ADJUSTING FLOUR QUALITY BY ENZYMES: CURRENT STATE, PROBLEM ANALYSIS, FUTURE DEVELOPMENT PROSPECTS

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Abstract. The article overviewes the issue of wheat flour modification by enzymes. The role of enzymes in the dough formation process is considered. Modern ways of providing the desired dough parameters for flour products in conditions of Ukraine are shown. Recommendations and suggested directions for further research are given. Flour is a complex multicomponent product and have to correspond with a number of requirements for its composition and properties. Different conditions of grain cultivation and storage result in significant deviations of its quality indicators when it comes to flour mills. The modification of flour going through adding several technological additives, in particular by enzyme products. The action of enzymes to a large extent allows to adjust the properties of the dough and of flour end-products. In addition, enzymes further affect the nutritional values of flour, which makes it possible for the flour production to use low-quality grain, while maintaining the planned quality indicators of flour. The functional properties of flour fractions obtained on different technological passages depend on the content of various anatomical parts of the grain from which they derived from. Particle size, starch damage, protein content, fat content and intensity of enzyme activity vary significantly depending on the type of grinding equipment. All this gives reason for recommending the introduction of enzymes not while manufacturing bakery end-products but still at the stage of flour production. The damage to the grain with a corn bug, grain germination in Ukraine puts grain-processing plants the task of assessing the activity of own grain enzyme systems. Indirectly, this can be estimated using the gluten deformation index and the grain Falling Number. But the estimation of enzyme systems by such methods does not allow precisely to calculate the amount and composition of enzyme products necessary to achieve maximum effect when adjusting flour properties. The issue of removing anti-nutrient factors in flour, which is largely inhibitors of the action of both their own grain enzyme systems and additionally introduced enzyme preparations, is also relevant.

Key words: flour, enzymes, functional products.
Introduction. Formulation of the problem

The dynamics of human development is directly related to its diet. Any technological or social breakthroughs depended directly on what a particular social group was eating. Indicative in this regard is the use of food made of grain processing products. The first successful attempts to domesticate wild wheat, barley, peas, nut, lentils etc. dated about 12000 BC in Fertile Crescent (Arabia), and findings of the first grain processing areas and objects dated about 8000 BC in Jericho. There were specially prepared areas for grain peeling and rubbing in the primitive mortars. Later, there was found the way of grain rubbing in flour using several mills. Due to the ease of implementation, this method can still be found today in a number of countries as part of their cultural legacy.

With the development of science and knowledge of grain naturally there was a need to use a more complicated approach to grain processing technology. That allowed to differentiate significantly the products obtained from the processing of grain, basing on their nutritional and technological properties. Later, the combination of such products made it possible for moving to complex formulations that were balanced over number of indicators.

Wheat is the main grain crop for the production of bread products in Ukraine. Grown in different climatic conditions, on different soils, wheat varieties differ significantly from each other by a number of technological properties [1]. Such diversity significantly affects the structure of the of wheat grain processing technological cycle – a set of preparation modes, the grinding of grain, and also determines the quality and output of end products [2].

Classic wheat grinding is a mechanical gradual reduction process, during which the endosperm is separated from the bran and embryos. During the production process of high-quality flour, a significant amount of nutrients is eliminated [1,3]. Because of this, the quality and quantity of streams in the production of flour significantly differ both in technological parameters and in the set of nutritional components [4]. The combination of different flour streams leads to the appearance of different types of flour for a variety of end-use products.

Despite the efficiency of combining technological flows and providing indicators of finished flour, observance of effective rheological parameters of the dough in some cases requires the introduction of additional technological additives. The feasibility of using them is substantiated, mainly, by the influence on the rheology of the dough and finished products [5]. However, we cannot ignore the effect of some technological additives, in particular, enzymes systems, on the anti-nutritional factors. This results to an increase of the bioavailability of a number of nutrients.

The study of enzyme systems of flour in the process of making flour products devoted to numerous publications by both home and foreign authors. Significant changes in the technologies of enzyme synthesis over the last 15 years have led to a significant differentiation of conditions and mechanisms for their action, as well as the emergence of a huge number of highly specialized products.

