Volume 24(1), 23 - 27, 2020
JOURNAL of Horticulture, Forestry and Biotechnology
www.journal-hfb.usab-tm.ro

Microbiological Analysis for Some Premixes Containing Red Blueberries

Borozan Aurica Breica[1*], Popescu Sorina[1], Raba Diana[1], Popa Mirela[1], Belu-Borozan R.[2], Molnar L. Șt.[1*], Madoșă E.[1]

[1*] Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timișoara, 119 Calea Aradului, RO-300645,Timisoara,Romania
[2] High School of Technological Food Industry from Timișoara, 32 A Calea Bogdăneștilor, Timișoara, Romania

*Corresponding author. Email: borozan_a@yahoo.com; mlocsi@gmail.com

Abstract The safety and health of consumers depends on the quality of raw materials and the complex of nutrients they contain. Premixes are increasingly used to meet these conditions. In the present paper premixes based on spelt wheat flour added with red blueberries were considered. In order to establish the microbial quality, the mesophilic aerobic bacteria, the number of viable spores, the number of fungi and coliforms were determined, applying classical methods. The results showed that the microbial content of the premixes and the spelt wheat flour sample (control) was lower than the limit set by the standard, which gives the analyzed biological samples a good microbiological quality. The bacterial and fungal microflora undergoes a progressive reduction with the increase of the concentration of blueberries in premixes. Coliforms are influenced by the presence of fruits, this fact being observed by reference to the control. Blueberries reduce the microflora in the tested mixtures and can be a source of natural additives, applicable in the food industry, but their effect depends on the proportion of blueberries in the premixes.

Key words

microbiota, premixes, red blueberries, spelt wheat

At global and national level, it is intended to obtain premixes (mixture of quality raw materials) that meet the nutritional and health safety requirements for consumers, in order to reduce the large number of diseases caused by the consumption of processed foods that contain synthetic additives. These consumer requirements are met on the basis of regulations related to the Code of Practice for Food Premix Operators [15].

Why premixes?

The answer results from the many advantages it has: starting with the possibility of continuously improving a product, using natural ingredients specific to the area, a complex chemical composition, eliminating certain steps, especially those related to storage of raw materials, reducing the number of equipment, etc.

Premixes for pastry-confectionery products have been studied in this work. They were based on spelt wheat flour added with berries, in our case blueberries were used.

The spelt wheat (*Triticum spelta*), present in the Middle East, but also in many European countries, is considered the ancestor of the common wheat [12] and is part of the *Gramineae* family. The chemical properties recommend it for obtaining bakery-confectionery products, namely: the highest starch content (65%), proteins with a value of 16-17%, supplemented with fiber, vitamins, tocopherols, bioactive compounds, minerals [8]. It should also be noted that some products based on spelt wheat flour retain their antioxidant capacity [6] and are tolerated by people with allergies [9].

Blueberries (*Vaccinum vitis idaea* L.), are woody plants, belonging to the family *Ericaceae,* genus *Vaccinum.* In our country, blueberries are found at high altitudes, along the Carpathians, and especially in Transylvania [16].

Blueberries have a high antioxidant capacity and a high content of polyphenols (566 mg/100 g), anthocyanins, vitamins, organic acids etc. [2, 7].

As the microbiota is present everywhere, it is not absent on raw materials or premixes.

In this case the microbiota is derived from the cereals from which the flour has been obtained to which is added the microflora present on the berries.

The number of microorganisms evolves upwards with increasing humidity and temperature. But, it must be added here that the type of cereals, the way of harvesting and storage is important [11, 14].

The aim of the paper is to evaluate some microbial groups that could influence the quality of the premixes and implicitly of the finished product.

Material and Method

Determinations were made on the premixes and the spelt wheat flour sample considered as the control sample, as follows: total number of germs, number of molds, number of bacterial spores and number of coliform bacteria.
Premixes based on spelt wheat flour (F) and dehydrated red cranberries (RA), were obtained by a team of teachers from the Faculty of Food Engineering, and microbiological determinations were performed at the discipline of Microbiology, Faculty of Horticulture and Forestry.
The proportion of red blueberries in premixes was: 5% (a1), 10% (a2), 15% (a3), 20% (a4) and 25% (a5). The control variant in the case of quantitative determinations was represented by spelt wheat flour (F).

Determination of the total number of aerobic mesophilic bacteria
Bacteria are a group of prokaryotes, heterogeneous, Gram positive or Gram negative, influenced by a number of factors present in the living environment. Aerobic mesophilic bacteria grow only in the presence of oxygen, in the thermal range 10-47ºC [3].
Nutrient agar was used to determine the total number of aerobic mesophilic bacteria in the premixes and the spelled wheat flour sample. Biological samples were homogenized and diluted 3x successively, for inoculation using 1/1000 dilution [3, 4, 13]. The incubation temperature was 30 °C for 48 hours.

