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Research Paper



Creation of multicomponent mix based on lactic bacteria consortium for flour enrichment

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Abstract

This article presents the results on creating the probiotic preparation with inclusion of microorganisms of highly productive lactic bacteria cultures with high biosynthetic and bactericidal properties. Conditions for production of bacterial preparation and multicomponent mix for flour enrichment and bread making were developed. Composition of the produced improving agent and premix for flour enrichment was studied and conditions for flour enrichment using the produced biological improving agent based on lactic bacteria were developed. Technological parameters of flour semi-products where determined on addition of the enriched flour. It was determined that produced improving agent plays a key role in transformation of protein-proteinase and carbohydrate-amylase flour complex, thereby improving the structural-mechanical properties of dough and further resulting in production of elastic crumb with thin-walled and even texture.

Keywords: *Flour Enrichment; Micronutrients; Probiotic Preparation; Multicomponent Mixes; Improver; Lactic Acid Bacteria Isolates.*

1. Introduction

It is known that human food rations in all regions of Kazakhstan incur deficit in vitamins, micro- and macroelements, and other physiologically necessary substances. It develops not only in spring, but also in summer-fall period, which seemingly is favorable period in the year, thereby being fixed disadvantage having negative impact to health, growth and viability of whole nation.

National research showed that in Kazakhstan more than 45.7% of women of reproductive age suffer from iron deficiency anemia (IDA), which has substantial influence to maternal and neonatal mortality level. Furthermore, this is a cause of delay in physical development and delay in mental development and cognitive skills of children. Related decreased indicators in school and work are associated with losses in national economy being evaluated by more than billion dollars per annum. Thus, anemia and vitamins deficit result in decreased working ability by 17% among manual workers and by 5% among brain workers [1].

Situation is complicated by the fact that food products, mainly flour and its derivatives, forming the basis of human food ration, are largely combined by these substances in the course of their production.

Technologic processing of cereal crops, including wheat and rye, into flour is followed by unavoidable loss of micronutrients – vitamins and mineral substances - removed together with glume. Making of bread, bakery products and flour confectionery goods using such flour results in additional loss of these important biologically active substances [2].

Considering the importance of this problem, articles related to fortification of first-grade and high-grade wheat flour are included to the Health Code approved by Mazhilis and Senate of Parliament of the Republic of Kazakhstan in November 2009 [3].

Currently, some food products are enriched with certain microelements, such as iodine preparations or preparations, containing some vitamins, which in whole does not solve the problem of making food products with higher nutritional and biological value.

Lucerne extract is studied in the Bashkir State Agriculture University for some years. On addition of lucerne extract to first-grade of high-grade flour in a dose of 0.1% of flour weight, the organoleptic indicators and physicochemical parameters of dough and finished products, and structural-mechanical properties of crumb and stability of shape improve [4].

Use of sea-buckthorn oil meal and its fermentation products when making bakery products also serve to improvement of bread quality by all basic parameters. Application of studied additives when making bread substantially intensifies the gasification process and dough maturation period shortens by 25-30 minutes, while preserving high qualitative indicators of the products [5].

Dry ready-to-eat semi-products – multicomponent mixes designated for short-time production of wide range of bakery products – are of particular interest.

According to the data provided by the Institute of Agricultural Marketing for 2008, ready mixes for production of bakery products are used by 76.7% of bakery plants of the Russian Federation [6].

In studies carried-out, addition of Vitazim preparations affected the protein-proteinase and carbohydrate-amylase complexes of wheat flour [7].

2. Materials and methods

Experimental studies were carried-out using the modern method provided below, which allow determination of characteristics of raw materials and finished goods based on the complex of indicators: sampling method (GOST 27668-88); acidity – by method of water mess titration (GOST 27493-87); determination of

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bread-making properties of flour – by method of test laboratory baking (GOST 27669-88); content of vitamin B_1 method (GOST 29138-91); content of vitamin B_2 (GOST 29139-91); iron content (GOST 26928-86); physical properties of dough using the farinograph (GOST P 51404-99 (ISO 5530-1-97); sampling for microbiological analysis (GOST 26668-85); methods for determination of quantity of mesophilic aerobic and facultative anaerobic microorganisms (GOST 10444.15-94).