This makes the purpose of this work as systematization and certain generalizations of modern scientific perspectives and practical approaches to the adjusting of flour properties with the enzyme products.

As the priority objectives, it will be expedient to allocate a number of existing problems typical for flour production in Ukraine, as well as to highlight the main factors affecting the bread-making properties of flour.

Analysis of recent research and publications

Relevance of research. Monitoring studies of wheat grain quality show a strong tendency for its decrease, and as a result – the production of flour with unsatisfactory baking properties [6-11]. In this regard, the flour mills face the problem to bring the bread-making properties of the flour to the standard level. Therefore, the technological additives implementation for flour bettering at ukrainian mills is relevant [5,12,13].

The expediency of using micro-ingredients is substantiated by the following aspects:

- difference of wheat varieties and types;
- agrotechnical and climatic conditions of grain growing [1,14];
- low quality (weak or strong) of gluten or its insufficient amount;
- decrease or increase of grain enzymes autolytic activity;
- the complexity of adjusting the bread-making properties of flour at bakery enterprises;
- unpopularity of chemical ingredients usage;
- the need for purpose flour depending on the range of produced products;
- economic feasibility by using low quality flour with improved properties and therefore reducing cost of end product.

Usage of micro-ingredients allows us to solve the following problems for flour mills:

- adjustment and stabilization of baking properties of flour;
- formation of flour properties depending on its intended purpose;
- lowering cost and making products more competitive [5].
The concept of adjusting the flour properties at flour mills depends on the following factors:
- original quality of wheat and rye flour (depending on regional, varietal, climatic and other conditions);
- the list of food additives and bakery improvers permitted for usage by law;
- special level of the analytical equipment in laboratories of flour mills;
- methods of dough making and finish products assortment at bakery enterprises;
- the traditions of using bakery improvers in bakery enterprises.

Special additives using is an effective direction to adjust the properties of wheat and rye purpose flour and to increase its range [4]. This led to the expediency of their adding at the final stages of flour production to adjust needed baking properties for customers.

**Components of flour that determine its baking properties**

Wheat flour is a complex multicomponent system that includes: protein-protease complex, carbohydrate-amylose complex and lipid complex, as well as various mineral components.

The above-mentioned complexes include, respectively, the protein, carbohydrate, lipid components, as well as activators or inhibitors of their lysis. In fact, technological properties of flour are determined by its composition [15].

The **protein-protease complex** of flour covers protein and proteolytic enzymes. Distribution of proteins in the structure of grain is irregular. Surface layers and aleurone layer is depleted by proteins, but contain high amounts of fiber and mineral substances. The endosperm and the embryo contain an increased amount of proteins and fats; in the endosperm the maximum amount of starch is concentrated. The aleurone layer and the germ mostly contain globulins and albumin, and the maximum number gliadins and glutelins are observed in the endosperm [16].

Some proteins (gliadins and gliadins) are combined by disulfide bonds into a complex spatial structure that is the basis of a gluten frame [17]. In fact, the technological properties of the flour are not determined by the whole protein, but only by proteins of the gluten frame [18,19]. Therefore, in order to improve the quality of flour it is necessary to apply technological additives which eliminate the main cause of low baking properties of flour (low gluten content in combination with its indicators of quality).

The rheological properties of flour dough (elasticity, resilience, extensibility) are also largely influenced by the amount of disulfide and hydrogen bonds: the more links occur between protein molecules, the higher is the elasticity of gluten and the lower its extensibility. Weak gluten has less disulfide and hydrogen bonds than strong [20]. The quality of gluten directly depends on the activity of proteolytic enzymes. The activity of grain enzymes depends on various factors, and for Ukrainian wheat the greatest effect actually is the grain damage by a corn bug (lat. Eurygaster integriceps).

The **carbohydrate-amylose complex** of flour cover carbohydrates (starch, pentosans, sugars) and amylolytic enzymes (amylose). Starch is the main chemical substance of grain, its content varies from 65% to 70%, depending on the several characteristics of wheat, climatic conditions of its ripening and grain size. The starch is structured in starch grains. It is a chemical compound consisting of two polysaccharides: 15–30% amylose, forming the inner part of starch grains, and 70–85% of amylpectin, which forms its outer part. Both components are heterogeneous, their molecular weight varies widely, depending on the origin of starch [21]. The structure of starch grains is very diverse and depends on the type of grain, the conditions of growth [22] and also the type of wheat. At the end of the 20th century the breeders received waxy wheat, whose starch consists only mostly from amylpectin [23].