Determination of the number of spores in aerobic bacteria
Bacterial spores are forms of conservation of bacterial species, which are resistant to environmental factors, have spherical and oval shapes and may have different positions in the sporangium [3].
Nutrient agar was used to determine the number of aerobic bacterial spores in premixes and the spelt wheat flour sample. Dilution no. 3 used for inoculation, was heated to 70°C for 10 minutes, followed by sudden cooling. Inoculation of the dilution was performed by the embedding technique. Thermostatization was performed at 35ºC for 48 hours [11].
Determination of the total number of molds
Molds are talophytes, mycelial, heterotrophic, which multiply vegetatively and by spores, present in different living environments, including agri-food products.
The determination of the total number of molds in the premixes and the sample of spelt wheat flour was performed by inoculating the 1/1000 dilution on Sabouraud Chloramphenicol Agar nutrient medium, followed by incubation at 28ºC for 3 days [11].

Determination of coliform bacteria from premixes
Coliform bacteria are bacilli or cocobacilli, with rounded extremities, Gram negative, unsporulated, optionally anaerobic, but also aerobic, have a high sugar fermentation capacity, producing acids with and without gases.
Under improper storage conditions, microorganisms evolve numerically. The same happens in the case of flour contaminated with rodent feces, which lead to the appearance and multiplication of coliform bacteria.
After homogenization of the biological samples, in a dispersing medium, three successive dilutions were obtained. The three dilutions were inoculated into 3 test tubes (1 ml per test tube), with liquid broth-lactose-lauryl-sodium sulfate liquid medium, in which there is one Durham tube. The incubation period of the inoculated tubes was 30ºC for 48 hours.

Results and Discussions

The flour contains the microbiota present on the cereals, but due to their processing processes, the microflora is reduced. Thus, the number of microorganisms in the flour varies between a few thousand, up to a few million per gram, but the maturation process of the flour and its preservation in optimal conditions can lead to the reduction of the microbial flora [11].
The European Union has provided the opportunity to exploit the biological activity of plant extracts and secondary metabolites (essential oils, tannins, flavonoid saponins) as natural alternatives [10].
Microbiological results obtained from the analysis of biological samples are illustrated in figures 1-3, and demonstrate that the increase in the concentration of red blueberries in flour derived from spelt wheat reduces the microorganism quantity, improves the quality and increases the safety of wheat flour and derived products intended for human consumption.

Results on the total number of aerobic mesophilic bacteria in premixes
After the 48-hour incubation period, the total number of aerobic mesophilic bacteria in the premixes and the flour sample was calculated, taking into account the dilution factor (Fig. 1).
According to Figure 1, the total number of aerobic mesophilic bacteria was lower in premixes than the flour sample, which is one of the main ingredients in the five premixes.
Blueberries have a negative influence on bacteria due to their chemical composition, high content of phenols, hydroxybenzoic acids and hydroxycinnamic acids, which have antimicrobial characteristics.

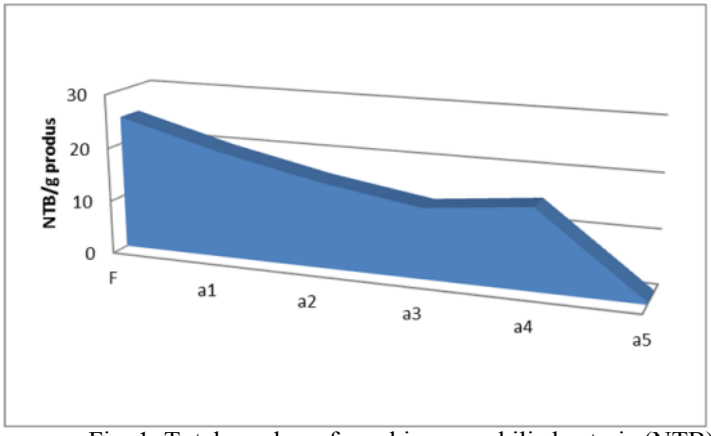

Fig. 1. Total number of aerobic mesophilic bacteria (NTB)

Legend: F - spelt wheat flour; a1 - premixes with 5% blueberries; a2 - premixes with 10% blueberries; a3 - premixes with 15% blueberries; a4 - premixes with 20% blueberries; a5 - premixes with 25% blueberries

Results on the total number of aerobic bacterial spores in premixes

After 2 days from the inoculation of the microbial suspension subjected to a heat treatment, the number of aerobic, viable bacterial spores from the premixes and the flour sample was established, taking into account the dilution factor.

