The Quality and Microbiological Stability of Quinoa-enriched Wheat Bread

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Abstract. Quinoa flour has high nutritional qualities and is a promising source of functional food ingredients for baked goods. Quinoa was successfully used for enriching wheat bread by domestic and foreign researchers. However, increasing the concentration of quinoa flour decreases the volume yield of wheat bread. This study ultimately aims to increase the organoleptic and structural indexes and the microbiological stability of bread enriched by 17% quinoa flour and 2% wheat protein isolate. We compared the qualities of bread baked using different traditional methods: direct method, indirect method (sponge and dough), and direct method with lactic acid bacteria starter added (LABs from now onward). Compared to other methods, the LABs method decreased the fermentation time, increased air pocket formation, and shaped stability. The addition of LABs starter to the direct quinoa-enriched bread baking method increased the microbiological stability, decreased the staling speed, and reduced rope spoilage risk.

Keywords: Bread · Technology · Rheological properties · Quality indexes · Microbiological stability · Rope spoilage

1. Introduction
High frequency of nutritional diseases in developed countries has increased the population’s demand in the “health & wellness” products. According to the WHO Nutrition and Food Safety reports [32], every third inhabitant of a large city has an unbalanced diet lacking several vital food elements. The survey by the Federal Research Center for Nutrition, Biotechnology, and Food Safety [7] revealed a deficiency of several micro- and macronutrients in the Russian population’s diet. The government of the Russian Federation sanctioned several public health food programs aimed at restoring the dietary structure of the population. As a result, the average protein consumption increased, while the consumption of saturated fats decreased. The available selection of enriched and functional foods has broadened. However, the frequency and levels of noninfectious nutritional diseases in Russian Federation still exceed those of the EU [31]. Thus, the RAS Presidium issued the decree No 178 “On present-day problems of optimizing the nutrition of Russian population,” which formulated the main tasks of mitigating micro- and micronutrient deficiency in adults and children [26]. The government undertook measures aimed at encouraging the scientific development of the agricultural industry. Special attention is paid to developing new recipes and methods of producing enriched and functional foods [27, 33].
Baked goods are a staple in the population’s diet. However, wheat bread has high caloric value, lacks incomplete proteins, and has an unbalanced composition of functional food ingredients. Therefore, enriching baked goods with rare available food ingredients is a priority research area [8, 12, 20, 25, 36]. The database of nutrition-rich and functional-ingredient-rich foodstuffs is diversified continuously. On behalf of the WHO, studies on quinoa, a perspective source of rare functional food ingredients, are conducted regularly [11, 15, 30].

Quinoa flour, high in nutritional value, can be potentially used to model the chemical composition of baked goods, due to its organoleptic, physical, chemical, and technological properties. Quinoa was successfully used in wheat bread enrichment by domestic and foreign researchers [5, 6, 10, 17, 21, 23].

Our previous works have experimentally confirmed that 17 to 20% of quinoa flour significantly increases the nutritional value of wheat bread [10, 17]. To prevent the decreased loaf volume, we recommended adding 2% wheat protein isolate (WPI from now onward) to the mixture. This recipe increases the protein content (from 7.0 to 12%), fiber, B vitamins, and micro- and macronutrients in bread.

We have noted a significant increase in nutritional value and biological efficiency of quinoa-enriched baked goods. However, their recipes and production methods needed to be improved so that the enriched bread could compete with the traditional wheat bread and comply with the technological standards.

This study aims to the properties and microbiological stability of quinoa-enriched bread, baked using three traditional methods: direct dough method, indirect method, and direct method with LABs. This study examined the effect of production methods on the structural properties, staleness, staling speed, and rope spoilage risk of bread.

2. Materials and Methods
The standard and specialized methods of researching baked goods were used in this study. The staleness of bread samples was determined using “Structurometer ST-2” device according to the STP-1703 “Baked goods staleness evaluation” method. This method was developed in the Food Rheology Center at Scientific Research Institute for the Baking Industry [SRIBI] according to “AACC 74-09.01” standard [1, 28]. The method is based on inserting the “Cylinder Ø 36” penetrator 6.25 mm deep into a 25 mm-thick loaf (at 1 mm/s with the initial force of 5 grams), determining the end force of the penetrator, and reversing it to 5 grams. The force indexes were measured at four points in the crumb, and the changes in pressure were then plotted on a graph. The average force was calculated using the graph data. The following indexes were then established:

- $F_{av}$, g – average penetrator force;
- $\Delta t$, days – the time after which the $F_{av}$ index was measured;
- $StSp$, g/day – staling speed (rate).

