

# СУЧАСНІ ТЕХНОЛОГІЇ ХАРЧОВИХ ПРОДУКТІВ

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## STUDY OF THE INFLUENCE OF DRINKING WATER QUALITY ON BAKERY PROPERTIES OF WHEAT FLOUR

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## ДОСЛІДЖЕННЯ ВПЛИВУ ЯКОСТІ ПИТНОЇ ВОДИ НА ХЛІБОПЕКАРНІ ВЛАСТИВОСТІ ПШЕНИЧНОГО БОРОШНА

**Objective.** *The purpose of the article is to investigate the effect of drinking water quality on the baking properties of wheat flour.*

**Methods.** *In the process of the research, methods for determining the activity of amylolytic and proteolytic enzymes of raw materials were used. The amylase activity of wheat flour in the presence of heavy metal cations was estimated by the amount of maltose formed in the reaction mixture, a product of deep saccharification of flour starch. The effect of heavy metal cations on the proteolytic activity of wheat flour enzymes was evaluated by the yield of raw and dry gluten by kneading dough, comparing the yield of gluten from dough, which was prepared in distilled water and water containing 0.05 g/l of lead or nickel cations. The relative viscosity of the gelatin solution under the action of proteolytic enzymes of wheat flour in the presence of cations of lead and nickel was found using a VPZH-2 capillary viscometer with a capillary diameter of 0.56 mm in a water thermostat.*

**Results.** *It is proved that heavy metal cations adversely affect the biological activity of proteolytic enzymes of flour, they deprive gluten of elasticity, which may adversely affect the protein frame of the dough by baking, and as a result, the quality of the finished product. It is proved that the water is contaminated by impurities of heavy metals, particularly lead and nickel, can worsen the gas-retaining ability of the semi-finished dough. The pollution of water, which is used for the preparation of the dough during the bread baking, particularly heavy metal cations, which are inhibitors of most enzymes, plays a very important role in ensuring the quality of the finished product that requires careful monitoring of its purity.*

**Key words:** *wheat flour, yeast dough, proteolytic enzymes, amylolytic enzymes, heavy metals, cations, water, gluten.*

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**A problem statement.** Various wheat flour products, in particular, yeast dough products and especially bread, still form the basis of human nutrition. Therefore, the quality and nutritional value of bread, as a daily consumption product, is of paramount importance [1, 2]. The problem of the nutritional value of bread becomes especially acute in those periods when, for any reason, the consumption of food products of animal origin — eggs, milk, cheese, meat, animal fats — significantly decreases, and the proportion of cereal products, primarily products, increases relatively from flour. It is clear that in these conditions of more uniform nutrition, the problem of the quality and nutritional value of bread and possible ways to increase it becomes especially urgent. Therefore, it is natural that for many years there have been studies in this area that have not stopped until now, and the problem does not lose its relevance in modern conditions [3–5]. All existing methods for increasing the nutritional value of bread can be divided into two large groups: enriching it with a complex of valuable biologically active substances and improving consumer qualities. In some cases, these two paths coincide, as in the case of an increase in the activity of the enzyme complex of wheat flour, in which amylolytic and proteolytic enzymes play a large role. They are responsible for the accumulation of free amino acids and sugars in the test semi-finished product, the formation of a crispy brown crust, and, in general, play a paramount role in ensuring the quality of the finished product.

**Analysis of recent research and publications.** It is known [6] that both amylolytic and proteolytic enzymes in various substrates are very sensitive to the most insignificant changes in the environment. Various chemicals have a special effect on their activity: some of them activate enzymes, while others inhibit, depriving enzymes of activity. Among the most famous inhibitors of most enzymes are heavy metal cations. If they get even in small quantities into the recipe during dough production, this can lead to poor quality of the finished product. When developing dough formulations for the production of various products from wheat flour, very little attention is paid to such an important recipe component as water, which is usually taken from the city water supply network. It is now known, that over the past decades there has been a constant deterioration in the quality of the water of surface water bodies, rivers and, as a consequence, a deterioration in the quality of drinking water. There are several reasons for this. First of all, there is an increase in freshwater consumption by industrial and agricultural enterprises, which, after contamination of used water, throw it into surface water bodies. Particularly dangerous is the ingress of heavy metals cations into water bodies as necessary components of the wastewater of galvanic shops, including a considerable amount of nickel, zinc, etc. [7–10].

About thirty years have passed after the huge disaster in Chernobyl, which led to intensive pollution of a large area of Ukraine. Along with radioactive, environmental pollution by heavy metals poses a very great danger, among which lead is one of the most toxic. The increase in lead in the atmosphere of Ukraine over the years is because they tried to use this metal at the very beginning of the Chernobyl accident to shield the destroyed reactor. At the same time, hundreds of tons of lead evaporated and entered the atmosphere, and subsequently into the soil.

