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STORAGE OF EXTRUDED CEREAL AND LEGUME GRAIN BASES IN ION-OZONE MEDIUM

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SUMMARY

The modern food industry seeks to produce ecologically healthy and clean food products. However, finding effective means of storing raw materials without losing their quality is still a major problem faced by the industry. One of the promising methods on long-term storage of grain bases for good quality and safety is the ion-ozone cavitation treatment. Therefore, this study aims to determine the influence of various ion-ozone treatment parameters on the quality and safety factors of millet, buckwheat, chickpea, and lentil grain bases at the Food Quality and Safety Assessment Laboratory, Almaty University of Technology, Almaty, Kazakhstan. With ozonation, the buckwheat grain bases showed a significant increase in fatty acids, the millet and chickpea grains showed a slight increase, while the lentil seeds exhibited a slight decrease in fatty acids compared to the control. Overall, by treating with a low concentration of ion-ozone, the buckwheat showed fewer microorganisms; in chickpea grains the molds and yeast did not develop; in millet grains the yeast was absent and molds were not higher than control; and in lentil grain bases, the permissible yeast growth with no molds was observed. The storage life of extruded grain bases after ion-ozone cavitation treatment increased by a minimum of 60 days (millet) and to more than 240 days (buckwheat). The optimum basic parameters were determined, ensuring a maximum shelf life of up to eight months without losing the quality of the cereals and legume grain products. The said procedure was found as an effective measure for long-term storage of the extruded grain bases with sustainable safety and quality.

Keywords: Chickpea, lentil, millet, buckwheat, storage, microorganisms, food quality and safety

Key findings: The optimal methods of safety and maintaining the quality of food grain bases have been determined in millet, buckwheat, chickpea, and lentils. Treatment with ion-ozone cavitation allows maintaining the stability of extruded bases of buckwheat grain for 240 days, while millet, chickpea, and lentil for 180 days.

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INTRODUCTION

Extruded cereal grains are promising in today's food industry with the many possibilities to create healthier and more nutritious food products added with innovative flavors. However, the maintenance of raw materials is of great importance for their production. Grains of legumes are high in nutrients and fiber, low in fat, and have a healthier lipid profile due to their mono- and poly-unsaturated fatty acids (Grela *et al.*, 2017; Ciudad-Mulero *et al*., 2020; Ferreira *et al.*, 2021). Containing desirable qualities, these food grains are of greater value in the food industry, along with high-value crops such as buckwheat and millet. Preliminary studies have identified the most promising crops in terms of extruded grain bases production i.e., buckwheat, millet, chickpea, and lentil, which are distinguished by several exceptional properties (Adedeji *et al.*, 2018; Bessada *et al.*, 2019; Faridy *et al.*, 2020). Using the cereals and legumes as extruded grain bases in different food products raise the issue of ensuring their safe and healthy storage (Woo and Adachi, 1997; Shaimerdenova *et al.*, 2019).

Contemporary methods of cereal grains storage, including extruded grain bases, involve the use of specially modified containers and rooms managed as artificial ecosystems (Mangkita *et al*., 2005; Pekmez, 2016). One of all these technologies is storage in a controlled gas environment. This technology was found not only successful to replace fumigants for insect control but also effective in preserving the quality of the commodity without residues after processing (Navarro and Navarro, 2016). Of all the regulated gas environments, storage using ozone (O_3) is the most interesting and an alternative method for pest control of cereal grains (Christ *et al.*, 2017; Zinhoum, 2019).

Ozone can penetrate large masses of grains, is highly oxidizing and unstable, and rapidly degrades to oxygen without leaving residues. It is also extensively used in drinking water purification and disinfection of medical devices, as well as, the removal of odors, dyes, pesticides, inorganic and organic compounds. In agriculture, ozonation is an inexpensive and environment friendly methodology, used to store and preserve fruits and vegetables, and decontaminate and disinfect surfaces of production equipment and packaging materials, water, and perishable food products (FDA, 2001).

Ozone is also classified by the U.S. Food and Drug Administration (FDA) as a "Generally Recognized as Safe Substance" (GRAS) and is widely used as an antimicrobial agent. However, in some cases, ozone contributes to lipid oxidation, protein modifications, grain color changes, and the development of undesirable flavors, thereby affecting the food and end-user qualities of the products. In addition, its effectiveness depends on factors such as the food matrix, ozone concentration, and exposure time (FDA, 2001; Mendez *et al.*, 2003; Tiwari *et al.*, 2010; Christ *et al.*, 2017). Therefore, we need to investigate the effectiveness and safety of ozone as an antimicrobial agent.