The staling speed ($StSp$) was calculated using Formula 1.1:

$$StSp = \frac{\Delta F}{\Delta t},$$

where $\Delta F_{(after 48 hours)} = F_{av_{(after 48 hours)}} - F_{av_{(after 24 hours)}}$

The penetrator tests were conducted 24 hours, 48 hours, and 72 hours after the bread was baked. The bread was stored at 23°C with 80% relative air humidity.

The water activity was measured according to GOST R ISO 21807-2015 “Microbiology of food and animal feeding stuffs. Determination of water activity” [14] using the AquaLab Pre device. This device measures relative air humidity in the enclosed volume with constant temperature and automatically determines the water activity.

The microbiological properties of quinoa-enriched bread were measured 24 hours after baking according to:

- GOST 10444.12-2013 “Microbiology of food and animal feeding stuffs. Methods for the detection and colony count of yeasts and molds” [13];
− GOST 10444.15-94 “Food products. Methods for determination quantity of mesophilic aerobes and facultative anaerobes”;
− GOST 10444.8-2013 “Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of presumptive Bacillus cereus. Colony-count technique at 30 °C”.

The rope spoilage level was evaluated using organoleptic and luminescent methods of rope spoilage analysis, according to “Instructions for the prevention of rope spoilage of bread at bakeries” approved by SRIBI [29]. An organoleptic analysis entails studying the bread, stored under thermostatic disease-inducing conditions for 24, 36, 48, and 72 hours. The bread samples are then sliced, and the presence of rope spoilage features (e.g., sliming and stickiness of the crumb, a peculiar smell, black spotting, etc.) is noted. The luminescent method relies on the ultraviolet-reactive luminescence of the rope spoilage colonies, allowing detecting the early stages of the disease.

The studied bread was baked using the mixture enriched with 17% quinoa flour and 2% WPI. The bread was baked using the following methods:
− Direct method (abbreviated DIR);
− Direct with lactobacilli starter (LABs);
− Indirect with quinoa flour added to the pre-dough (PreQ from now onward);
− Indirect with quinoa flour added to the dough (PostQ from now onward);
− Control group (abbreviated Ctrl) without quinoa, using the direct method.

According to SRIBI recommendations, concentrated LABs (acidity of 14.0–18.0 degrees, pH 3.6 ÷ 3.9) were added to the dough as 10% of the total mass of flour [29].

The control group was baked using standard method and recipe, under SRIBI Instructions [29] and Collected volume [19]. The dough was mixed in the BEAR Varimixed TEDDY lab kneader (Denmark). The fermented dough was manually sliced and placed into baking trays with the bottom dimensions of 10×16 cm, maximum dimensions of 12×17 cm, and 10 cm height. The dough was formed and leavened in the UNOX leavening cell (Italy). After leavening, the bread was baked in the UNOX convection oven at 165°C.

The following properties of bread were calculated:
− specific volume of bread and its form-stability (GOST 27669–88);
− porosity (GOST 5669–96);
− acidity (GOST 5670–96);
− moisture content (GOST 21094–75);
− baking loss (GOST 32677–2014);
− organoleptic properties (GOST R 58233–2018).

According to GOST 5670-96, the degree of acidity is the volume, in cubic centimeters, of the 1 mol/dm³ solution of sodium hydroxide or potassium hydroxide needed to neutralize the acidity in 100 g of the end product.

3. Results
Baking method significantly affected the organoleptic, physical, and chemical properties of bread. It also determined the duration, labor-intensiveness, and prime cost of production. The indirect method is the most labor-intensive and costly. The comparison of quinoa-enriched bread baking properties is presented in table 1.

Using the LABs method decreased the fermentation time, compared to other methods.

The quality and organoleptic properties of bread are critical when choosing a baking method. The comparison of organoleptic qualities of quinoa-enriched bread is presented in figure 1. The bread had a more pronounced smell and flavor; the crumb was uniform and developed.
Table 1. The effect of different baking methods on process performance and duration.

| Process parameter       | Baking method |
|-------------------------|---------------|
|                         | indirect      | direct | LABs |
| Fermentation time, min  | 180           | 90     | 60   |
| Leavening time, min     | 20            | 30     | 30   |
| Pre-dough acidity, deg  | 3.2           | -      | -    |
| Dough acidity, deg      | 3.5           | 3.2    | 3.3  |
| Baking temperature, °C  |               | 170    |      |
| Baking time, min        | 30            | 20     | 20   |

*Source:* Compiled by authors.