**The purpose of the article** is to study the effect of drinking water quality on the baking properties of wheat flour.

**Statement of the main research material.** Were conducted experiments to study the effect of heavy metals on the action of both amylolytic and proteolytic enzymes of wheat flour. As objects of study were chosen two metals — lead and nickel in the form of their salts. The activity of wheat flour amylases in the presence of heavy metal cations was evaluated by the amount of maltose formed in the reaction mixture, a product of deep saccharification of flour starch. The experimental data are shown in table 1.

**Table 1** — Amylolytic activity of wheat flour in the presence of heavy metal cations

Metal cation	The content of maltose, %
Without metal (control)	3,9
Ca <sup>2+</sup>	4,5
Pb <sup>2+</sup>	1,4
Ni <sup>2+</sup>	2,1

The experiment indicates that lead cation, as is known from the literature [1], is the most potent inhibitor of amylase enzymes — it almost ceases their activity. Nickel cation also inhibits amylases, but less actively. In contrast, calcium cation, a well-known activator of enzyme systems, accelerates the hydrolysis of starch with flour amylases. These data allow us to conclude that water contaminated with impurities of heavy metals, in particular, lead and nickel, can cause a deterioration in the gas-forming ability of the test semi-finished product.

The effect of heavy metal cations on the proteolytic activity of wheat flour enzymes was evaluated by the yield of raw and dry gluten during kneading, comparing the yield of gluten from the dough, which was prepared on distilled water and water containing 0.05 g/l lead or nickel cations. It should be noted that the protein complex of gluten dough during kneading is exposed to protease enzymes that hydrolyze proteins to free amino acids enriched the dough, give nitrogen nutrition to yeast, and contribute to the Maillard reaction. The last results in the formation of a brown crispy crust of cooked bread. Gluten was washed from the dough after it was cured for 1.5 hours. The experimental data are shown in table 2.

**Table 2** — The yield and quality of gluten when washing it from the test, made on water mixed with lead and nickel cations

Metal cation	Gluten yield, %		Gluten extensibility, cm
	Raw	Dry	
Without metal (control)	33,0	10,2	6,8
Ca <sup>2+</sup>	24,0	7,4	13,5
Ni <sup>2+</sup>	36,0	11,1	6,8
Pb <sup>2+</sup>	38,8	12,0	6,8

The experimental data given in the table indicate that the yield of crude gluten in the test, which was made on the water with an admixture of heavy metals — lead and nickel, increased significantly compared to the test made on distilled water. This indicates the inhibition of proteolytic enzymes of wheat flour by these cations. The mechanism of action of cations on protease enzymes is associated with their reaction with active lateral functional groups of protein molecules of enzymes, most often, with sulfhydryl groups of SH, which violates the tertiary structure of the enzyme and leads to its denaturation and loss of activity. It can be seen from the experimental data that lead is a stronger protease inhibitor, which is probably because it is a stronger oxidizing agent than nickel, and, therefore, it interacts more actively with SH groups that have reducing properties. For comparison purposes, has been conducted an experiment with gluten washed from dough prepared on the water with the addition of a well-known enzyme activator — calcium cation — at a concentration of 0.05 g/l in terms of metal. The gluten yield sharply decreased, which indicates the acceleration of the action of proteolytic enzymes under the action of calcium, which takes part in the stabilization of the tertiary structure of the enzyme and the formation of an active enzyme-substrate complex.

Along with the raw yield, the yield of dry gluten and its extensibility was controlled, which predicts the elasticity of the protein framework of bread when baking a dough piece. The yield of dry gluten is very important for evaluating the processes occurring in the protein complex of wheat flour, since under the influence of certain substances the ability of protein molecules to aggregate water molecules around them may increase. This increases the hydration of gluten proteins, it becomes able to retain more bound water and the yield of raw gluten increases. In the technology of production of wheat flour products, such a process is very useful for the quality of the finished product. In this case, when the washed raw gluten is dried, all the bound water eliminates, and the yield of dry gluten does not differ from usual. When the raw gluten obtained in the experiments was dried, its yield shows the same dependence as the crude yield, which completely excludes the possibility of increased hydration of dough proteins under the influence of heavy metals, and leaves only their enzyme-inhibiting effect.