The present research considered the recommendations of Iztayev *et al.* (2018) on the effectiveness of ion-ozone cavitation treatment of the cereal grains. The present method is also based on the combination of quantum-physical processes and disinfection, which leads to preventing the development of undesirable processes in the stored cereal grains, by increasing their technological and biochemical properties, and the qualities of the food products. This study aimed to develop a modern storage method for extruded grain bases using ozone and study its effectiveness, as well, in ensuring the safety and preservation of product quality during long-term storage.

MATERIALS AND METHODS

In this study, the effectiveness of long-term storage for four types of extruded grain bases made from buckwheat, millet, lentil, and chickpea grains was investigated through the ion-ozone medium.

Ion-ozone cavitation treatment

The ion-ozone cavitation treatment of extruded grain bases was conducted on a semi-industrial processing line at the Food Quality and Safety Assessment Laboratory, Almaty University of Technology, Almaty, Kazakhstan (Figure 1).

Determination of effective parameters through ion-ozone cavitation

The effectiveness of ion-ozone cavitation treatment was determined and evaluated in a factorial design. The factors tested were i.e., Ion concentration (500,000 vs. 500 units cm^{-3}), Ozone concentration (3000 vs. 1000 g m^{-3}), Processing time (minutes), and Pressure (atm). In total, 32 components of test batches resulted from the factorial experiment of eight test batches for each extruded grain base (Table 1). As a negative control group, four samples of untreated extruded grain bases were put into long-term storage.

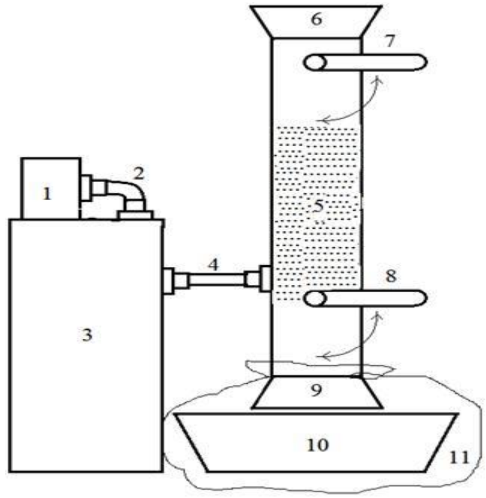

Figure 1. Semi-industrial ion-cavitation process line. 1: compressor, 2: air piping, 3: ion-ozone unit, 4: piping of ion-ozone mixture, 5: cavitation unit, 6: grain filling pipe, 7: grain filling valve, 8: pressure relief valve, 9: grain discharge pipe, 10: tank - grain collector, 11: grain trap.

Table 1. Conditions for ion-ozone cavitation treatment before long-term storage of extruded grain bases.

Test Batch	Ion concentration (units cm^{-3})	Ozone concentration (g m^{-3})	Processing time (min.)	Pressure (atm)
1	500000	3000	15	3
2	500	1000	15	3
3	500000	3000	5	3
4	500	1000	5	3
5	500000	3000	15	1
6	500	1000	15	1
7	500000	3000	5	1
8	500	1000	5	1

To determine the effectiveness of the ion-ozone mixture, the number of colony-forming units of yeast and mold fungi, as indicators of microbiological safety, and the fatty acids, as an indicator of the qualitative condition of grain bases, were assessed. The study was conducted following the normative-methodological documents GOST 31700-2012 "Grain and products of its processing. Method for determination of fatty acids" and GOST 10444.12-2013 - Microbiology of food products and animal feed. Methods for detecting and counting yeasts and molds (GOST, 2013; 2014).

Determination of duration and shelf life of extruded grain bases

After ion-ozone cavitation treatment, the extruded grain samples were stored for eight months. Biochemical and microbiological parameters of stored grain samples were checked every two months, following the GOST 26791-89 "Products of grain processing, packing, marking, transportation and storage" (GOST, 2010).

RESULTS

Effectiveness of the ion-ozone cavitation treatment in extruded grain bases

The extruded buckwheat grains were treated with minimum ion-ozone concentration at maximum pressure and exposure time, as well as, with maximum ion-ozone concentration at minimum pressure and exposure time (Figure 2). After the treatment of buckwheat grain bases under these conditions, the increase in fatty acids compared to the control sample was detected. Microorganisms appeared in