3. Results and discussion

Based on performed patent-information researches and investigation of properties of first-grade and high-grade wheat flour, the following components were selected for complex improver composition forming: thiamin (B_1), riboflavin (B_2), iron in the form of sulfate heptahydrate.

Consortium of lactic acid bacteria - Lactobacillus plantarum-2, Lactobacillus casei var.alactosus-22, Lactobacillus brevis-67, Lactobacillus fermentum-104 – was used as a base for dry concentrate designated for enrichment of wheat flour. Cultivation was performed using the nutrient medium of fixed composition at a temperature of 35±2°C within 24 hours. Iron in the form of sulfate heptahydrate, vitamin B_1 (thiamin hydrochloride) and vitamin B_2 (riboflavin) were used to increase titre of lactic acid bacteria designated for preparation of dry preparation.

Experiment performed 24 hours after cultivation by method of multifactorial experiment design with three variables of the process showed that 1-2 order increase of biomass by 1-2 was observed; other quality indicators of starter: active and titratable acidity, antagonist activity is at the level of control values.

It was noted that when determining antagonist activity of ready starter using the wort agar media, red contour occurs around the zone of test culture growth inhibition, which can be indicative of formation of complex ferric iron compounds with B vitamins [8].

To improve biochemical and technological properties of consortium of lactic acid bacteria to be used for production of multicomponent improver, method of application of different additives (vitamin B_1, vitamin B_2, ferric sulfate heptahydrate) and neutralization of waste products of lactic acid bacteria using calcium carbonate. Quantity of calcium carbonate required for neutralization of waste products of lactic acid bacteria was determined based on acidity of the starter. Neutralization was performed 8 hours after cultivation.

According to the obtained data, method of neutralization allows 1 order increase of titre of lactic acid bacteria. The same increase of cells quantity results in introduction of complex of additive in the course of cultivation. Then determination of micronutrients was performed in liquid improver prepared on the basis of consortium of lactic acid bacteria.

Ferric sulfate was added in quantity of 600 mg/L or 60 mg/100g. Molecular weight of ferric sulfate heptahydrate is 278 g, it contains 56 g if iron, thus, 60 mg of ferric sulfate contain 12.1 mg of iron. Method fixes increase of iron by 8.98-15.22 mg, which is quite consistent with the error of method.

When vitamins were added in quantity of 35 mg/L or 3.5 mg/100g, increase amounted just to 0.34 mg/100g. When vitamins were added in quantity of 28 mg/L or 2.8 mg/100g, increase amounted just to 0.24 mg/100 g. These data can be indicative of consumption of vitamins by lactic acid bacteria in the course of cultivation, which corresponds to literature data [8, 9].

Therefore, it is possible to use the abovementioned consortium of lactic acid bacteria for production of complex improver. Cultivation was performed using the flour medium with 58-62% humidity during 24-48 hours. Drying was performed using the sublimation drier manufactured by "Jouan" company during 10-12 hours, up to residual humidity of 2.5-3.5%. Vitamins can be added in the course of cultivation of consortium, or directly to finished improver. To obtain available form (lactate) of iron, it is necessary to add lactic acid bacteria in the course of cultivation in quantities recommended by nutritional standards [10].

Carried-out patent-information researches and results of in-house studies of microbiological composition of Kazakhstani wheat grain showed that quantity and species composition of microflora vary depending on soil-climatic conditions, and condition harvesting and storage of grain and flour.