The extensibility of gluten does not change in the test made on distilled water and water mixed with lead and nickel, and only in the presence of a calcium cation does gluten become twice as elastic. These results support the conclusion that heavy metals inhibit the action of protease en-

zymes that do not break down gluten proteins. Calcium cation strongly activates enzymes, which at the same time begin to actively hydrolyze proteins to amino acids, reduce their amount and molecular weight, which makes gluten weak. An important criterion for the quality and baking properties of wheat flour is the ability of a gluten ball weighing 10 g to spreadability after an hour of tracking. Data on the spreadability of gluten, washed from dough with an admixture of heavy metals, are shown in table 3.

**Table 3** — The dependence of the spreadability of the gluten ball, washed from wheat flour, from impurities of heavy metal cations

Metal cation	Ball diameter, mm
Without metal (control)	41
Pb <sup>2+</sup>	37
Ni <sup>2+</sup>	39
Ca <sup>2+</sup>	95

The given experimental data are completely consistent with the previous ones — gluten under the action of heavy metal cations is fixed, becomes less elastic.

Measurement of the relative viscosity of gelatin solutions under the action of substances whose activity is studied is a very convenient way to establish the activity of proteolytic enzymes in raw materials. As part of the study, an experiment was conducted to study the relative viscosity of a gelatin solution under the action of proteolytic enzymes of wheat flour in the presence of lead and nickel cations. The relative viscosity of gelatin solutions was determined using a VPZh-2 capillary viscometer with a capillary diameter of 0,56 mm in an aqueous thermostat. The solution was studied using a thermostat with an accuracy of 0,1 °C. The aging of the system before the start of the measurement was at least 15 minutes. Before the experiment, the solutions were filtered through Schott filters. The relative viscosity was calculated by the formula (1):

$$\eta = \frac{t_{solution}}{t_{solvent}} \quad (1)$$

where  $\eta$  is the relative viscosity;  $t_{solution}$  is the flow time of the solution, sec.;  $t_{solvent}$  — is the flow time of the solvent, sec.

The experimental data are shown in table 4.

**Table 4** — Change in the relative viscosity of 2 % gelatin solutions under the action of wheat flour proteases depending on the solvent

Solvent	Relative viscosity (h)
Water	1,6
Aqueous solution of lead salt ( $C_{Pb}=0,05$ g/l)	1,95
Aqueous solution of nickel salt ( $C_{Ni}=0,05$ g/l)	2,05
Aqueous solution of calcium salt ( $C_{Ca}=0,05$ g/l)	1,3

The data obtained as a result of the experiment are consistent with one previously done — the relative viscosity of the gelatin solution with the addition of wheat flour without the addition of heavy metal cations is significantly lower compared to that obtained with the addition of lead and nickel cations. These cations likely inhibit the action of wheat flour proteases, which become less active and more slowly hydrolyze gelatin macromolecules. Calcium cation, as in previous experiments, exhibits a very large activating ability, it accelerates the process of hydrolysis of gelatin, as a result of which the viscosity of its solution decreases. But in this experiment, impurities of lead cations significantly slow down the hydrolysis process compared to nickel cations. This fact is explained not by the greater activity of nickel cation as an inhibitor of wheat flour proteases, but by an additional complexation process between the gelatin macromolecule and this cation, which leads to stabilization of the tertiary structure of gelatin and, as a consequence, to the viscosity of its solutions. Nickel refers to transition metals having vacant d-orbitals, which makes it possible to form additional coordination bonds with substrate molecules, that is, increases its complexing

ability. This explains the even greater increase in the viscosity of gelatin solutions in comparison with impurities of lead cations. The lead cation refers to p-elements that do not have electronic levels with vacant d-orbitals, which deprives it of its ability to form coordination bonds with electron-enriched sections of gelatin protein molecules.

Experiments conducted indicate that protein molecules of proteolytic enzymes undergo denaturation under the influence of heavy metal cations. Denaturation is understood to mean any process that violates the quaternary, tertiary, and even secondary structure of the protein molecule changes its spatial spiral configuration without touching the covalent peptide bond. But, despite the preservation of the basic skeleton of the molecule, its biological properties are lost. To study protein denaturation under the influence of these metal cations, we developed a technique and conducted a model experiment in which we observed precipitation in 2 % aqueous solutions of egg albumin under the influence of these metals. The precipitation characterizes the course of the protein denaturation process, when it's tertiary and, partially, the secondary structure is violated, the molecule loses its subordinate spiral configuration and becomes a chaotic pile of tangles and loops. Precipitation is very convenient to quantitatively control by changing the optical density of the solutions, which was measured on a KFK-2 photoelectric colorimeter in cuvettes with a layer thickness of 3 cm at a wavelength of 400 nm. The experimental data are shown in table 5.