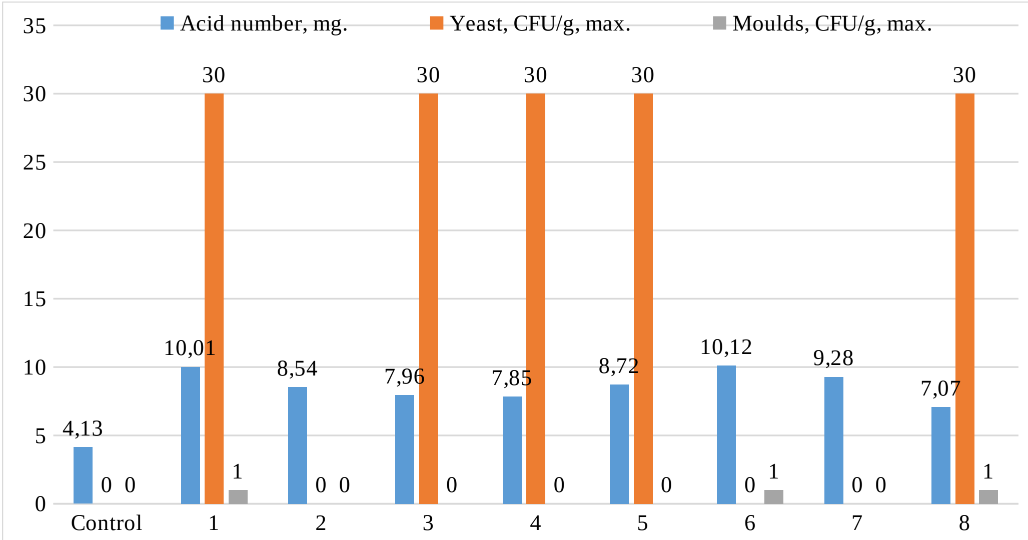

Figure 2. Biochemical and microbiological parameters of extruded buckwheat grain bases.

individual tests, and the fatty acids were about two times higher than the control. The best results were observed mostly at the lowest gas concentration.

For millet grain base, the optimum treatment parameters were also the maximum ion-ozone concentration at minimum pressure and exposure time. After this treatment, the fatty acids increased slightly compared to the control, and yeast was absent altogether (Figure 3). The molds were not higher than the control after the treatment. This can be explained by the high concentration of ions and ozone which contributed to the destruction of molds and yeast despite minimum exposure.

In the case of the chickpea grain base, prolonged exposure to low concentrations of ion-ozone at low pressure was optimal. After this treatment, molds and yeast did not develop in the chickpea grain bases, and the fatty acids were slightly increased compared to the control (Figure 4). The best results were achieved with high gas concentration, although the sixth test also showed good results. The ion-ozone cavitation treatment proved to be the most favorable for chickpeas. The minimum concentration of ion-ozone with maximum exposure time caused significant penetration of ion and ozone molecules, resulting in inhibition of bacteria, spores formation, and reduction in the physicochemical processes.

The optimum treatment parameters for the lentil grain bases were found at the short-term exposure to high/low concentrations of ion-ozone at low pressure. Thus, with high ion-ozone concentrations, the complete absence of yeast and a slight decrease in fatty acids were observed (Figure 5). However, in the fourth test at low gas concentrations, a high amount of molds was detected (up to 60 units). In total, 3 CFU g^{-1} of mold fungi were detected, however, it did not exceed the maximum permissible standard defined by the technical regulations of the Customs Union (50 CFU g^{-1}). After treating with a low concentration of ion-ozone, lentil grain bases recorded no mold, withmaximum permissible yeast growth, and a slight decrease in the fatty acids compared to the control.

Thus, the present results established the interdependence of the fatty acids, yeast, and mold content on the cavitation treatment of the extruded grains. Furthermore, individual optimal biochemical and microbiological parameters for the ion-ozone cavitation treatment of the extruded grain bases (buckwheat, millet, chickpea, and lentils) were established to determine their storage life tenacity and longevity.

Resistance of extruded grain bases in long-term storage

Studying the effects of ion-ozone cavitation treatments on the quality and safety parameters of embedded test samples stored for eight months revealed an increase in the shelf life of extruded grain bases as compared to untreated control samples (Figures 6, 7, 8 and 9).

