Biochemical characteristics of new varieties of flour from a binary mixture of wheat and flax

I S Vitol, G N Pankratov and E P Meleshkina

All-Russian Scientific and Research Institute for Grain and Products of its Processing – Branch of V.M. Gorbakov Federal Research Center for Food Systems of RAS, 11, Dmitrovskoye Sh., Moscow, 127434, Russia

E-mail: vitolis@yandex.ru

Abstract. Characteristics of the distribution of proteins, lipids, carbohydrates and basic enzyme systems (amylase, protease, lipase) in the formed varieties of wheat-flax flour obtained from a binary mixture consisting of 93% of wheat and 7% of flax seeds were revealed. It was indicated that the total lipids content in flour from two-component mixtures increases about 4 times, and the total protein content in the studied samples increases by 1-2%. An increase of the ratio of the albumin-globulin fraction content in wheat-flax flour samples to alcohol and alkali-soluble proteins content, as well as to its content in wheat flour, in which the part of gluten proteins prevails, was registered. The activity of proteases and amylases in the formed samples of wheat-flax flour changes, but not significantly, and the activity of alkaline lipases (cereal lipases) remains unchanged, while the activity of acid lipases (oilseed lipases) is approximately 1.5 times higher than the activity of alkaline lipases in the studied samples of wheat-flax flour. The content of linoleic acid (ɷ-6) in wheat-flax flour samples is 1.6...3.3 times higher than in wheat flour; the content of linolenic acid (ɷ-3) in wheat-flax flour samples is 36.8...57.2 times higher than in wheat flour (taking into account the total lipids content in the samples). The enrichment of wheat flour due to the entire phytopotential of flax seeds allows to make up the deficiency of PUFA family in the diet of a modern person and to obtain products on a grain basis of a balanced composition.

1. Introduction
One of the ways to obtain balanced grain-based food products is the creation of multicomponent grain mixtures, the composition of which can be corrected in order to obtain specialized food products, including medical and preventive ones [1,2,3,4,5,6].

The inclusion of flax seeds into the mixture allows one to significantly enrich and balance the composition of the obtained cereal products, and to correct the fatty acid composition of polyunsaturated fatty acids (PUFAs) of the ω-3 and ω-6 family [3,6,7,8,9,10].

Flax seeds are a valuable food component, characterized not only a high protein and lipids content, but also a unique composition of these macronutrients, primarily polyunsaturated fatty acids. However, the high nutritional and biological value of the protein complex of flax seeds, which is characterized by a high albumin-globulin fraction content and by the presence of essential amino acids, also should not be forgotten.
The use of whole flax seed in the composition of multicomponent grain mixtures is justified by the fact that in this case the entire phytopotential of flax seeds is used, since it is known that the main source of PUFA and protein is the embryo and endosperm of flax seed and the main source of dietary fiber and lignans is the episperm [5,6,8,11].

The purpose of the study is a biochemical assessment of the newly formed varieties of wheat-flax flour obtained by the joint milling of the wheat-flax mixture.

2. Object of study

The object of the study was wheat-flax flour, obtained as a result of laboratory milling of a two-component grain mixture of wheat (93%) and flax seeds (7%) using the RSA-5 reduction and sorting unit, the laboratory plansifter and the laboratory bran finisher.

The principle of the formation of flour varieties is based on the belonging of the separated flows of flour, obtained from various technological systems, to different anatomical parts of the grain. Thus, the flows of flour from the central part of the endosperm from the first break system, the second break system, the sizing system, the first reduction system, the second reduction system, the third reduction system, are characterized by low ash content and high whiteness index. The A variety of flour was formed by mixing these streams. The B variety of flour was obtained by mixing flour streams from the third break system, the scratch system, the fourth reduction system and the fifth reduction system and represents crushed peripheral parts of the grain. The C variety of flour was obtained as a result of combining all flows of flour.

3. Research methods

The total protein content was determined by the Kjeldahl method (N × 6.25); fat according to Soxhlet; cellulose according to Kuschner and Hanek; starch according to Evers; reducing sugars according to the Bertrand method; soluble protein - according to the Lowry method. Determination of the fractional composition of proteins according to Osborne: albumins were isolated using distilled water, globulins - using a 10% NaCl solution, prolamines - using 70% ethanol, and glutelins - using a 0.2% NaOH solution. Enzymatic activity of proteases was determined by the modified Anson method using bovine serum albumin as a standard substrate; amylase activity – by the colorimetric method according to the amount of starch hydrolyzed based on an assessment of the color intensity of the starch-iodine complex; the activity of alkaline and acid lipases was determined by the titrometric method by the amount of fatty acids formed [12]. Fatty acid composition – by gas chromatography (gas chromatograph 6890N with mass-selective detector Agilent 5975C, USA).