**Table 5** — Change in optical density of aqueous solutions of the egg albumin in the presence of metal cations

Metal cation	Optical density, D
Without metal (control)	0,05
Ca <sup>2+</sup>	0,03
Ni <sup>2+</sup>	0,25
Pb <sup>2+</sup>	0,38

The experimental data confirm the fact of protein denaturation with cations of heavy metals, especially lead, and are completely consistent with the data obtained during experiments with gluten. Calcium cation helps to stabilize the tertiary structure of the protein, and therefore the transparency of the egg albumin solution increases.

All tests, carried out, confirmed the fact that heavy metal cations adversely affect the biological activity of proteolytic enzymes of wheat flour, they deprive gluten of elasticity, which can adversely affect the protein frame of the dough during baking, and as a consequence, the quality of the finished product.

**Conclusions.** Studies have shown that the pollution of water used to make the dough in bread baking processes, especially heavy metal cations, which are inhibitors of most enzymes, plays a very important role in ensuring the quality of the finished product, which requires careful control of its purity.

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**Мета** — дослідження впливу якості питної води на хлібопекарні властивості пшеничного борошна.

**Методи.** У процесі дослідження використано методи визначення активності амілолітичних та протеолітичних ферментів сировини. Активність амілаз пшеничного борошна в присутності катіонів важких металів оцінювали за кількістю утвореної в реакційній суміші мальтози — продукту глибокого оцукрювання крохмалю борошна. Вплив катіонів важких металів на протеолітичну активність ферментів пшеничного борошна оцінювали за виходом сирові та сухої клейковини при замішуванні тіста, порівнюючи вихід клейковини з тіста, яке готувалося на дистильованій воді і на воді, яка вмішувала 0,05 г/л катіонів свинцю, або нікелю. Відносну в'язкість розчину желатину під дією протеолітичних ферментів пшеничного борошна у присутності катіонів свинцю та нікелю знаходили за допомогою капілярного віскозиметра ВПЖ-2 з діаметром капіляра 0,56 мм у водному термостаті.

**Результати.** Доведено, що катіони важких металів згубно впливають на біологічну активність протеолітичних ферментів пшеничного борошна, вони позбавляють клейковину еластичності, що може негативно позначитися на білковому каркасі тіста при його випіканні і, як наслідок, на якості готового виробу. Доказано, що вода, забруднена домішками важких металів, зокрема, свинцю та нікелю, може спричинити погіршення газоутворювальної спроможності тістового напівфабрикату. Забруднення води, яку використовують для приготування тіста у процесі випікання хліба, особливо катіонами важких металів, які є інгібіторами більшості ферментів, відіграє важливу роль у забезпеченні якості готового виробу, що потребує ретельного контролю її чистоти.

**Ключові слова:** пшеничне борошно, дріжджове тісто, протеолітичні ферменти, амілолітичні ферменти, важкі метали, катіони, вода, клейковина.

**Цель** — исследование влияния качества питьевой воды на хлебопекарные свойства пшеничной муки.

**Методы.** В процессе исследования использованы методы определения активности амилолитических и протеолитических ферментов сырья. Активность амилаз пшеничной муки в присутствии катионов тяжелых металлов оценивали по количеству образованной в реакционной смеси мальтозы — продукта глубокого осахаривания крахмала муки. Влияние катионов тяжелых металлов на протеолитическую активность ферментов пшеничной муки оценивали по выходу сырой и сухой клейковины при замесе теста, сравнивая выход клейковины из теста, которое готовилось на дистиллированной воде и на воде, содержащей 0,05 г/л катионов свинца или никеля. Относительную вязкость раствора желатина под действием протеолитических ферментов пшеничной муки в присутствии катионов свинца и никеля находили с помощью капиллярного вискозиметра ВПЖ-2 с диаметром капилляра 0,56 мм в водяном термостате.

**Результаты.** Доказано, что катионы тяжелых металлов отрицательно влияют на биологическую активность протеолитических ферментов пшеничной муки, они лишают клейковину эластичности, что может негативно сказаться на белковом каркасе теста при его выпекании и, как следствие, на качестве готового изделия. Доказано, что вода, загрязненная примесями тяжелых металлов, в частности, свинца и никеля, может способствовать ухудшению газодерживающей способности тестового полуфабриката. Загрязнение воды, которую используют для приготовления теста в процессе выпекания хлеба, особенно катионами тяжелых металлов, которые являются ингибиторами большинства ферментов, играет важную роль в обеспечении качества готового изделия, что требует тщательного контроля ее чистоты.

**Ключевые слова:** пшеничная мука, дрожжевое тесто, протеолитические ферменты, амилолитические ферменты, тяжелые металлы, катионы, вода, клейковина.