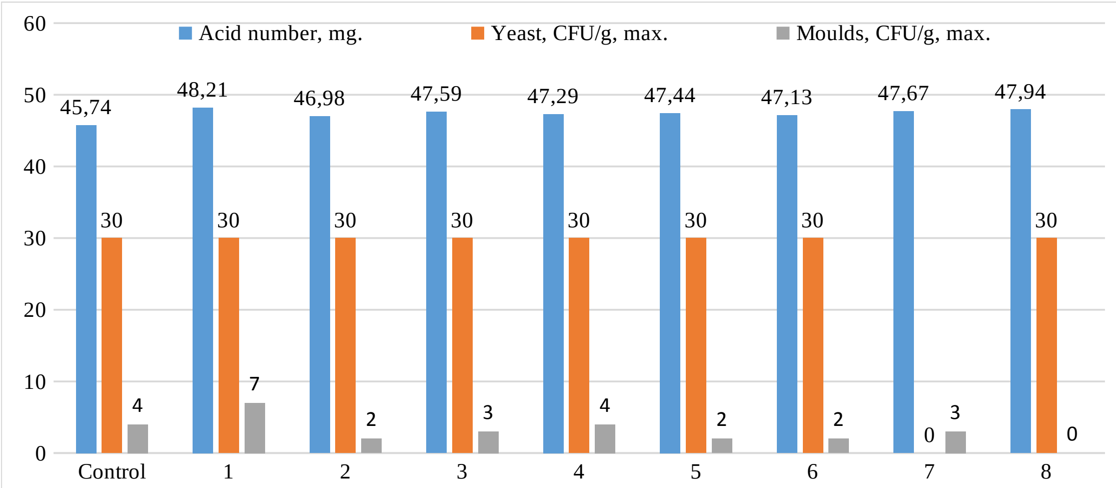

Figure 3. Biochemical and microbiological parameters of extruded millet grain bases.

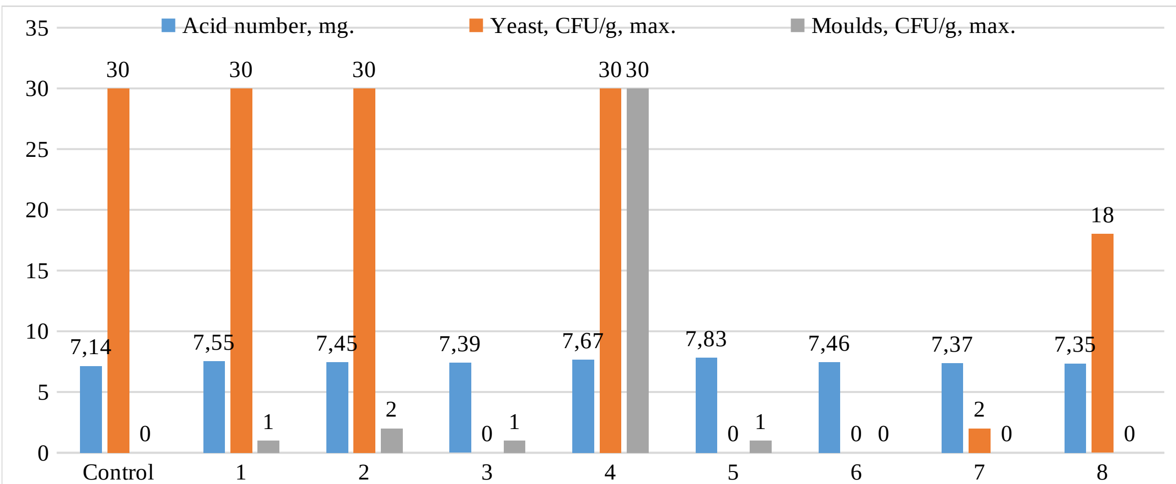

Figure 4. Biochemical and microbiological parameters of extruded chickpea grain bases.

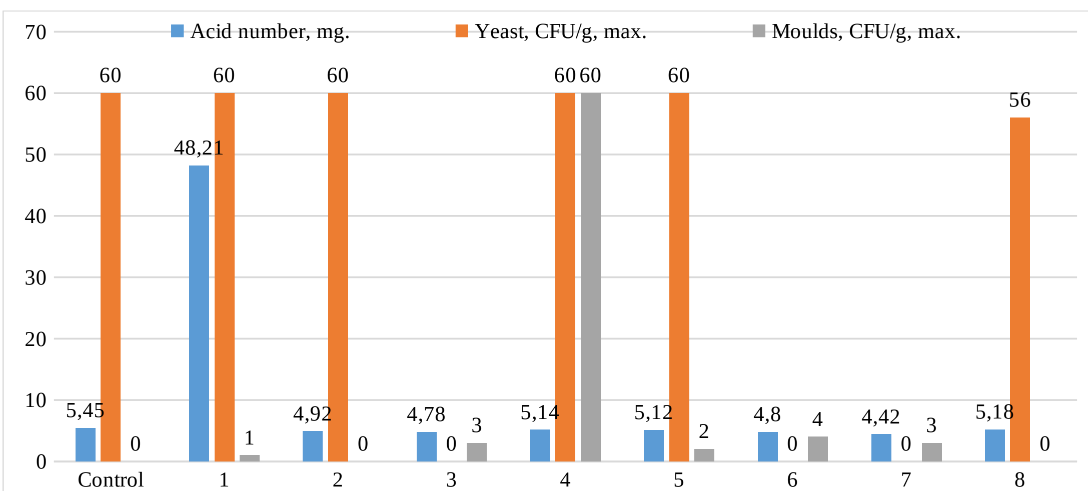

Figure 5. Biochemical and microbiological parameters of extruded lentil grain bases.