Using special microbiological methods, isolates of lactic acid bacteria were obtained from first grade flour "Tsesna" manufactured by "Concern Tsesna-Astyk" LLP; second-grade flour "Adil" manufactured by "Almaty flour milling combine" LLP; first-grade flour "Adil" manufactured by "Almaty flour milling combine" LLP; first-grade flour "Sultan" manufactured by "Sultan-Elevator-Melnichno-Makaronnyi Komplex" JSC, Petropavlovsk city; high-grade flour "HILAL" manufactured by "Hilal" IE LLP; high-grade flour "Aksai nan" manufactured by "Vostochno-Kazakstanskyi mukomolnyi combinat" JSC, Semipalatinsk city; high-grade flour "Korona" manufactured by "Kostanai Melkombinat" JSC. 35 more active isolate were selected from the obtained 172 isolates (by value of titrable acidity and antagonist activity).

For further investigations, isolates of lactic acid bacteria extracted from the following flours were used:

1) "Tsesna", "Concern Tsesna-Astyk" LLP, first-grade flour – Isolate No. 1, acidity number 35.0;
2) "Adil", "Almaty flour milling combine" LLP, first-grade flour – Isolate No. 2, acidity number 22.8;
3) "Sultan", "Sultan-Elevator-Melnichno-Makaronnyi Komplex" JSC, Petropavlovsk city – Isolate No. 3, acidity number 26.0

Consortium was composed of three most active isolates of lactic acid bacteria - Лц 1, Le 12, and Ls 7. Various experiments were performed: culture ratio 1:1:1; culture medium - optimized; temperature - 35±2°C; period of cultivation - 24-72 hours (Table 2).

Table 1:.Characteristics of Extracted Isolates

Source	Number of isolate	Antagonistic activity, d zone, mm
First grade flour "Tsesna" manufactured by "Concern Tsesna-Astyk" LLP	Лц 1	24±2.5
	Лц 2	22±0.5
	Лц 3	21±0.5
	Лц 4	19±1.0
First-grade flour "Adil" manufactured by "Almaty flour milling combine" LLP	Le 10	21 ±0.5
	Le 12	24±3.0
	Le 13	20±1.0
	Le 15	21±0.5
	Le 16	19±2.0
	Le 17	20±1.0
	Le 18	21±0.5
	Le 20	22±1.0
first-grade flour "Sultan" manufactured by "Sultan-Elevator-Melnichno-Makaronnyi Komplex" JSC	Ls6	20±1.5
	Ls7	22±2.0
	Ls8	19±1.0

As could be expected, consortium composed of three isolates of lactic acid bacteria has higher technologic-biochemical properties if compared to separate cultures. However, antagonistic activity in this case is absent, irrespective of the fact that it is quite high in some isolates: 22-24 mm.

Different additives stimulating the development of lactic acid bacteria – B vitamins, soya flour and sodium citrate – were used for the purpose of improvement of biochemical and technological properties of consortium of lactic acid bacteria to be used for production of multicomponent improver.

Table 2:.Results of Coculture of Obtained Isolates of lactic Acid Bacteria

Variant of experiment	pH	Acidity number	Antagonistic activety, mm
I.Isolate Лц 1;	5.93	5.6	0
2. Isolate Лц 1 + Isolate Le 12;	5.72	6	0
3. Isolate Лц 1 + Isolate Le 12, + Isolate Ls 7	5.5	6.4	0

Substantial increase of titrable acidity was observed when soya flour was added. Two order decrease of biomass occurred when soya flour was added – by 20% of weight of wheat flour and by 0.5% of sodium citrate to weight of cultivated mix. Considering availability of tested additives, the most acceptable one is soya flour, which contributes not only to bacterial growth, but also reduces drying process of the obtained improver.

Ferrous sulfate and ascorbic acid (vitamin C) also were used as enriching additives. It is known that vitamin C increases iron solubility through its oxidation from ferrous (Fe^{2+}) to ferric form (Fe^{3+}) and formation of soluble compound [11].

For the purpose of enrichment of consortium of lactic acid bacteria, ferrous sulfate and ascorbic acid were added to nutrient medium in the beginning of the process of cultivation. Experiments were carried-out using the known consortium of lactic acid bacteria as a basis for complex improver. Variants of experiments were the following:

1) Control – known consortium of lactic acid bacteria: Lactobacillus plantarum-2, Lactobacillus casei var.alactosus-22, Lactobacillus brevis-67, Lactobacillus fermentum-104.
2) Consortium composed of three cultures: Lactobacillus plantarum-2, Lactobacillus casei var.alactosus-22, Lactobacillus fermentum-104.
3) Consortium composed of two cultures: Lactobacillus plantarum-2, Lactobacillus fermentum-104.
4) Known consortium (4 cultures with addition of iron and vitamin C). Additives – variant No.4: ferrous sulfate - 600 mg/L; ascorbic acid - 500 mg/L.