The results in Figure 2 indicate a reduction in aerobic bacterial spores from premixes, especially in the sample in which red blueberries have a concentration of 25%.

Reducing bacterial spores is very important, because their presence in large numbers leads to poor quality of finished products [11].

Results on the total number of molds in the premixes

After 3 days of incubation, the total number of molds was determined, taking into account the dilution factor, as in the case of bacteria.

The fungal microflora from the analyzed samples (Fig. 3) falls within the limits required by the standards in force, with the mention that the tested commercial flour sample has a higher mycological content.

However, there are results that have shown that the fungal flora, but also of the coliform bacteria (which were determined in this paper) is higher in traditional flour samples, compared to commercial flour samples, and in premixes it is relatively low [5].

The reduction of the mold population in wheat flour is closely related to storage factors, especially temperature and humidity [1].

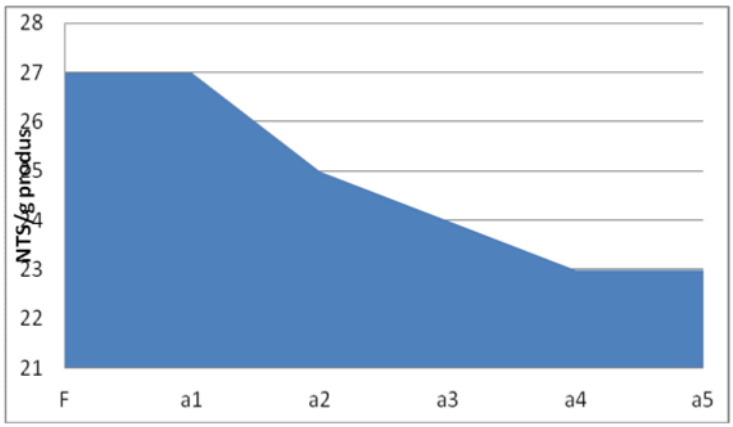

Fig. 2. Total number of spores (NTS)

Legend: F - spelt wheat flour; a1 - premixes with 5% blueberries; a2 - premixes with 10% blueberries; a3 - premixes with 15% blueberries; a4 - premixes with 20% blueberries; a5 - premixes with 25% blueberries

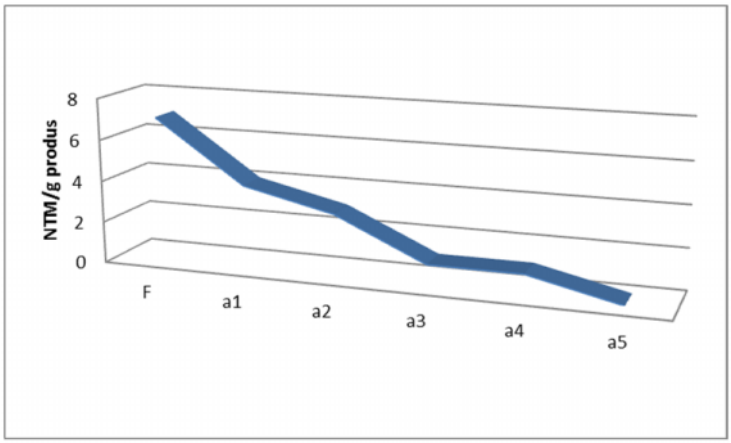

Fig. 3. Total number of molds (NTM)

Legend: F - spelt wheat flour; a1 - premixes with 5% blueberries; a2 - premixes with 10% blueberries; a3 - premixes with 15% blueberries; a4 - premixes with 20% blueberries; a5 - premixes with 25% blueberries

Results on the total number of coliform bacteria

The determination of coliform bacteria is made on the basis of tables for the interpretation of this bacterial group, reported to 1 g of product.

The coliform bacteria are numerically reduced in samples a4, a5, compared to the other 3 samples (Fig. 4). There are no numerical differences between the last two samples that contained higher red cranberries.

The microbial load, from the samples subjected to microbiological analysis, is lower than the standard limit allowed [11, 14] whether it is vegetative forms, bacterial spores and molds.