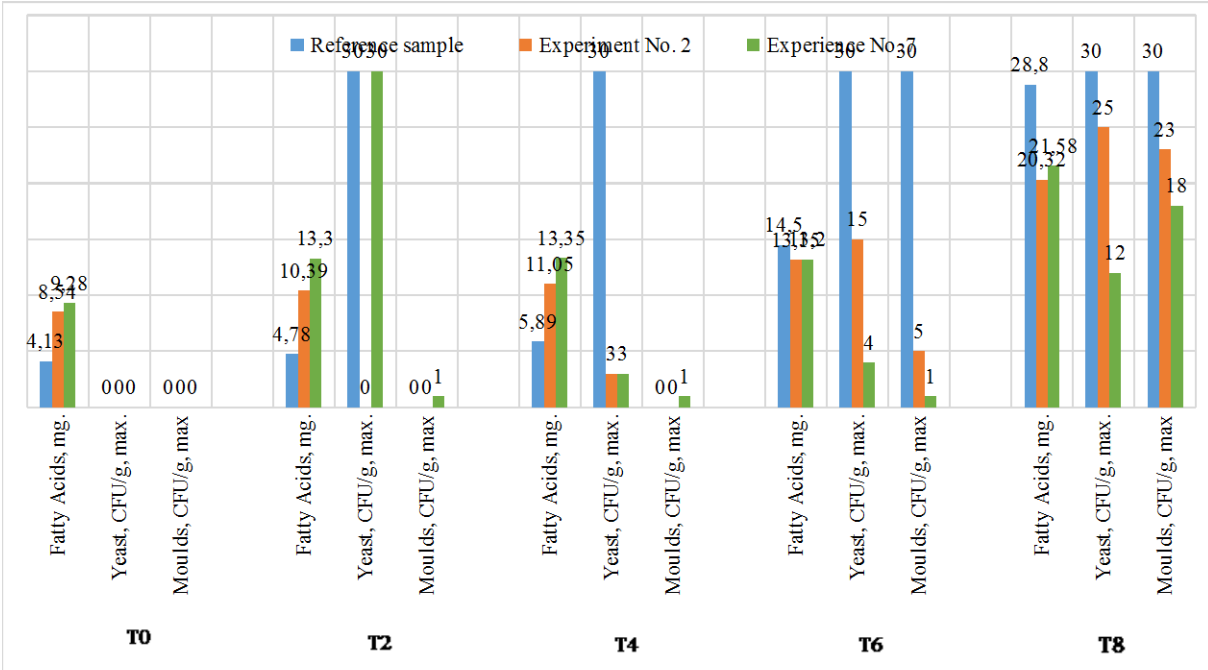

Figure 6. Quality parameters of the prototypes of extruded buckwheat grain bases when stored for up to 240 days. T0: 0 days, T2: 60 days, T4: 120 days, T6: 180 days, T8: 240 days.