Amylose differs from amylpectin with less molecular weight and simpler structure of the molecule. During heating of starch in water amylose passes into colloidal solution, and amylpectin swells, forming a paste. Complete pasteurization of flour starch, in which its grain loses shape, is carried out at a mass ratio of starch and water 1:10. When undergoing pasteurization, starch grains increase significantly in volume, become loose and more vulnerable enzymes action. The temperature and origin of the pasteurization process depend on the starch origin, the pH of the medium, damage rate of starch grains when grinding, etc. [24,25]. The starch is characterized by a significant adsorption capacity, which can cause a large amount of water to bind even at a temperature of 30 °C, which corresponds with the temperature of dough making.

Enzymatic hydrolysis of starch is carried out with amylase presence. There are three types of amylase: α-amylase, β-amylase and glucoamylase. Each of these types has its own specific mechanisms of action. With increased amylolytic activity of the hydrocarbon-amylose complex, flour properties change due to the large amount of accumulated dextrin and free, unbound moisture starch [26,27].

The carbohydrate complex of wheat flour also includes non-starch polysaccharides – pentosans. Researchers distinguish further insoluble pentosans in water – hemicelluloses, and water-soluble pentosans – mucus [28].

The **lipid complex** of flour covers simple and complex lipids, fat-soluble pigments, sterols, vitamins, lipolytic enzymes, etc. [28,29].

Significant influence on the technological properties of wheat flour is enzymatic hydrolysis of lipids, carried out using lipase enzymes and lipoxgenase. The result of hydrolysis is the formation of hydroperoxides that cause oxidation of disulfide.
bonds of protease, glutathione and cysteine residues in the protein polypeptide chain. As a result, structure of the proteins became stronger and denser, decreasing their vulnerability to proteases, and increases water absorption of flour [30,31]. Along with the positive role of the lipid complex of flour, a significant increase in its activity is undesirable, since during storage, it can lead to oxidative damage to finished products containing a large amount of fat in the formulation.

**Technological additives that is being used in the flour and baking**

Depending on the functional purpose and technological properties, various food additives and bakery enhancers are used [21,32]. The variety of food additives and baking improvers determined by a different set of functions they perform. They are classified in the following groups:

- Oxidizing and reducing agents;
- Modified starches;
- Enzyme preparations;
- Surfactants (emulsifiers);
- Organic acids;
- Mineral salts;
- Preservatives;
- Aromatic and flavoring additives.

Oxidizing and reducing agents allow to correct the rheological properties of the dough and the intensity of biochemical and colloidal processes in the dough. Such additives strengthen the gluten, increase the gas-retaining ability of the dough, thereby increasing the dough ability to be processed, reduce the indistinctness of hearth bread products [33-35].

Modified starches (oxidized, extruded) improve the structural and mechanical properties of the dough, the structure of bread porosity and the color of the crust. Modified starches help to improve the porosity and color of the crumb and slow down the bread staling. For correction of flour with reduced baking properties in Ukraine, starches (E-1404) that oxidized in various ways are widely used.

Surfactants (emulsifiers) improve the properties of the dough and the quality of bread, extending its freshness.

The organic acids (citric, acetic, lactic, wine acid, etc.) are acidity regulating agents of dough, especially rye made.

Mineral salts containing calcium, magnesium, phosphorus, sodium, manganese activate enzymes of yeast cells.

Dry wheat gluten regulates the rheological properties of the dough, its water absorption and quality of the end products. For complex technological processes, multicomponent baking additives are widely used.

Enzyme Preparations of different action allows to regulate the alcohol fermentation intensity in the dough, improve the color of brown bread crust, increases dough water absorption, intensify ripening of dough, etc. Enzyme-based improvers for the production of flour are widely used in western countries [36]; in Ukraine they have not yet become significantly widespread. An important role in the technology of bread production is played by enzymes that correct dough rheology. Enzyme preparations of various composition have a wide range of effects on components such as starch, proteins, lipids, non-starch carbohydrates etc. [37-41].