Figure 1. The effect of baking method on organoleptic properties of quinoa-enriched bread *Source:* Compiled by the authors.

Table 2 presents the comparison of physical and chemical indexes of quinoa-enriched bread.

Table 2. The effect of baking method on physical and chemical indexes of quinoa-enriched bread.

| Index                          | Control group | LABs | Indirect | Direct | PostQ | PreQ | Direct |
|--------------------------------|---------------|------|----------|--------|-------|------|--------|
| Specific volume, cm\(^3\)/g    | 2.4           | 2.6  | 2.5      | 2.3    | 2.3   |
| Porosity, %                    | 62            | 72   | 66       | 68     | 67    |
| Acidity, degrees               | 1.6           | 3.8  | 3.4      | 3.5    | 3.6   |
| Water content, %               | 37.0          | 42.5 | 41.8     | 42.5   | 42.3  |
| Final product weight, g        | 392           | 385  | 390      | 391    | 387   |
| Baking loss, %                 | 6.7           | 8.3  | 7.14     | 6.9    | 7.86  |
| Form-stability                 | 0.43          | 0.63 | 0.75     | 0.58   | 0.57  |

*Organoleptic properties*

| Bread appearance               |                |
|--------------------------------|-----------------|
| Shape                          | That of the baking tray, no overflowing sides |
| Surface                        | Smooth, no fissures and cracks |



Crust color | Light brown | Golden brown | Golden brown | Light brown | Golden brown
--- | --- | --- | --- | --- | ---
Crust shape | | Domed | | | |
Crumb properties
Color | White | White-gray |
Porosity | Developed texture; no large air pockets, or dense areas; thin and smooth; no detachment from the crust |
Taste | Of wheat bread | Of wheat bread with quinoa hints |
Smell | Of wheat bread | Of wheat bread with quinoa undertones |

Source: Compiled by authors.

LABs was the only method that did not decrease the bread quality if compared to the control group. Adding a lactobacilli starter to the dough increased the porosity, form-stability, and specific volume of bread.

The staling rate of the end product is an essential factor to consider when choosing a baking method. Rheological changes in the crumb cause staling. The changes in the rheological properties of baked goods were examined using the “Structurometer ST-2” device. The dynamics of the penetrator force were plotted on the graph presented in figure 2.

![Figure 2](image)

Figure 2. The effect of baking method on the penetrator force in crumb deformation of quinoa-enriched bread. Source: Compiled by the authors.

After 24 hours, the penetrator force was lower in bread baked using the direct method (1,376 grams) and LABs method (1,154 grams), compared to the control group (1,633 grams) and other forms. Thus, the crumb of direct-baked and LABs bread was significantly softer.

To evaluate the penetrator force dynamics, we introduced ΔF index, denoting the change in force over one day (see Table 3).

| Index | ΔF (after 48 hours), g | ΔF (after 72 hours), g |
|-------|----------------------|----------------------|
| Control group | 499 | 314 |
| PreQ | 544 | 567 |
| DIR | 591 | 279 |
| LABs | 408 | 282 |
| PostQ | 451 | 351 |

Source: Compiled by authors.
The LABs bread demonstrated minimal changes in penetrator force (408 g and 282 g after 48 hours and 72 hours, respectively). In the PostQ bread, the increase in force was 23% and 25% higher than in LABs bread. In the direct method, the crumb firmed up significantly after 48 hours (591 g), but then, after 72 hours, the increase was lower than in other methods (279 g). The PostQ bread had a softer crumb after 48 hours, but firmer after 72 hours. The latter increase in force was 351 grams, higher than indirect and LABs bread. The PreQ bread firmed much faster than the others (544 g after 48 hours, and 567 after 72 hours).

After 72 hours, we calculated the staling speed using Formula 1.1 (see figure 3).

![Figure 3](image)

**Figure 3.** The effect of baking method on quinoa-enriched bread staling speed (72 hours after baking) Source: Compiled by the authors.

The LABs and PostQ bread staled slower (324 g/day and 401 g/day, respectively) than the control group bread (407 g/day). The direct bread and PreQ bread staled faster (by 12% and 37% respectively) than the control group bread.

In choosing a baking method, one must keep in mind the microbiological stability and resilience of the end product, especially its ability to resist rope spoilage. The contamination rate is increased by the microbe content of raw materials and new unconventional raw materials. Table 4 presents data on microbiological contamination of quinoa-enriched bread.