The analyses were performed in the samples of wheat-flax flour, presenting the results as average arithmetic ones. The discrepancy between parallel assays did not exceed 3% of the average arithmetic value with the confidence probability P=0.95.

4. Discussion of the results

The chemical composition of the formed flour varieties A, B, C, presented in table 1, indicates the enrichment of wheat flour with protein and fat components, as well as fiber due to the inclusion of flax seeds in the binary grain mixture.

An analysis of the chemical composition of the formed flour varieties indicates an increase in the mass fraction of protein mass fraction by 1.0-2.0%, fat mass fraction 1.5-3.5 times; fiber mass fraction - 3.4-4.0 times and a decrease in the mass fraction of starch by about 2-4%.

The fractional composition of soluble proteins, the ratio of different fractions is important both for evaluating technological properties (gluten formation, its quantity and quality), and for the biological value of proteins, their assimilation degree [5,8,12,13]. The data presented in table 2 demonstrate the ratio of soluble proteins fractions in the formed varieties of wheat-flax flour.
Table 1. The chemical composition of the formed varieties of flour

| Sample                        | Protein (N × 6.25), % | Lipids, % | Starch, % | Cellulose, % | Reducing sugar, % |
|-------------------------------|-----------------------|-----------|-----------|--------------|------------------|
| Wheat-flax flour, A variety   | 13.16                 | 3.6       | 69.52     | 1.60         | 0.16             |
| Wheat-flax flour, B variety   | 14.38                 | 5.6       | 64.85     | 1.92         | 0.18             |
| Wheat-flax flour, C variety   | 13.58                 | 4.3       | 68.11     | 1.86         | 0.16             |
| Wheat flour top grade         | 12.65                 | 1.6       | 72.10     | 0.46         | 0.14             |

The significant increase of the ratio of the albumin-globulin fraction content in wheat-flax flour samples to alcohol and alkali-soluble proteins content, as well as to its content in wheat flour, in which the part of gluten proteins prevails, should be marked.

When grain is processed into flour, the cell structure is destroyed, and as a result, oxidative and hydrolytic processes are enhanced [13]. In this regard, it is of interest to evaluate the activity of the main hydrolytic enzymes in samples of the formed varieties of wheat-flax flour. Thus, the value of proteolytic activity, along with other biochemical parameters, has fundamental importance, as proteinases are able to actively hydrolyze their own proteins, including the gluten ones, which, ultimately, affects the technological process and the finished product. In addition, proteolytic enzymes are involved in the regulation of the activity of other enzyme systems, for example, of amylases.

Amylases also assume major significance in assessing the quality of flour and products made from it. High amylolytic activity negatively affects its baking advantages.

In wheat flour, the substrate for the action of lipases is the flour’s own lipids, the content of which can reach up to 1.5-2% of its mass, and in the studied samples of wheat-flax flour up to 3.6–5.6%. It is known that the use of lipase specimen leads to an improvement of the rheological properties of the dough, an increase of the specific volume of products, and an improvement of the crumb structure and color [2,5]. There is also evidence that lipases contribute to the retardation of the bread crumb, which can be explained by the action of hydrolysis products - monoglycerides and fatty acids, which, forming complexes with amylose, slow down its retrograde. It is supposed that lipases modify the interactions between proteins and lipids of flour, improving the gluten quality [16].

Table 2. The fractional composition of soluble proteins of the formed varieties of flour from a grain mixture based on wheat grain and flax seeds

| Sample                        | albumins | globulins | prolamins | glutelins | insoluble part |
|-------------------------------|----------|-----------|-----------|-----------|----------------|
| Wheat-flax flour, A variety   | 15.8     | 18.8      | 30.8      | 28.6      | 6.0            |
| Wheat-flax flour, B variety   | 13.2     | 18.5      | 29.6      | 29.8      | 7.8            |
| Wheat-flax flour, C variety   | 14.8     | 20.2      | 28.8      | 30.2      | 6.0            |
| Wheat flour top grade         | 8.4      | 17.0      | 35.8      | 30.8      | 8.0            |
Moreover, lipolytic enzymes indirectly affect the oxidation processes in the dough during kneading, which is due to an increase of the availability of unsaturated fatty acids for the action of the lipoxygenase enzyme that is present in flour or introduced into the dough as part of improving agents. Plant lipases are characterized by an optimum pH: cereal lipases mainly show their activity in the alkaline region - pH 8.0; oilseed lipases - in the acid region - pH 4.7 [17].

