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Evaluation of the Microbiological and Sensory Quality of Amaranth-based Biscuits

Ocjena mikrobiološke i senzorne kakvoće keksa na bazi amaranta

Bernadette Hozová, J. Zemanovič and R. Chorvátová

Department of Milk, Fats and Foods Hygiene, Faculty of Chemical Technology of the Slovak Technical University, Bratislava, Slovakia

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Summary

The microbiological and sensory evaluation of newly-developed cereal amaranth-based products (snacks and crackers) during the six-month storage in laboratory conditions (20 ± 2 °C, RH = 62 ± 1 %) were studied. Besides the quantitative evaluation (aerobic mesophilic bacteria, aerobic sporogenic bacteria, coliform bacteria, yeasts and moulds) of final products and basic raw-materials used for their production the experimental works involved also the identification of the respective microflora.

The microbiological control confirmed no presence of microorganisms in more than 50 % of the basic raw-material samples; in the rest of the samples the aerobic mesophilic bacteria ranged between 10^2–10^3 CFU/g; sporulants were detected only in 4 samples (dried milk, amaranth grain, cocoa powder and caraway); the number of moulds ranged between 10–10^3 CFU/g; yeasts and coliform bacteria were not found in any of the samples investigated.

The microbiological quality of snacks and crackers was unchanged up to the fifth month of storage; after this time an increase in the aerobic mesophilic bacteria (10–10^3 CFU/g) and aerobic sporogenic bacteria (10^2–10^3 CFU/g) was observed.

The sensory quality of snack and crackers was kept at a high standard level during the whole time of storage.

Sažetak

Ocjena mikrobiološke i senzorne kakvoće novih proizvoda na tržištu (keksa na bazi amaranta: krekeri i proizvodi »snack«) provedena je tijekom šest mjeseci njihova skladištenja u laboratorijskim uvjetima (20 ± 2 °C, pri relativnoj vlažnosti od 62 ± 1 %).

U gotovim proizvodima i osnovnim sirovinama za njihovu proizvodnju, osim kvantitativne ocjene (aerobnih mezofilnih bakterija, aerobnih sporogenih bakterija, koliformnih bakterija, kvasaca i plijesni) provedena je i identifikacija odgovarajuće mikroflore.

Mikrobiološkom kontrolom osnovnih sirovina nisu utvrđeni mikroorganizmi u više od 50 % uzoraka; u ostalim uzorcima nađeno je 10^2–10^3 CFU/g aerobnih mezofilnih bakterija; u četiri uzorka (mlijeko u prahu, zrnca amaranta, kakao u prahu i kumin) utvrđene su spore; količina plijesni bila je između 10–10^3 CFU/g. Ni u jednom uzorku nisu nađeni kvasci i koliformne bakterije.

Mikrobiološka kakvoća proizvoda »snack« i krekera ostala je nepromijenjena tijekom pet mjeseci skladištenja. Nakon toga opaženo je povećanje broja aerobnih mezofilnih bakterija (10–10^3 CFU/g) i aerobnih sporogenih bakterija (10^2–10^3 CFU/g).

Senzorna su svojstva proizvoda »snack« i krekera tijekom skladištenja ostala nepromijenjena i nisu izgubila na svojoj kakvoći.

Introduction

The production of biscuits is an important component of the agricultural and alimentary complex which is occupying today a well-established position in the nutrition of the population.

Both the demand and the popularity of this type of cereal products are growing also in connection with some allergic reactions to certain sorts of food, as for instance in the case of the coeliac disease requiring a gluten-free diet, which means practically permanent consumption of foods without any allergenic component (α-gliadine) contained in the gluten. The preparation of the shelf-stable baked products for this group of population needs, mostly, to leave traditional procedures and look for the new kinds of raw- materials consumable without and injury to human health.

These raw-materials include also amaranth (*Amaranthus* sp.), the application of which has not been very extensive so far. However, its special properties (such as

adaptability and meager cultivation conditions, significant nutrition properties demonstrated by a higher content of lysine, minerals and vitamins, mainly of riboflavine versus other cereals) are expected to be utilized in the food industry *(1,2)*. The amaranth leaves can be used as a vegetable and the whole plant as a fodder or grain for industrial processing.

The amaranth flour is added very often to the mixture of wheat or soya flour *(1,3)*. The mixture is used according to literature for the production of omelettes *(4)*, sponge-biscuits *(5)*, snacks *(6)*, bread *(7)* and various extruded *(1,3)* and fermented *(8)* products.