**Characteristics of flour enzymes**

Baking properties of flour directly depend on the properties of protein-protease and carbohydrate-amylase complexes. Insufficient protein content, excessive elasticity or extensibility of gluten do not promote the formation of a continuous dough structure. The condition of protein-protease and carbohydrate-amylase complexes depends not only on the amount of protein and starch, but on the activity of enzymes that act on them during dough making [37-41]. The maximum action on the substrate occurs in the solution, therefore, while storage of dry grain and flour, enzyme has no action. After the mixing of half-stuff, most enzymes begin to catalyze the reaction of substrate hydrolysis.

The autolytic activity of flour is an important indicator of its baking properties. Both low and high autolytic activity of flour negatively affects the quality of dough and bread. It is important that the autolytic process of protein and starch degradation in dough occurs at a certain, moderate rate. In order to regulate autolytic processes in the production of bread, it is necessary to know the properties of enzymes groups that act on proteins, starch and other components of flour. This will allow to control the stage lysis direction.

Amylolytic enzymes (amylases).

The main substrate for the action of amylolytic enzymes is starch, which is hydrolyzed to dextrin and maltose. Depending on the type of amylase, the amount of maltose can vary significantly; at the simultaneous action of various types of amylase, the starch in flour paste is hydrolyzed almost completely [37,40]. The sensitivity of alpha and beta amylase to environment conditions is different. Alpha-amylase is more sensitive to the acidity of the medium and is less sensitive to temperature than beta amylase. The technological significance of amylase is also different: beta-amylase reduces the starch contained in the dough, promotes the accumulation of sugars needed for alcohol fermentation, and while alpha-amylase is converting starch into dextrin, impairs the quality of bread products. Beta-amylase is found in flour of all kinds and varieties; alpha-amylase is found in flour from unripe or sprouted grain. Rye flour of normal quality always contains alpha amylase, which greatly affects its baking properties [41,42].

Proteolytic enzymes (proteases). Proteolytic enzymes act on proteins and products of their hydrolysis. Proteases are widespread in animal and
plant tissues, as well as in many microorganisms. They are the most common industrial enzymes and can have plant, animal or microbiological origin. Depending on the protease origin, their heat-stability and pH-optimum of their action vary in wide range. Unlike fungal proteases that break down certain limited bonds in the gluten protein molecules, bacterial protease and papain affect the structure of gluten more intensively; as a result, the dough is more malleable.

By the way of action on the protein compounds proteases can be separated into 2 subclasses: endoproteases and exoproteases. Endoproteases break peptide bonds within the polypeptide chain, forming small peptides and free amino acids. Depending on the type of catalysis mechanism, the following groups of endoproteases can be identified: serine, cysteine, asparagine and metal-proteases. Exoproteases (peptidases) break peptide bonds at the edge’s protein chain with the formation of free amino acids and short peptides. Depending on what amino acids are cleaved by hydrolysis from the substrate with peptidases, carboxypeptidases and aminopeptidases are identified.

Since the most important functional component of wheat flour is gluten, any modification of the gluten frame or individual gluten proteins has a significant effect on the quality of the dough and bread. Hydrolysis of gluten proteins immediately affects at covalent interactions in the gluten framework. Proteases have long been used in bread making, mainly in the processing of thick and dry dough that comes from a very strong flour. Firstly, proteases were used to improve the softness and improve the technological properties of the dough, but their effect is wider. They reduce the duration of dough kneading, increase gas-retaining ability (due to improved extensibility of the dough) improve disclosure of dough billets while making buns and rolls, better crumb texture, water absorption, color, flavor and aromas of products [43].

The grain and flour always contain proteases, usually having low activities. It is believed that cereal protease does not completely destroy the protein molecule, but changes its complex structure, which changes the properties of proteins and dough. In the sprouted, unripe and especially in affected by the corn-bug grain, the activity of proteases is significantly increased. In this case, it impairs the quality of gluten, reduces its elasticity, resilience and ability to swell [44]. Grain protease act most actively in the low acid medium at a temperature of 45–47°C. Protease activity greatly reduced in the presence of oxidants, and increases in the presence of reducing agents. Depending on the type of flour products, the different resilience and elasticity of the dough are required for their preparation. The use of enzyme additives allows you to adjust the properties of the dough to the desired parameters.