The activity of the main hydrolytic enzymes in the samples of the formed varieties of flour from a grain mixture based on wheat and flax seeds is presented in table 3.

**Table 3. The activity of the main hydrolytic enzymes in the formed varieties of flour from a grain mixture based on wheat and flax seeds**

| Sample                        | UA\(^a\) protease, units / mg protein | UA amylase units / mg protein | UA lipases, units / g |
|-------------------------------|--------------------------------------|-----------------------------|----------------------|
|                               | neutral     | acid     | neutral     | acid     | neutral     | acid     | neutral     | acid     |
| Wheat-flax flour grade A      | 0.110       | 0.080    | 0.45        | 3.8      | 5.2         |
| Wheat-flax flour grade B      | 0.120       | 0.090    | 0.60        | 3.8      | 6.0         |
| Wheat-flax flour grade C      | 0.110       | 0.080    | 0.55        | 3.8      | 5.6         |
| Wheat flour top grade (control) | 0.100       | 0.070    | 0.50        | 3.8      | 0           |

\(^a\) UA – unit activity

**Table 4. The fatty acid composition of the formed varieties of flour from a two-component grain mixture consisting of 93% of wheat grain and 7% of flax seeds**

| Indicator                  | The content of high fatty acids, % |
|----------------------------|------------------------------------|
|                            | wheat flour, top grade | wheat-flax flour, grade A | wheat-flax flour, grade B | wheat-flax, flour grade C |
| C14:0 myristic             | < 0.1                     | < 0.1                      | < 0.1                      | < 0.1                      |
| C16:0 palmitic             | 19.64 ± 1.57              | 18.79 ± 7.50               | 12.54 ± 1.00               | 15.44 ± 1.24               |
| C16:1 palmitoleic          | < 0.1                     | < 0.1                      | < 0.1                      | < 0.1                      |
| C17:0 margarine            | < 0.1                     | < 0.1                      | < 0.1                      | < 0.1                      |
| C17:1 margaroleic          | < 0.1                     | < 0.1                      | < 0.1                      | < 0.1                      |
| C18:0 stearic              | 1.21 ± 0.13               | 5.79 ± 0.46                | 4.81 ± 0.53                | 5.26 ± 0.42                |
| C18:1 oleic                | 17.54 ± 1.40              | 28.50 ± 1.43               | 22.54 ± 1.8                | 25.15 ± 0.02               |
| C18:2 linoleic             | 57.95 ± 2.90              | 41.21 ± 3.06               | 55.57 ± 2.78               | 49.97 ± 2.46               |
| C18:3 linolenic            | 2.95 ± 0.32               | 48.80 ± 0.54               | 39.23 ± 0.43               | 45.10 ± 0.45               |
| C20:0 arachin              | < 0.1                     | 0.25 ± 0.03                | 0.17 ± 0.02                | < 0.1                      |
| C20:1 gondoin              | 0.73 ± 0.08               | 0.58 ± 0.06                | 0.39 ± 0.04                | 0.31 ± 0.03                |
| C20:2 eicosodienoic        | < 0.1                     | < 0.1                      | < 0.1                      | < 0.1                      |
| C22:0 behennaya            | < 0.1                     | 0.29 ± 0.03                | 0.15 ± 0.02                | 0.15 ± 0.02                |
| C22:1 erucia               | < 0.1                     | < 0.1                      | < 0.1                      | < 0.1                      |
| C22:2 docosodienic         | < 0.1                     | < 0.1                      | < 0.1                      | < 0.1                      |

The activity of proteases and amylases in the studied samples of wheat-flax flour changes, but not significantly, and the activity of alkaline lipases (cereal lipases) remains unchanged, while the activity of acid lipases (oilseed lipases) is approximately 1.5 times higher than the activity of alkaline lipases in the studied samples of wheat-flax flour. As noted above, it occurs due to the presence of flax seed...
milling products and may affect the shelf life of this type of flour. However, the test samples storage in the laboratory at 4-6 °C for 14 weeks led to an insignificant increase of the acid lipases activity by 1.8-2.5%.

The fatty acid composition data (table 4) of the formed flour varieties from a two-component grain mixture consisting of 93% of wheat and 7% of flax seeds allows us to draw the following conclusion: the content of linoleic acid (ω-6) in the wheat flour sample is 1.6...3.3 times less than in the wheat-flax flour samples (0.93% against 1.51...3.14%, taking into account the total lipids content in the samples); the content of linolenic acid (ω-3) in the wheat flour sample is 36.8...57.2 times less than in the wheat-flax flour samples (0.047% against 1.73...2.69%, taking into account the total lipids content in the samples).