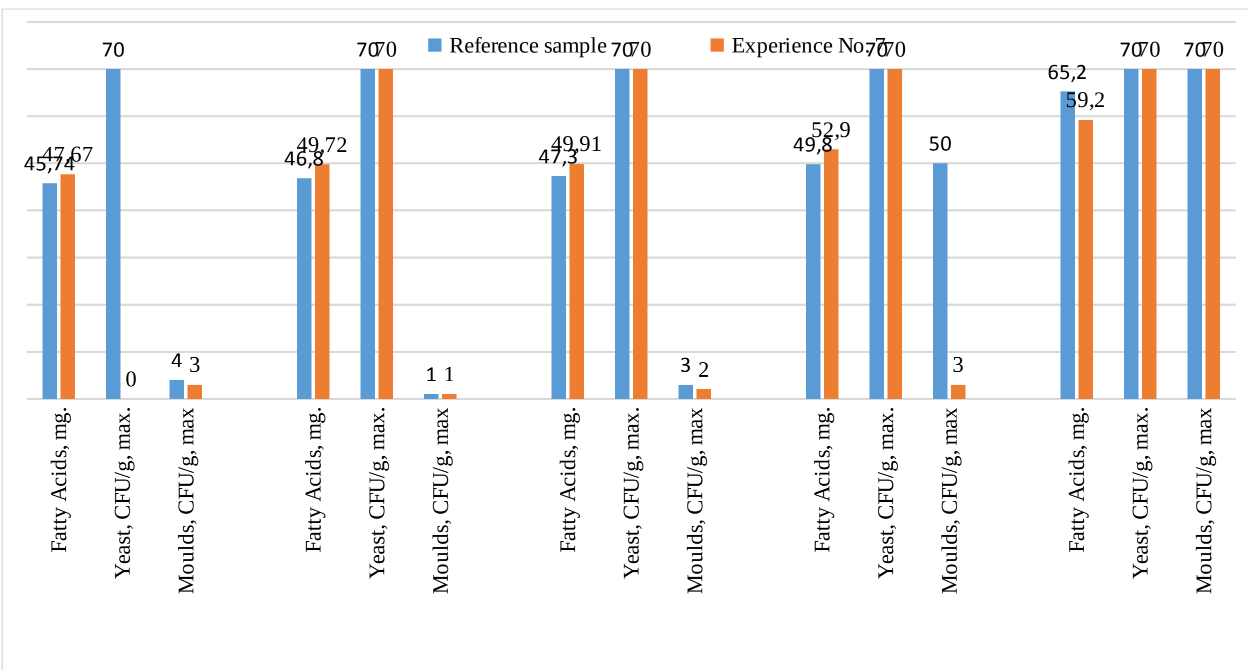

Figure 7. Quality parameters of the prototypes of extruded millet grain bases when stored for up to 240 days. T0: 0 days, T2: 60 days, T4: 120 days, T6: 180 days, T8: 240 days.

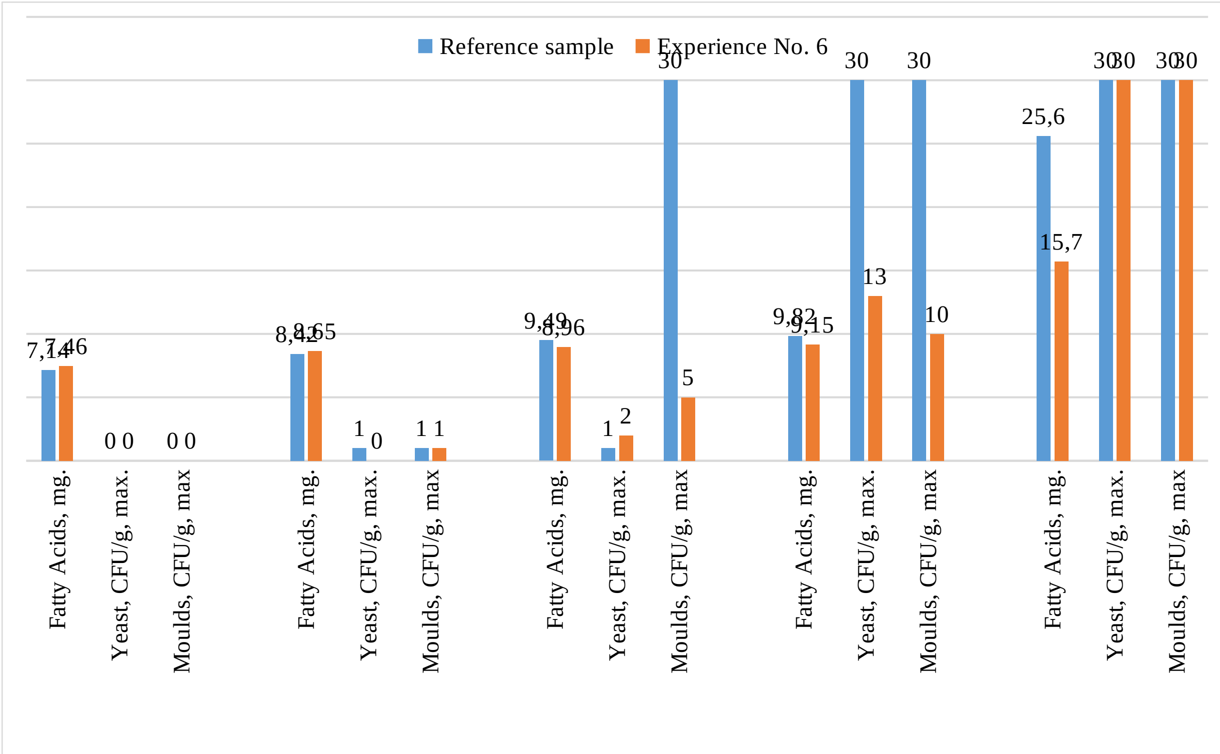

Figure 8. Quality parameters of the prototypes of extruded chickpea grain bases when stored for up to 240 days. T0: 0 days, T2: 60 days, T4: 120 days, T6: 180 days, T8: 240 days.