Lipases always contained in the flour, they hydrolyze fats to glycerol and fatty acids. Lipase is very important while flour storing, since increasing of flour acidity during storage is primarily result of the lipase presence. Lipase involved in the formation of gluten frame in and influence on the interaction of gluten proteins with starch and lipids [42]. The enzymes of this group also help to increase the specific volume of bread, obtaining the thin bread crust, increasing crumb elasticity and the improvement of bread structure.

Enzymes of oxidative action are enzymes of oxidase, peroxidase. They improve the gas-retaining ability and stability of the dough during treatment, resulting in increased quality of finished products. Earlier, as a source of enzymes, malt (source of α-amylase, proteolytic and other enzymes) was used, now enzymes of microbial origin are widely being used [45].

The use of enzymes in bread-making: advantages and problems

The current tendency of replacing chemical additives in flour with analogues from natural sources is determined more to the need for enrichment of food products with components that have a beneficial effect on human health. Numerous studies have shown the necessity of using enzymes to obtain bread-rich cellulose [46,47], to develop of gluten-free products [48], and products with high arabinoxylan content with high prebiotic potential [47].

Enzyme additives have a number of advantages compared with other food additives. The main ones are natural origin and high specificity of action. That ensures ecological compatibility of end products and the lack of negative effects that occur in the latest stages of production. In addition, in practice, enzymes allow bakeries to expand the products range of their enterprise and save both raw materials and energy.

Today, the rapid development of biotechnology has made enzyme preparations indispensable participant in many of food technologies. Usage of enzymes can increase the speed of technological processes, significantly increase the output of finished products, improve its quality, save valuable raw materials and reduce the amount of waste.

For industrial manufacture of enzymes for food purposes, sources of animal, plant and microbiological origin are used. Different origin of enzymes determines the conditions of their application. The modern market of enzymes is largely represented by enzymes of bacterial genesis, since the rate and volume of production of enzyme preparations for bacterial genesis are significantly higher compared to fungal genesis enzymes. The enzymes of the bacterial genesis are more narrowly specialized in relation to the substrate comparing to enzymes of fungal origin. This is to a certain extent a useful feature for using them in the flour-mill industry, since it allows adjust the production process to be more predictable. In addition, in some cases, bacterial enzymes can be more convenient for usage because the optimum of their
action is closer to the physical and chemical conditions in the production of dough [49].

We should also mention about the usage of enzyme preparations which is derived from of gene-modified producents. Despite the different points of view concerning the lawfulness of the use of such organisms in the issue of human food security, nowadays the high rate of purification during production actually excludes the presence of residues of microorganisms with a modified genome in their composition, and therefore the use of such enzyme products is completely safe and expedient [50,51].

The use of enzyme mixes is a widespread practice, since a properly selected combination of enzymes can act synergistically and have a better effect on the various components of flour [52].

Some authors reported the use of enzyme combination made of amylase, xylanase and lipase [46,52,53]. Such a specific mixture is said to increase the volume of bread and the duration of its storage. The use of α-amylase and glucose oxidase to replace potassium bromate leads to a significant improvement in the breadth and breadth of bread [54].

The addition of commercial enzyme mixtures containing α-amylase and lipase activity in bread samples in experiments also increased the thermostable properties of the amylose-lipid complex as compared to control samples of bread [55].

Enzymes that are synthesized by polar organisms usually have optimum range performance and stability at lower temperatures than their mesophilic counterparts [56]. Due to the fact that the temperatures most commonly used in mixing and maintaining the dough are approximately 35°C or lower, it was assumed that such enzymes may be potential candidates for use as additives in the baking industry [57,58]. In the context of such assumptions, researchers have shown that much lower concentrations of xylanase, effective at low temperatures, can be used to reach the maximum amount of bread, to achieve maximum bread levels [58,60]. Nevertheless, the issue of the long-term storage of such enzymes after their isolation from producer organisms was not resolved. On the other hand, directed evolution is a powerful tool for protein engineering to develop and modify the properties of enzymes [61]. This technology can be used for a wide range of proteins, most of which are of interest to biocatalytic processes. Over the past 10 years, evolution has become a standard methodology in protein engineering and can be used in conjunction with rational protein design and other standard methods that meet the requirements for bio-catalysts in industrial production: high activity, high temperatures and long-term stability, as well as the desired specificity and/or selectivity [62]. Recent studies report the combined use of directed evolution and high-yield screening to improve the performance of the use of maltogenic α-amylase from Bacillus sp. in bread with lower pH conditions [63].