Results of the experiments are provided in Table 3

According to the results, consortium of lactic acid bacteria composed of four cultures has the best physiologic-biochemical properties. Introduction of iron ions and ascorbic acid in the process of cultivation results, correspondingly, in increased content of vitamin C and bisulfite binding agents participating in formation of aromatic complex of finished product - bread.

Test laboratory baking were performed using the improver. Baked bread was characterized by high qualitative indicators. Addition of micronutrients to composition of the improved results in their increased content in finished product. Bread did not become ropy during 72 hours being stored in provocative condition.

Furthermore, model experiments were carried-out for determination of behavior of iron ions in water-flour medium with present lactic acid bacteria (Table 4). Obtained date can be indicative of the fact that used methodologies response to addition of ferrous sulfate to flour medium and complex interactions take place in the process of bacteria cultivation resulting in increased content of ferrous lactate in the medium containing lactic acid bacteria and in some consumption or binding of iron ions by waste products of lactic acid bacteria

Thus, to stimulate accumulation of biomass of lactic acid bacteria at the last stage of cultivation it is necessary to add soya flour in the amount of 20% of wheat flour weight to be used for preparation of nutrient medium or to add sodium citrate in the amount of 0.5% to nutrient medium mass. Drying is to be performed by method of sublimation with prior freezing or by spraying method using "soft" dehydration for the purpose of preservation of high titre of lactic acid bacteria cells.

Sample quantity of dry improver were produced in laboratory conditions using sublimation drier "Jouan" with addition of ferrous sulfate and micronutrients. Quality of dry improver was determined (Tables 5, 6)

Ferrous sulfate was added to quantity of 600 mg/L or 60 mg/100g. Molecular weight of ferrous sulfate heptahydrate is 278 g; iron content is 56 g, thus, 60 mg of ferrous sulfate contains 12.1 mg of iron. Method provides increasing of iron content by 9.26-15.69 mg, which is quite consistent with method error.

When vitamin B_1 was added in quantity of 35 mg/L or 3.5 mg/100g, increase amounted just to 0.36 mg/100g. When vitamin B_2 were added in quantity of 28 mg/L or 2.8 mg/100g, increase amounted to 0.96 mg/100 mL. Obtained results confirm data on consumption of vitamins by lactic acid bacteria in the process of their cultivation.

Response to iron corresponds to added quantity, vitamin C was added in quantity of 500 mg/L or 50 mg/100g, however, decrease amounted just to 2 mg/100 g. Obtained data also may be indicative of consumption of vitamin C by lactic acid bacteria in the process of their cultivation.

By experiment in laboratory conditions, vitamin-mineral flour premix was prepared for enrichment of wheat flour. As the basis, vitamin-mineral additive (VMA) "KAP Komplex No.1" manufactured by "Biomedpreparat" LLP (Stepnogorsk city) and dry improver containing lactic acid bacteria, prepared in laboratory conditions using the sublimation drier "Jouan" were used. Vitamin-mineral additive was added to wheat flour in the ratio of 1:20 and then dry improver was added in quantity providing the expected content of lactic acid bacteria of 10^6- 10^7 CFU/g of mix. MVA addition rate was regulated by Technological Instruction developed by Kazakh Academy of Nutrition dated March 14, 2003, and amounted to 120 g VMA per ton of flour for first grade flour and 150 grams VMA per ton of flour for high-grade flour. Mixture of VMA with high-grade flour in certain ratio results in production of vitamin-mineral flour premix containing the less vitamins and minerals the more ration of VMA and flour is. Physical properties – color, flow ability and odor – are the same as that of VMA, but more smooth and soft

Results of experimental studies showed that iron content in the obtained improver for bread and premix corresponds to quantity added with ferrous sulfate, but content of vitamins B is less than it was expected, which can be indicative of consumption of these micronutrients by lactic acid bacteria in the course of their cultivation.