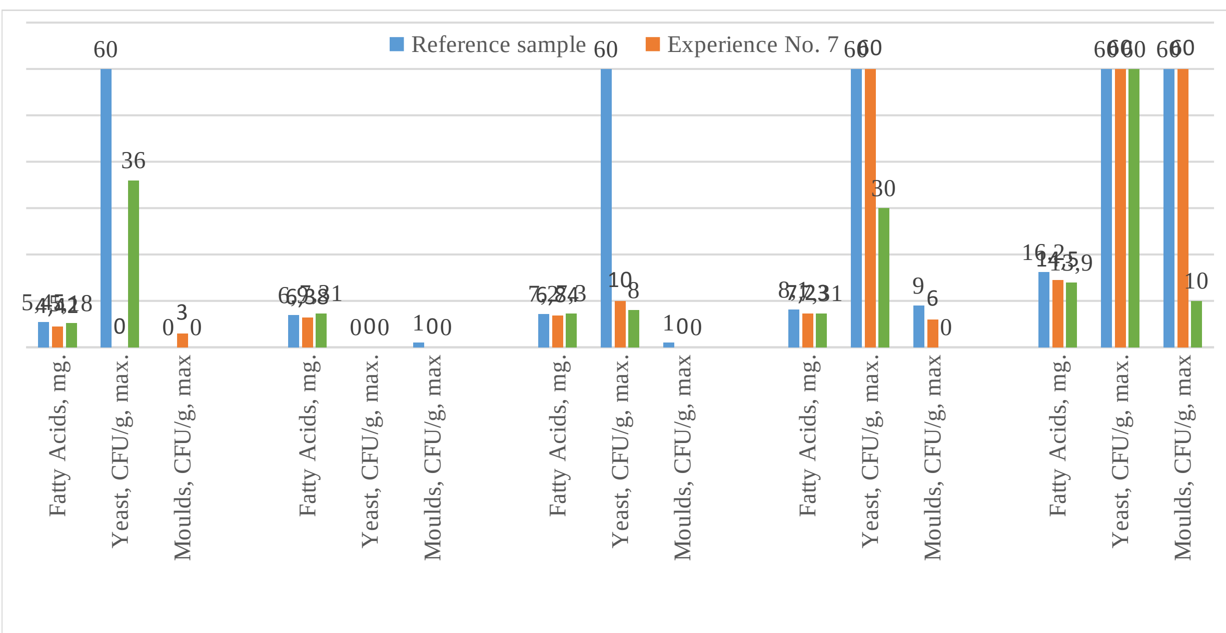

Figure 9. Quality parameters of the prototypes of extruded lentil grain bases when stored for up to 240 days. T0: 0 days, T2: 60 days, T4: 120 days, T6: 180 days, T8: 240 days.

After long storage of extruded buckwheat grain bases for 180 days treated with minimum ion-ozone concentration at maximum pressure and exposure time, and with maximum ion-ozone concentration at minimum pressure and exposure time, a slight increase was observed in the fatty acid, yeasts, and molds as compared to the control grain samples. After 240 days of storage, the number of molds and yeasts did not reach the maximum permissible safety standards as defined by the Customs Union technical regulation (50 CFU g^{-1}). However, in the controlled buckwheat grain samples, there was a continuous growth of microorganisms after 180 days of storage (Figure 6). The highest resistance to microorganisms was observed in buckwheat grain bases after a short-term treatment with high concentrations of ion-ozone at low cavitation, allowing them to be stored for more than 240 days.

After 180 days storage of extruded millet grain bases, the grain samples showed an increase in the fatty acids, yeasts, and molds growth which validated that millet grain bases cannot be stored for more than 180 days (Figure 7). During 120 days storage period, the chickpea grain bases showed slight changes in the fatty acids, yeasts, and mold numbers. At 120 days of aging, the mold does not show prolonged growth but it does at 180 days. Storage conditions in 15 minutes treatment with low concentrations of ion-ozone at low pressure, the chickpea grain bases stored for 180 days were observed with microbial growth but within normal limits (Figure 8). However, after 240 days of chickpea grains storage, the experimental samples showed an increase in fatty acids, yeasts, and mold growth.