5. Conclusion
The use of whole flax seed in a binary grain mixture, consisting of 93% of wheat and 7% of flax seeds, allowed one to balance the chemical composition of composite wheat-flax flour by the protein and lipids components, and also to enrich it with fiber, which means to use the entire phytopotential of flax seeds. Primarily, as the studies showed, the obtained wheat-flax flour contains the sufficient amount of PUFA in accordance with the recommended standards for consumption of grain-based food products [18], and the products made from it will help to make up the deficiency of ω-3 family PUFA in the diet of a modern person.

References
[1] Tsyganova T B, Minevich I E, Zubtsov V A and Osipova L L 2010 Nutritional value of flax seeds and promising areas for their processing (Kaluga: Eidos)
[2] Kolomnikova Y P, Derkanosova A A, Manukovskaya M V and Litvinova E V 2015 Effect of non-traditional vegetable raw materials on the properties and biotechnological structure pastry. Proceedings of the Voronezh State University of Engineering Technologies 3 157-160 https://doi.org/10.20914/2310-1202-2015-3-157-160
[3] Koneva S I 2016 Features of the use of flax seed processing products in the production of bakery products Polzunovsky bulletin 3 35-37
[4] Sigareva M A, Mogilny M P and Shaltumaev T Sh 2015 Use of flax seed processing products for the production of products of increased nutritional value News of higher educational institutions. Food Technology 5-6 42-45
[5] Rabetafika H N, Van Remoortel V, Danthine S, Paquot M and Blecker C 2011 Flaxseed proteins: food uses and health benefits International Journal of Food Science and Technology 46 221-228 http://dx.doi.org/10.1111/j.1365-2621.2010.02477.x
[6] Gutte K B, Sahoo A K and Ranveer R C 2015 Bioactive components of flaxseed and its health benefits International Journal of Pharmaceutical Sciences Review and Research 31(1) 42-51
[7] Goyal, Sharma V, Upadhyay N, Gill S and Sigag M 2014 Flax and flaxseed oil: an ancient medicine & modern functional food Journal Food Science Technology 51(9) 1633–1653 https://doi.org/10.1007/s13197-013-1247-9
[8] Zaitseva L V and Nechaev A P 2014 Balance of polyunsaturated fatty acids in food Food industry 11 56-59
[9] Pankratov G N, Meleshkina E P, Vitol I S, Kandrokov R X and Zhitsova N S 2018 Features of the processing products of two-component mixtures of wheat and flax Bread products 12 42-46 https://doi.org/10.32462-0235-2508-2018-0-12-42-46
[10] Machikhina L I, Meleshkina E P, Priezzevha L G, Smirnov S O, Zhuchenko A A, Rozhmina T A 2012 Creation of a technology for the production of new food products from flax seeds Bread products 6 54-58
[11] Bakumenko O E and Shatnyuk L N 2017 Technological aspects of the use of flax flour in functional food concentrates Bread products 6 56-59
[12] Nechaev A P, Traubenberg S E, Kochetkova A A, Kolpakova V V, Vitol I S and Kobeleva I B 2006 Food Chemistry. Laboratory workshop (St. Petersburg: GIORD)

[13] Kuhn K R, Netto F M and Cunha R L D 2014 Assessing the potential of flaxseed protein as an emulsifier combined with whey protein isolate Food Research International 58 89–97

[14] Tyurina I A, Nevskaya E V, Tyurina O I and Borisova A E 2019 Development of a high protein baking composite mixture for fortified bakery products Bread products 9 53-55 https://doi.org/10.32462/0235-2508-2019-31-9-53-55

[15] Gridina S B, Zinkevich E P, Vladimertseva T A and Zabusova K A 2014 Enzymatic activity of grain crops Proceedings of the Krasnodar State Agrarian University 8 57-60

[16] Dubtsova G N, Nechaev A P and Molchanov M I Vegetable protein: new perspectives 2000 (Moscow: Pishchepromizdat)

[17] Demchenko Yu A 2018 Lipase: properties, sources, production methods, application Science: complex problems, available at: http://www.nigniikp.adygnet.ru

[18] Guidelines MP 2.3.1.2432–08 2009 Norms of physiological requirements for energy and nutrients for various population groups of the Russian Federation (Moscow: Federal Center for Hygiene and Epidemiology of Rospotrebnadzor)