The object of this work was to perform the microbiological and sensory evaluation of the indicated newly-developed cereal amaranth-based products* during the six month in laboratory conditions (20 ± 2 °C, RH = 62 ± 1%). Besides the quantitative evaluation (aerobic mesophilic bacteria, aerobic sporogenic bacteria, coliform bacteria, yeasts and moulds) of final products and basic raw-materials used for their production the experimental works involved also the identification of the respective microflora.

Materials and Methods

For the evaluation of the microbiological quality were used:

A: *Basic raw-materials*

Wheat flour (fine- and coarse ground), corn meal, amaranth grain, amaranth flour, fresh eggs (one-day), dried egg yolks, water, fat, salt, sugar powder, vanillin sugar, dried milk, starch, fructose syrup, lecithin, caraway, cocoa powder, NH_4HCO_3, $NaHCO_3$, crushed snacks.

B. *Amaranth-based biscuits*

Snacks (250 g) – composition: wheat flour, amaranth flour, sugar, fat, eggs, aromatic ingredients.

Crackers (75 g) – composition: corn meal, salt, whole-grain amaranth flour.

The microbiological evaluation of basic raw-materials was carried out immediately after sampling. The final products (snacks and crackers) were analyzed every 30th day during the six mont storage at 20 ± 2 °C and RH = 62 ± 1% (the mass of all analyzed samples was 10 g).

1. Determination of aerobic mesophilic and aerobic sporogenic bacteria by the plate count method, on the tryptone glucose extract agar (Šarišské Michal'any, Slovak Republic) *(9)*,
2. Determination of coliform bacteria by the plate count method, on the VRB agar (Šarišské Michal'any, Slovak Republic) *(10)*,
3. Determination of yeasts and moulds by the plate count method, on the chloramphenicol glucose extract agar (Šarišské Michal'any, Slovak Republic) *(11)*,
4. Determination of slimeforming bacteria of the Leuconostoc genus, on the saccharose agar (Šarišské Michal'any, Slovak Republic) *(12)*,
5. Qualitative evaluation made by microscopy and macroscopy *(13)*: morphology, biochemical classification, formation of spores; determination of non-fermenting coliform G^- bacteria by NEFERMTEST, ENTEROTEST I and II, and G^+ bacteria by STAPHYTEST (Lachema Brno, Czech Republic). The examination of the cultural characteristics of moulds: the type of growth, the reproductive structures – the degree and kind of sporulation, the pigment produced on the surface and on the reverse side of the colony, the extent and rate of growth at the incubation temperature, etc. *(14)*.
6. Sensory evaluation: point-scale evaluation by 8 panel members according to 4–5 points of hedonic scale *(15)*,
7. Mathematical and statistical calculations of results ($\bar{x}$, s, s_r) *(16)* were made from three samples (each 10 g), with two parallel determinations (n = 6).

Results and Discussion

A. *Basic raw-materials*

As it follows from the results in Fig. 1, the analyzed basic raw-materials such as water, fresh eggs, lecithin, starch and baking powders ($NaHCO_3$ and NH_4HCO_3) did not contain any microorganisms or the microorganisms occurred only in the amount below 10 CFU/g.

Water activity – a_w of the samples was measured before the end of the storage period (Thermoconstanter – Defensor AG – Novasina, Switzerland) when bacteria appeared; a_w of snacks was 0.38 and of crackers 0.30 respectively (which corresponds to the values of 30–33 % for the RH samples).

The vanillin sugar contained $2.65 \cdot 10^2$ CFU/g ($s = \pm 35$) of the aerobic mesophilic bacteria (AMB), the aerobic sporogenic bacteria (ASB) only in the amount below 10 CFU/g and no moulds and yeasts. The prevailing microflora was *Leuconostoc* sp. that manifested itself by the slime formation over the period of growing on the selective medium.

The sugar powder sample contained $1.7 \cdot 10^2$ CFU/g ($s = \pm 41$) of AMB, the aerobic sporogenic bacteria only below 10 CFU/g, and 60 CFU/g ($s = \pm 19$) of moulds; yeasts and coliform bacteria were not present in the sample. During the identification the prevailing microorganism was *Bacillus* sp.; moulds were represented by *Aspergillus* sp. and *Fusarium* sp.