**Table 4.** The effect of baking method on microbiological indexes of quinoa-enriched bread (24 hours after baking)

| Microbiological properties, CFU/g | Baking methods |
|----------------------------------|----------------|
|                                   | Control group  | DIR | LABs | PreQ | PostQ |
| Mesophilic aerobic and facultative anaerobic microorganisms | 50 | 52 | 25 | 60 | 55 |
| Yeast | <10 | <10 | <10 | <10 | <10 |
| Mold | 38 | 40 | 20 | 48 | 45 |
| *Bacillus* bacteria | 35 | 40 | 20 | 60 | 40 |

*Source: Compiled by authors.*

Adding quinoa flower to the mixture contaminates bread with microorganisms. The indirect method, with its slower fermentation, increases the microbe content of the crumb. Using the LABs method decreases the contamination with all types of microorganisms, including spore-forming bacteria of *Bacillus* genus, which cause rope spoilage.

According to the Instructions [29], the cold bread samples were wrapped with clean porous water-soaked paper, put into alcohol-disinfected plastic bags, and stored in the thermostat at 38 ± 1 °C. To
determine the contamination, we used the organoleptic and luminescent methods. The luminescent colonies were identified using the “Luminoscope” device (see figure 4).

After 36 hours, the first signs of rope spoilage were identified in DIR bread (32 CFU) and PostQ bread (24 CFU). PreQ bread had the early signs of rope spoilage after 48 hours (36 CFU). The control group was more resilient to rope spoilage than quinoa-enriched DIR, PreQ, and PostQ bread. LABs bread was the most resilient sample, as it had only 15 CFU after 60 hours of thermostat storage.

Simultaneously, we examined the samples visually, using the organoleptic method, at 24, 48, and 72-hour marks.

According to the Method [28], the organoleptic signs of rope spoilage consist of sliming, stickiness of crumb, black spotting, and a peculiar smell. The first signs of rope spoilage were identified in PreQ and PostQ samples at the 48-hour mark. DIR bread had the first signs of the disease after 50 hours, and LABs after 76 hours. Thus, the application of the organoleptic and luminescent method confirmed that the LABs samples were the most resilient to rope spoilage.

An essential criterion of microbiological stability is the “water activity” index of baked goods. Water activity (A_W) controls the shelf life and the risks of rope spoilage in the end product. It is an index of water association with other ingredients in the bread mixture [34]. Figure 6 presents data on water activity in quinoa-enriched bread. Adding quinoa flour to the mix decreases water activity if compared to the control group (A_W of 0.956.) The LABs sample had the lowest A_W index.
4. Discussion

In this study, we examined the effect of baking method on the microbiological stability and other bread properties. We compared the organoleptic, physical, chemical, and microbial properties of bread samples baked using different methods. The direct baking method is less labor-intensive; it decreases the fermentation time of bread. Adding quinoa flour to the bread mixture reduces the specific volume and rheological properties of the end product, which was confirmed by this experiment and the works of I. G. Belyavskaya [6]. The decreased physical and chemical qualities of quinoa-enriched bread predetermined the need for baking method modification. The results of a research by I. G. Belyavskaya [4] confirmed the efficiency of using quinoa flour in the bread starter. To apply the direct method to baking quinoa-enriched bread, we studied the effect of adding lactobacilli starter to the bread mixture (LABs method). The direct method with the addition of LABs allowed us to decrease the fermentation time of quinoa-enriched bread and enhance the properties of the end product. LABs and yeast were added to the mixture according to SRIBI recommendations [29].

The results of this experiment confirmed the effectiveness of using LABs method in baking quinoa-enriched bread. The end product was on par with the indirect-baked bread by organoleptic qualities, but it had better smell and taste.

Baking process introduces complex changes to the biochemical, chemical, and mechanical structure of bread. The used ingredients and baking method predetermine the specifics of these changes. Adding quinoa flour and LABs to the direct-baked bread mixture changed the structural and mechanical qualities of crumb. To assess the qualitative changes occurring in dough formation and baking, we used the integral method that describes the effect of technological factors on the qualitative and quantitative properties of bread. Chernykh et al. [9] recommended choosing a baking method according to the rheological properties of the semi-finished and end product. The analysis of structural and mechanical properties is widely used for food texture evaluation at different stages of production and storage [9]. Various researchers use the structural-mechanical properties (i.e., porosity, specific volume, form-stability, and crumb deformation) in optimizing the recipes and methods of producing bread enriched by quinoa flour, flaxseed flour, and other raw materials [5, 6, 18].

