, 5 fractions with molecular weights from 5,500 to 57,000 were obtained.

Figure 7. Dependence of the relative path length on the logarithm of the MM of whole proteins of wheat grain, flax seeds and flour on G-100, \( \beta = 180, \text{pH } 8.0 \).

◊ - whole proteins of wheat grain (10 fractions)
♦ - whole proteins of flour (9 fractions)
☆ - whole proteins of flax seeds (7 fractions)

Fractional proteins. There was carried out extraction of fractional proteins of wheat grain and wheat flour (2 g of a dry sample) according to the modified Osborne scheme: albumins were extracted with distilled water (6 ml), globulins - 0.5 M NaCl solution (3.5 ml), prolamins - 70% ethanol solution (4.0 ml), glutelins - 0.1 M acetic acid solution (4.0 ml) or 0.2% sodium hydroxide solution (3.5 ml). The resulting solution was purified from starch by centrifugation (5000 rpm), the resulting centrifuge served as a material for research [11,12].

Analysis of fractional proteins of wheat grain and flour of the premium grade showed the following results. On Sephadex G-50, there were obtained 4 albumin fractions, their molecular weights range from 8,000 to 21,500; 6 fractions of globulins, with a molecular weight from 10,000 to 32,000; 6 fractions of prolamins (gliadins), with molecular weights from 19,800 to 78,000; 4 fractions of glutelins (glutenins), with molecular weights from 40,000 to 100,000. Elution was carried out in phosphate buffered saline (pH 4.5). The separation time was 1 hour 15 minutes at an angle of inclination of 12° [13].

On Sephadex G-150, under the same conditions for protein extraction from flour, the results were: 5 fractions of albumin with a molecular weight from 6,200 to 26,000; 5 fractions of globulins with a molecular weight from 7,000 to 28,500; 6 fractions of gliadins with molecular weights from 18,500 to 56,000 and 4 fractions of glutenins with molecular weights from 40,000 to 100,000. Duration of separation is 2 hours 30 minutes. Tilt angle 15°.

Chromatographic analysis of wheat flour proteins of different solubility was carried out on Sephadex G-50 and G-100 in phosphate buffer solution (pH 6.86). Sephadex G - 50 yielded 4 albumin fractions with molecular weights ranging from 9,000 to 21,500; 5 fractions of globulins with molecular weight from 10,000 to 31,000; 7 fractions of gliadins with a molecular weight of 18,000 to 76,000; 4 fractions
of glutelins with a molecular weight from 40 000 to 100 000. The separation time was 1 hour 15 minutes, tilt angle 15°.

Figure 8. Chromatogram of fractional proteins of wheat flour of the premium grade for G-150. pH 6.86, \( \beta = 12^\circ \), \( t = 65 \text{ min} \). Option 3.

Discussion of a large amount of material on the analytical study of whole and fractional proteins of wheat grain, wheat flour and flax seeds is based on the main conclusion that all the studied samples have the same character in terms of fractional composition - in a qualitative sense. The differences lie in the quantitative ratio of fractions.

In whole proteins, there were found from 8 to 10 fractions, and when using differentiating solvents, up to 35 fractions were extracted. Thus, it is obvious that the TSEC method can replace a set of 2-3 other known methods of protein analysis and give a complete qualitative description of the samples under study.

The results of the quantitative analysis presented in Table 2 showed that there is a noticeable similarity in the assessment of the proportions of high molecular weight proteins in wheat and wheat flour and a noticeable difference in the proportion of these fractions in flax seeds (about 10%).

Table 1. Quantitative distribution of protein fractions of wheat, wheat flour and flax seeds.

| №  | Molecular weight | Wheat flour, % | Wheat, % | Flax seeds, % |
|----|-----------------|----------------|----------|---------------|
| 1  | 6 000 – 15 000  | 5.9            | 6.8      | 6.7           |
| 2  | 16 000 – 30 500 | 7.5            | 9.6      | 11.2          |
| 3  | 31 000 – 40 500 | 9.2            | 11.9     | 15.6          |
| 4  | 41 000 – 58 000 | 15.6           | 16.7     | 18.9          |
| 5  | 59 000 – 78 000 | 25.0           | 22.7     | 20.8          |
| 6  | 80 000 – 100 000| 36.8           | 32.5     | 25.8          |

The estimation of measurement errors was carried out on the basis of the standard deviation (for each series of measurements). The absolute error was determined taking into account the student’s coefficient at the selected level of confidence \( P = 0.95 \).

Table 3 presents the results of statistical processing of wheat grain data. Polyethylene glycols (PEGs) were used as a test material. The molecular weights of PEGs are determined by their synthesis conditions and are reference characteristics. Statistical processing of the experimental results was carried out in relation to direct experimental data in primary units of chromatographic path lengths. The data of the qualitative analysis (by the molecular mass of grain and flour proteins) were processed according to the materials of the final estimates based on the calibration curves and are expressed in units of molecular weight.
Summarizing the results of statistical processing, it can be stated that the relative error is maximum for relatively low molecular weight components, where it can reach 20% (for soluble proteins such as albumin and globulins) and below 10% for high molecular weight fractions (proteins such as prolamins and glutelins). Quantitative analysis, the error of which is no longer due to the chromatographic procedure itself, but precisely to the method of determination, is much more accurate and gives an error in the range of 2 - 5%.

| №  | Average molecular weight | Standard deviation S | Relative error Sr, % | Confidence interval + -Δ |
|----|--------------------------|----------------------|---------------------|--------------------------|
| 1  | 8 500                    | 500                  | 5.8                 | 620                      |
| 2  | 13 500                   | 400                  | 2.9                 | 490                      |
| 3  | 16 900                   | 400                  | 2.3                 | 490                      |
| 4  | 24 000                   | 750                  | 3.1                 | 930                      |
| 5  | 32 000                   | 750                  | 2.3                 | 930                      |
| 6  | 45 600                   | 690                  | 1.5                 | 850                      |
| 7  | 56 200                   | 520                  | 1.0                 | 640                      |
| 8  | 64 900                   | 840                  | 1.3                 | 1040                     |
| 9  | 81 480                   | 800                  | 1.0                 | 900                      |
| 10 | 98 700                   | 1300                 | 1.3                 | 1600                     |

4. Conclusion
The study allowed us to reveal the regularities of the preparation and grinding of binary grain mixtures and then there were modeled the main mixing methods to obtain composite types of flour with given technological properties and increased nutritional value. Based on the analysis of the results of grinding the grain mixture by coarse systems of the break process, a technological scheme of grinding was developed, which ensured the flour yield of 70 - 75%.

Detailed analytical studies of plant proteins for obtaining qualitative and quantitative characteristics by the TSEC method are presented in the paper. Analytical characteristics of the method have been studied, which makes it possible to obtain reproducible results of a kind of qualitative analysis of biopolymers by quantitative characteristics of molecular weights and quantitative analysis of proteins by optical densitometry. The developed methods for determining the molecular mass of proteins are characterized from the metrological point of view.

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