In the fat sample (margarine), $1.01 \cdot 10^3$ CFU/g ($s = \pm 28$) of AMB were detected and the presence of ASB was observed only below 10 CFU/g. Moulds and yeasts or coliform bacteria were not present. The identification of the prevailing microflora confirmed the presence of *Staphylococcus* sp., excluding the presence of *Staphylococcus aureus*.

In analyzing the dried milk it could be ascertained that the amount of $8.5 \cdot 10^2$ CFU/g ($s = \pm 1.1 \cdot 10^2$) of AMB and 67 CFU/g ($s = \pm 15$) of aerobic sporogenic bacteria was present. The moulds and yeasts or coliform bacteria were not present. The microflora of dried milk was rep-

* Bohemia Amaranth Ltd., Olomouc, Czech Republic

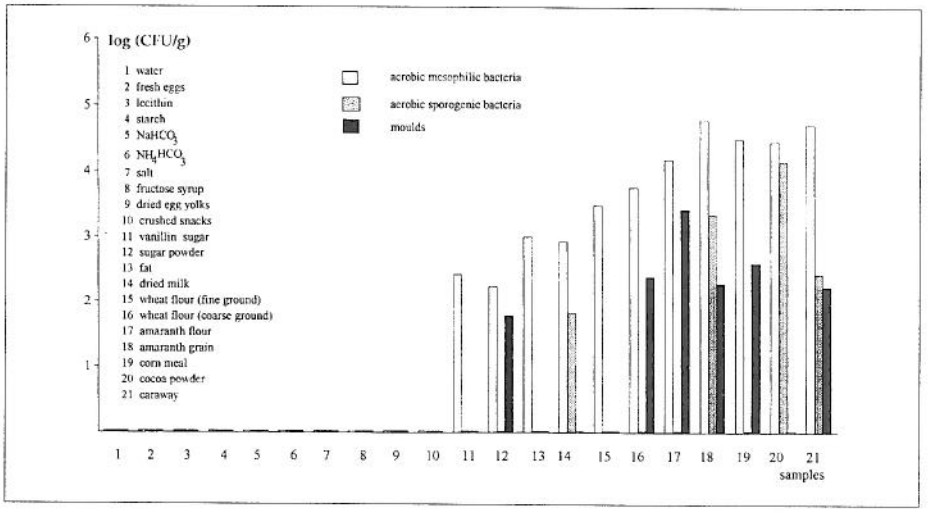

Fig. 1. Microbiological evaluation of basic raw-materials
Slika 1. Mikrobiološka ocjena kakvoće osnovnih sirovina

resented mainly by *Bacillus* sp., particularly by genera involving *Bacillus subtilis* and *Bacillus brevis*.

During the microbiological analysis of wheat flours (fine- and coarse-ground) the large numbers of AMB (in the density of 10^3 CFU/g) were assessed. The prevailing microorganism was *Erwinia* sp. which is characterized by the production of large yellow colonies and is usually present in the high-quality flours *(17)*. From among moulds, the presence of *Penicillium citrinum* and *Cladosporium* sp. was registered. The wheat coarse-ground flour obtained from a producer contained on the average $3 \cdot 10^3$ CFU/g ($s = \pm 3.5 \cdot 10^2$) of AMB; moulds were not detecctd in the sample, but in the fine-ground flour there appeared $5.6 \cdot 10^3$ CFU/g ($s = \pm 1.6 \cdot 10^3$) of AMB and $2.4 \cdot 10^2$ CFU/g ($s = \pm 49$) of moulds; other groups of microorganisms under investigation were not present in any of the samples.

Both the amaranth flour and the amaranth grain were considerably contaminated, namely by *Bacillus subtilis, Bacillus cereus, Bacillus sphaericus* and by genera of *Erwinia* sp. The moulds were represented by *Penicillium* sp., *Aspergillus niger* and *Alternaria* sp. The AMB in the amaranth flour was in densities corresponding to $1.5 \cdot 10^4$ CFU/g ($s = \pm 1.6 \cdot 10^4$) and in the amaranth grain to $6 \cdot 10^4$ CFU/g ($s = \pm 1.3 \cdot 10^4$). The aerobic sporogenic bacteria occurred mainly in the amaranth grain in the amount of $2.2 \cdot 10^3$ CFU/g ($s = \pm 2.1 \cdot 10^2$); moulds occurred in the amount of $2.6 \cdot 10^3$ CFU/g ($s = \pm 2.1 \cdot 10^2$) in the flour, and in the amount of $1.9 \cdot 10^2$ CFU/g ($s = \pm 38$) in the grain. Naturally, the presence of *Bacillus cereus* is undesirable because of its possibly pathogenic character.