The lentil extruded grain bases were treated with five-minute low-pressure ion-ozone treatment and stored for 180 days, small changes were observed in the fatty acids and molds as compared to the control samples. On the other hand, there was a significant increase for yeast at 180 days (Figure 9). However, after 240 days of storage, the experimental samples of lentil grains showed an increase in the fatty acids, and growth of yeasts and molds, which makes it impossible to be stored for more than 180 days.

The present results revealed that after 120 days (four months) of storage, all the controlled samples (without ion-ozone cavitation treatment) showed an increase in the fatty acids and the growth of yeasts and molds (Figures 6 - 9). However, a high concentration of ion-ozone (ion 500000/ozone 3000) in the cavitation field with a minimum treatment of five minutes had a reducing effect on the microorganisms and promote the prolonged healthy storage of extruded buckwheat and millet grain bases. However, for extruded chickpea and lentil grain bases, a low concentration of ion-ozone (ion 500/ozone 1000) in the cavitation field with 15 minutes treatment contributed to improved storage stability, compared to the control samples (Figures 8 and 9).

DISCUSSION

To ensure the efficient use of cereal products for intended purposes, the cereal grain bases must be stored safely before their use. Currently, in developing countries, the high losses are mostly associated with weaker storage technologies and gaps in food supply chains (TR CU 021/2011, 2011). The present results confirmed that high concentrations of ion and ozone, despite \short exposure, contribute a destructive effect for microorganisms on the surface of the grain bases. The destruction of microorganisms was due to the action of cavitation within the microorganisms themselves, and on the surface, where a thin layer of air is absorbed, these particles entering the zone at reduced pressure, serve as defense with overpressure due to the formation of cavitation (Iztaev *et al.*, 2017).

This is particularly important in the storage of cereal grains and their products, which play a key role in economic and social progress. When storing grain and grain products properly, it is necessary to take into account possible significant qualitative and quantitative losses due to factors such as environmental parameters (temperature, grain moisture, pH, moisture, etc.), the type of storage structure used, duration and purpose of storage, storage method and biological factors (insects, pests, microorganisms, and rodents) (Pekmez, 2016; Saadat *et al.*, 2020). In buckwheat grain bases, the present results are validated from past studies that the combination of maximum exposure time and pressure causes effective penetration of ion and ozone molecules, which were capable of inhibiting the growth and development of bacteria, spores formation, as well as, inhibition of physiological and physicochemical processes (Iztaev *et al.*, 2017).

For extruded chickpea grain bases after 240 days of storage, the increase in fatty acids concentrations was followed by the growth of yeasts and molds, thus the impossibility to store extruded chickpea grain bases for more than 180 days. This result can be explained with the high-fat content (0.68%) in terms of dry matter in chickpea grains, while in other crop samples it varied from 0.25 to 0.48% (Shaimerdenova *et al.*, 2019). High-fat content is known to be one of the main causes for the volatility of grain products during storage. The present results also exhibited instability during the storage of extruded lentil grain bases. This result correlates to its fat content, where the mass fraction of fat in terms of dry matter was 0.48% (Akhtar *et al*., 2015; Shaimerdenova *et al.*, 2019). Previous studies also showed similar insecurity during the storage of lentil grain bases (Priezzheva, 2013).

The increase in fatty acids of millet grain bases is due to lipid hydrolysis by lipase enzyme and the resulting accumulation of free fatty acids (Priezzheva, 2013). According to M.G. Gordon's studies, the rate of lipid oxidation depends upon the fatty acids composition i.e., the higher the content of polyunsaturated fatty acids (linolenic acid), the more products are formed, which are perceived as extraneous flavors (Gordon, 2006). According to Nechaev (1975), millet lipids contain up to 10% linolenic acids compared to other cereal crops (1-5%). The total amount of unsaturated fatty acids in millet is 92.1% which might be one of the main reasons that millet grain bases were not stable during storage.

The present findings have shown that the application of ion-ozone cavitation treatment during the storage of extruded grain bases lead to significant changes in their chemical composition, which was also fairly proven by past research data (Nechaev, 1975; Ciudad-Mulero *et al.*, 2020; Bessada *et al.*, 2019; Faridy *et al.*, 2020; Guldiken *et al.*, 2020).

CONCLUSIONS

The current research determined the optimal methods to preserve the quality of cereal base production in four selected kinds of cereal and legumes i.e., buckwheat, chickpea, millet, and lentil. The ion-ozone cavitation treatment helped to preserve the persistence of extruded buckwheat grain bases for 240 days, while chickpea, millet, and lentils for 180 days. This technology could be recommended as an effective tool for ensuring long-term storage of extruded grain bases while maintaining the grain product's safety and quality.

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