It was determined, that optimal quantity of added dry biological improver containing lactic acid bacteria is 0.75 – 1.5% of flour weight.For the purpose of uniform distribution of components, the improver shall be added in the course of production of flour premix. When biological improver is added to flour semi-products (sponge, dough), physical properties of that semi-product improve. Furthermore, addition of dry improver to the sponge contributes to accumulation of semi-products' acidity, content of yeast and lactic acid bacteria shows the one order increase of titre of lactic acid bacteria if compared to the control.

Gluten extensibility decreases under the action tested improver additives, which is consistent with data of farinograph analyses on improvement of physical properties of flour when the improver is added. Moisture content of gluten decreases when the improver is added and, tis hydration capacity decreases correspondingly. Elastic properties of gluten increase, presumably due to the improver,

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but addition of only VMA "KAP KOMPLEX No.1" results in decrease of the elastic properties of gluten, which limits the use of this additive when processing the flour with weak gluten. Thus, it was demonstrated, that produced improver plays important role in transformation of protein-proteinase complex of flour, thereby improving the structural-mechanical properties of dough and resulting in further production of elastic crumb with thin-walled and even texture.

Table. 3:.Qualitative Indicators of Improver 24 Hours after Cultivation

Composition of consortium	pH	Titrable acidity number	Content of vitami, %	Content of bisulfite binding agents, mg-equv/100g	Antagonistic activity, mm
1) Control - 4 cultures	3.89	19.8	0.2	0.35	20
2) cultures	3.95	17.4	0.1	0.25	21
3) cultures	3.9	19	0.05	0.3	17
4) Control with additives	3.93	18.6	0.4	0.6	18

Table 4:. Content of Ferrous Lactate

Variant of experience	Content of ferrous lactate, %
Control flour + water	0.177
Experiment flour + water + lactic acid bacteria	0.338
Experiment flour + water + Fe sulfate	1.0495
Experiment + water + lactic acid bacteria + Fe sulfate	0.570
Experiment flour + water + Fe sulfate + lactic acid	0.847

Table 5:.Content of Micronutrients in Dry Improver for Bread

Variant of experience	Iron content, mg/100g	Content of vitamins, mg/100g	
		B_1	B_2
Control, without additives	2.98	0.23	0.12
Experiment, with addition of ferrous sulfate	12.24	0.25	0.14
Experiment, with addition of ferrous sulfate and vitamin B_1	16.54	0.58	0.15
Experiment, with addition of ferrous sulfate and vitamin B_2	18.67	0.25	0.37

Table 6: Qualitative Indicators of Dry Improver for Bread

Variant of experiment	pH	Titrable acidity number	Content of vitamin C, mg/100g	Iron content, mg/100g	Antagonistic activity, mm
1.Control without additives	3.89	20.4	2.1	2.98	20
4. Experiment with addition of iron and vitamin C	3.93	19.2	4.1	12.24	18

4. Conclusion

Wheat flour enrichment technology was established under production conditions. It was demonstrated that flour produced with addition of 0.75 – 1.0% of dry improver contains 10^4 - 10^5 lactic acid bacteria and provides improved microbiological resistance of bread to "potato disease".

Bread production modes were established with use of complex baking improver by sponge dough and straight dough methods. Addition of complex baking improver to the sponge at the rate of 5.0% completely exclude possibility of bread disease even in case of additional introduction of Bacillus subtilis spore suspension. In case of straight dough process, the complex baking improver to be added in the rate of 2.5 – 5.0%, depending on the type of bakery products, and prior to dough kneading the improver shall be reactivated during 30-40 minutes.

Thereby, as a result of performed investigations, the whole new multicomponent baking mixes were developed based on microorganisms, mineral substances, microelements and other components for production of mass-consumption bakery products.

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