During the analysis of corn meal the dominant position was occupied by *Bacillus* sp. and *Alcaligenes* sp. The moulds were represented by *Fusarium alveolanum*. The microorganisms reached the following values; the AMB corresponded to $3.1 \cdot 10^4$ CFU/g ($s = \pm 5.2 \cdot 10^3$); the ASB were found only below 10 CFU/g; moulds reached the value of $3.9 \cdot 10^2$ CFU/g ($s = \pm 80$); yeasts and coliform bacteria were not detected.

The cocoa powder sample contained also the great numbers of microorganisms. It was confirmed that the AMB amounted to 2.8 ± 10^4 CFU/g ($s = \pm 4.8 \cdot 10^3$) and the ASB to $1.4 \cdot 10^4$ CFU/g ($s = \pm 3.8 \cdot 10^2$). The majority of the present microorganisms were classified among *Bacillus* sp., mainly *Bacillus subtilis*.

The caraway sample contained $5.1 \cdot 10^4$ CFU/g ($s = \pm 9.1 \cdot 10^3$) of AMB as well as $2.6 \cdot 10^2$ CFU/g ($s = \pm 58$) of ASB, and $1.7 \cdot 10^2$ CFU/g ($s = \pm 26$) of moulds. The other investigated groups were not found. By identifying the most frequent microorganisms the presence of *Flavobacterium* sp. and *Corynebacterium* sp. was proved. The moulds were represented by *Penicillium* sp. and *Fusarium* sp. In evaluating twenty-one basic raw-materials used for the production of biscuits it may claimed that they comply with prescribed standards *(18,19)*.

B. *Snacks and crackers*

The results of the microbiological evaluation of snacks, which are listed in Fig. 2, show the presence of microorganisms in the product as late as in the fifth month of storage (April). During the previous four months there appeared no groups of microorganisms. This points to the sufficient efficiency of the technological process (baking for 7 min at 220 °C), favourable barrier properties of the packaging (cellophane) and suitable storage conditions (temperature, relative humidity).

As can be seen in Fig. 2, after the five-month storage the first microorganisms which started their propagation were aerobic mesophilic bacteria, the numbers of which reached 58 CFU/g ($s = \pm 21$). The AMB grew significantly in the sixth month of storage (May) when it corresponded to the value of $1.4 \cdot 10^3$ CFU/g ($s = \pm 1.3 \cdot 10^2$) and when the ASB *(Bacillus subtilis)* amounted to $1.5 \cdot 10^3$ CFU/g ($s = \pm 39$). The presence of moulds, yeasts or coliform bacteria was not ascertained in the product. The presence of microorganisms in the next months of storage (5th–6th) could have been brought about by the secondary air contamination over the storage period, mostly due to the insufficient tightness of the packaging. It is also possible that the spores present in the raw material (e.g. in the amaranth grain), which survived the baking process, have proliferated. The other reason for a sudden increase in the number of bacteria could be the sample variability. According to standards *(18,19)* biscuits must not contain any pathogenic, facultatively pathogenic and toxigenic microorganisms, and their tox-

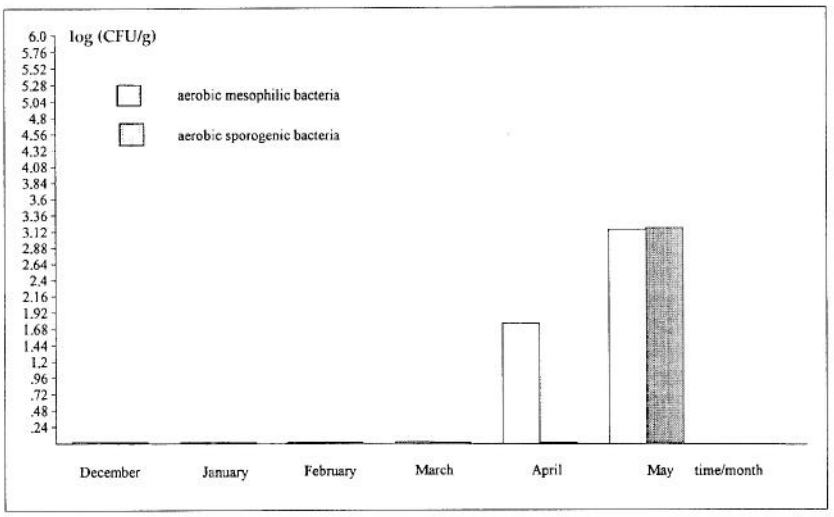

Fig. 2. Microbiological evaluation of snacks during the six-month storage
Slika 2. Mikrobiološka ocjena proizvoda »snack«