The staling rate is an important criterion to consider when evaluating baking methods. Staling decreases the compressibility and elasticity of crumb, increases crumbling during slicing. The rheological qualities of bread were studied in the “Structurometer ST-2” device using the “Baked goods staleness evaluation STP-1703” method, developed at the Food Rheology Center of SRIBI. The LABs samples have demonstrated the best rheological properties. After 24 hours, the penetrator force in crumb deformation was 20% lower than in direct-baked models, and 50% lower than in indirect-baked samples. Therefore, the crumb of the LABs bread was much softer than all other samples. Adding LABs to the bread mixture may encourage the accumulation of water-soluble quinoa flour substances and lactobacilli fermentation by-products, which support yeast growth and bread leavening.

Adding quinoa flour to LABs and indirect mixtures decreased the staling rate by 20% and 2%,
respectively. The two direct methods had faster staling (14% faster). Therefore, the combined alcohol-lactic acid fermentation in the quinoa-wheat flour mixture creates hydrolyzates that increase the quinoa protein structurization and gluten matrix development in the dough formation process. Adding LABs to the dough mixture creates smaller air pockets caused by a stronger wrapping of starch granules by gluten films. It leads to a more resilient gluten matrix and slower staling.

Koneva et al. established the same patterns in the rheological properties of flaxseed-enriched wheat bread. They suggested that the porosity and specific volume of wheat-flaxseed bread changed due to the lipid oxidation in flaxseed flour. Hydroperoxides of unsaturated fatty acids oxidize the sulfhydryl proteins and produce new disulfide bonds that affect the gluten matrix [15].

We conclude that the LABs method of quinoa-enriched bread baking decreases the staling speed and enhances its consumer properties.

The microbiological stability is an essential quality of bread. Microbial contamination of raw materials significantly affects the quality of the end product and its shelf life. Enriching the bread with non-conventional raw materials promotes end product contamination. The experiment has shown that the direct and indirect method of quinoa-enriched bread baking increased the microorganism contamination by 4% and 10–20%, respectively. Adding LABs decreased overall contamination by almost 50%. LABs method also decreased mold contamination by 40%, while the direct and indirect methods increased by 33% and 18%, respectively. Increased raw material contamination also promotes rope spoilage caused by Bacillus genus bacteria. These bacteria synthesize proteolytic and amylolytic enzymes that reduce the quality of dough and end product, introduce unpleasant smell and taste, and spoil the appearance of bread [2, 29]. Studies [16, 24] have shown that not only Bacillus subtilis causes rope spoilage, but also other Bacillus genus bacteria:

- Bacillus licheniformis;
- Bacillus pumilus;
- Bacillus cereus;
- Bacillus firmus;
- Bacillus clausii;
- Bacillus megaterium;
- Bacillus polymyxa.

Bacillus contamination increased in indirect-baked and direct-baked bread by 14% compared to the control group. LABs method decreased by 57%. The SRIBI Instructions [29] recommends lowering the pH of dough to prevent rope spoilage. The experiment has shown that adding lactic acid bacteria starters reduces the contamination by all microorganism groups, including Bacillus bacteria. Lactobacilli are antagonistic to many pathogenic microorganisms. Moreover, the fermentation process lowers the dough pH, reducing the contamination risks further.

The luminescent and organoleptic examination of quinoa-enriched bread samples has shown that the combined alcohol-lactic acid fermentation reduces the risks of rope spoilage. Mashkin et al. [22] confirmed the effectiveness of lactic starters in preventing microbiological spoilage of bread, including molding and rope spoilage.

The water activity index ($A_w$) also affects the microbiological stability of foodstuffs, including baked goods. The $A_w$ index is used in several countries, including Belarus and Ukraine, for evaluating the expiration date (shelf-life), the storage conditions of raw food resources, and end products. The index is also being introduced to food regulation in Russia [3]. The recommended water activity index for bread must not exceed 0.95. Water activity may be decreased by various raw materials and techniques that bind free moisture in the end product. Adding quinoa flour to the bread mixture decreased $A_w$ from 0.956 to 0.941 in the direct-baking method and 0.948 in the indirect method. Adding LABs further lowered $A_w$ to 0.879. Therefore, we may conclude that combined alcohol-lactic acid fermentation significantly reduces water activity in the end product. Vetokihn et al. [35] also
noted this fact, explaining it by the increased production of water-binding substances, caused by alcohol-lactic acid heterofermentation.

5. Conclusion
In this study, we examined the effect of adding 17% quinoa flour and 2% WPI to the bread mixture, baked by indirect, direct, and direct with lactobacilli starter methods. We conclude that the lactobacilli starter method was the best by organoleptic, physical, chemical, rheological, and microbiological indexes. The indirect-baked bread samples were in second place.

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