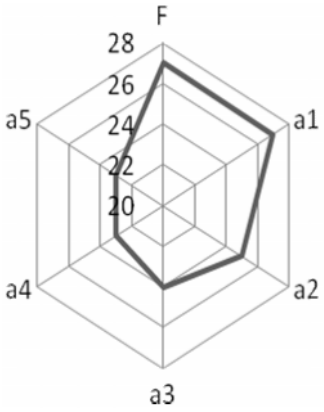

Fig. 4. Total number of coliform bacteria (NTC)

Legend: F - spelt wheat flour; a1 - premixes with 5% blueberries; a2 - premixes with 10% blueberries; a3 - premixes with 15% blueberries; a4 - premixes with 20% blueberries; a5 - premixes with 25% blueberries

Conclusions

The microbial content of the premixtures and the spelt flour sample was lower than the limit establishedby the standard, the biological samples analyzed are therefore of good microbiological quality. The bacterial and fungal microflora undergoes a progressive reduction as the concentration of red blueberries in premixes increases. The lowest number of micro-organisms was highlighted in the A5 mixture, where the highest content of red blueberries is found, which shows the anti-bacterial and anti-fungal effect of these forest fruits.

No differences in value were reported for coliform bacteria in premixes a4 and a5, but the differences between these samples and premixes a1-a3 and the flour sample were obvious.

Blueberries reduce the microflora of the tested mixtures and may be a source of natural additives, applicable in the food industry, but it should be specified that this effect depends largely on the concentration of berries in premixes.

References

1. Al-Defiery M.E., Merjan A.F. 2015. Mycoflora of mold contamination in wheat flour and storage wheat flour. Mesop. Environ. J, 1, 2: 18-25.
2. Bakowska-Barczak A.M, Marianchuk M., Kolodziejczyk P. 2007. Survey of bioactive

components in Western Canadian berries. Canadian Journal of Physiology and Pharmacology, 85, 1139–1152
3. Borozan A.B. 2006. Microbiologie, înfrumător de lucrări practice. Editura Mirton Timișoara.
4. Borozan A.B., Raba D., Popa M. ., Sărac I., Dobrei A., Popescu S., Cojocariu L. 2019. Variation of the bacterial community in the rhizosphere of the *Lotus corniculatus* L. in the four years of culture. JOURNAL of Horticulture, Forestry and Biotechnology, 23, 1 : 22- 28.
5. Ennadir, J., Hassikou R., Ohmani F.,Hammamouchi J.,Bouazza F., Qasmaoui A., Mennane Z., Ouazzani A., Charof T-R., Khedid K. 2012. Qualité microbiologique des farines de blé consommées au Maroc, *Canadian Journal of Microbiology*, 58(2): 145-150
6. Fatrcová-Sramková K., Kolesárová A., Nôková J., Babinská K. 2010, Nutrition habits and anthropometric parameters of slovak children, ecological, chemistry and engineering, 17, 1: 33-43.
7. Kähkönen M.P., Hopia A.I., Heinonen M. 2001. Berry phenolics and their antioxidant activity. J. Agric. Food Chem., 49: 4076–4082.
8. Kohajdova Z., Karovicova J. 2008. Nutritional value and baking applications of spelt wheat, Acta Scientiarum Polonorum. Technologia Alimentaria, 7, 3: 5–14
9. Marques C.D., Auria L., Cani Pd, Baccelli Ch., Rozenberg R., Ruibal-Mendieta N.L., Petitjean G., Delacroix D.L., Quetin-Leclercq J., Habib-Jiwan J.L., Meurens M., Delzenne M. 2007. Comparison of glycemic index of spelt and wheat bread inhuman volunteers. Food Chemistry, 100: 1265-1271.
10. Makkar H.P.S., Norvsambuu T., Lkhagvatseren S., Becker K. 2009. Plant secondary metabolites in some medicinal plants of Mongolia used for enhancing animal health production. Tropicultura, 27:159–167
11. Mișcă C.D. 2001. Microbiologie agroalimentară-îndrumător lucrări practice. Editura Solnes Timișoara.
12. Sabo M., Teklić T., Vidović I. 2002. Photosynthetic productivity of two winter wheat varieties. *(Triticum aestivum L.*). Rostlinná Výroba, 48, 2: 80–86
13. Zarnea Gh., Mihăescu Gr., Velehorschi V. 1992. Principii şi tehnici de microbiologie generală, vol. I, Bucureşti
14. Ordinul nr. 27/2011 privind aprobarea criteriilor microbiologice şi de igienă care se aplică produselor alimentare
15. PAHO HQ Library Cataloguing-in-Publication Pan American Health Organization, 2005, Family and Community Health Area. Nutrition Unit Code of practice for food premix operations, Washington, D.C., ISBN 92 75 12589 9.
16. ***https://ro.wikipedia.org/wiki/Meri%C8%99or_(plant%C4%83)#Utilizare.