Beside enzymes of microbiological origin in baking, enzyme-active plant materials are used – e.g., malt, malt flour or preparations based on them, soy flour with active lipooxygenase. Surfactants (emulsifiers) are used in bread making as additives for improving the quality of food products. Dough emulsifiers are used for a better quality of fatting, forming a gluten frame, increasing the water absorption of the flour.

The strength of wheat flour is also affected by the presence of fats and lipases in the grain. Enzyme systems aimed at interaction with grain lipids also play an essential role in correcting the properties of flour. According to [19,64], additional action of lipase leads to compact the tertiary and quaternary structure of the protein. Such transformations reduce its enzymatic vulnerability, which increases the strength of flour and strengthens gluten.

Mineral salts are activators of the fermentation ability of baking yeast, and increase the rate of accumulation of carbon dioxide in half-stuffs. Mineral salts are used as a complex additive – on the one hand, they regulate osmotic pressure in the yeast cell, on the other hand, they can act as inhibitors or activators of enzymatic action. In some cases, the use of mineral salts can simultaneously lead to the activation of certain enzymes and in the same time to be as inhibitor for others. The addition of mineral salts also improves the consistency and elasticity of the dough, as well as intensifies the formation of the dough. Also, inhibitors of enzymes in the grain differ for their origin. The inhibitor action is very often complex, which significantly impairs the control of the enzyme systems behavior of during dough process. However, the issue of reducing the natural inhibitors impact is extremely relevant for whole-wheat flour, which is considered more useful than white flour. Although quantitative indicators, forms of vitamins and minerals in such grain shells are often undigestible for human. One of example is undigestible phosphorus, calcium, magnesium, and zinc in wheat grains, which are bound tightly in the structure of the myo-inosithexafosforic acid salts molecules, serving as a kind of "nutrient depot" in the grain and almost not digested by humans. In order to convert the useful components into easily digestible forms, it is expedient to develop enzyme complexes that contribute to this process.

Another problem in the use of complex enzyme systems in the production of flour is the complexity of evaluating of own grain enzyme system activity. While making different purpose flour from Ukrainian grain, you need to know how much external enzyme activity to add. That requires knowing own grain enzyme systems activity. Currently, such an assessment requires either specialized laboratory and appropriate methods of analysis or to use some indicators (e.g., grain falling number, gluten deformation index, etc.)
that characterize indirectly protein-protease, carbohydrate-amylase and lipase complexes in grain. The development of inexpensive methods for express estimation of own enzyme systems of grain would be extremely relevant, especially in cases of damage to the grain from corn bug. Given the assessment of the quality of the grain, the introduction of enzyme preparations should be carried out at the stage of flour production, to ensure stable indicators of its quality.

Conclusions

1. Monitoring studies of grain quality of wheat show a steady tendency towards its decrease, and as a result - the production of flour with unsatisfactory baking properties.

2. Flour baking properties primarily depend on protein-protease and carbohydrate-amylase complex. But adding enzymes of other classes may significantly improve nutrition indicators of end product.

3. Own enzyme activity estimation of flour cannot be made by direct methods at flour mills. Indirectly, the amylolytic activity of the flour can be estimated by the falling number, the proteolytic activity – by gluten deformation index.

4. The enzymatic activity of the flour may be corrected by adding external enzyme preparations. Enzyme-based improvers for flour production are widely used in western countries; in Ukraine this promising direction has not yet become significantly widespread.

5. In order to improve the baking properties of flour, it is advisable to use enzyme preparations of complex action, but neither their formulation nor the dosage for purpose flour from Ukrainian grain is not scientifically based yet.

6. Input of complex enzyme additives based on enzyme preparations should be carried out directly on flour mills. It requires the development and substantiation of the structure, and also regimes of the technological process of flour manufacturing.

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