ins and other microorganisms must not be present in such amounts which would initiate any changes in the organoleptic propetics of final products. In addition, in snacks the acceptable amount of moulds is $2 \cdot 10^2$ CFU/g and of coliform bacteria $1 \cdot 10^2$ CFU/g. Our results complied with requirements of the indicated standards which resulted in the adequate microbiological quality being observed during the whole time of storage, i.e. up to the date of guarantee – 8th April, 1995.

From the results summarized in Fig. 3 it follows that during the five-month storage crackers were practically free of microorganisms, which indicates a remarkable effect of the extrusion process (1 s/150 kPa). After this time (in the 6th month), the occurrence of sporulants, particularly of *Bacillus subtilis* was registered in the amount of $8.1 \cdot 10^2$ CFU/g ($s = \pm 58$); this number was almost identical to $7.9 \cdot 10^2$ CFU/g ($s = \pm 49$) (vegetative cells of *Bacillus subtilis*). The moulds, yeasts and coliform bacteria demonstrated zero values up to the end of the storage.

From the indicated observations it is obvious that the detected numbers of microorganisms were lower than those in snacks, which might be caused by a decreased supply of the nutrition basic raw-material substances required for growth and propagation. Despite this fact it is possible to claim that during the three-month guarantee period for crackers (till 8th February, 1995), declared by the producer on the packaging, the perfect quality and the shelf life of this type of product was maintained.

Sensory evaluation

Changes in the organoleptic properties of snacks and crackers were evaluated at the beginning (in December, 1994) and at the end of the six-month storage (in May, 1995) at the laboratory temperature of 20 ± 2 C and RH of 62 ± 1 %.

The panel consisted of 8 members. The following sensory parameters were used; shape, surface, colour, consistency, odour and taste of products.

For the evaluation of the sensory attributes of amaranth snacks was used the five-point scale and for the evaluation of the characteristics of amaranth crackers – the four-point scale. The panel members were evaluating 6 quality parameters with the highest degree of the evaluation marked 5 or 4 corresponding to the fulfillment of all claims for organoleptic properties and the lowest degree of the evaluation marked 1 implying major qualitative deficiencies of the product.

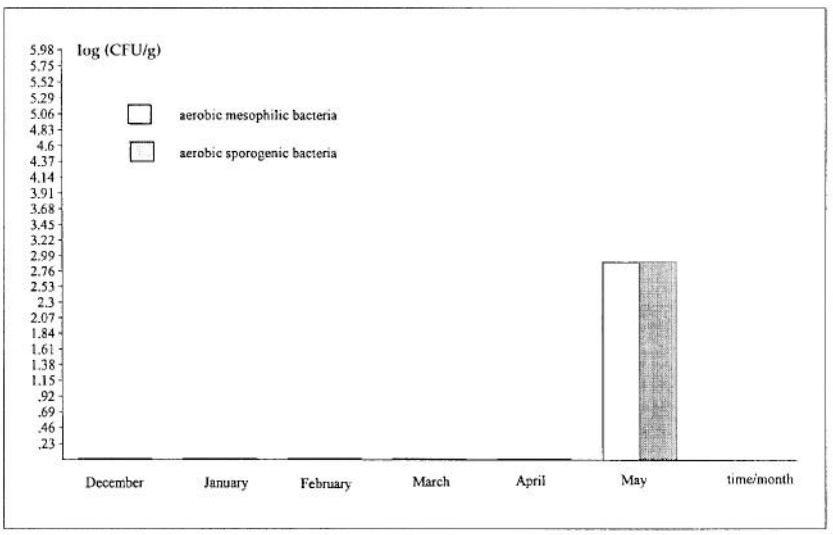

Fig. 3. Microbiological evaluation of crackers during the six-month storage
Slika 3. Mikrobiološka ocjena kakvoće krekera tijekom šestomjesečnog skladištenja

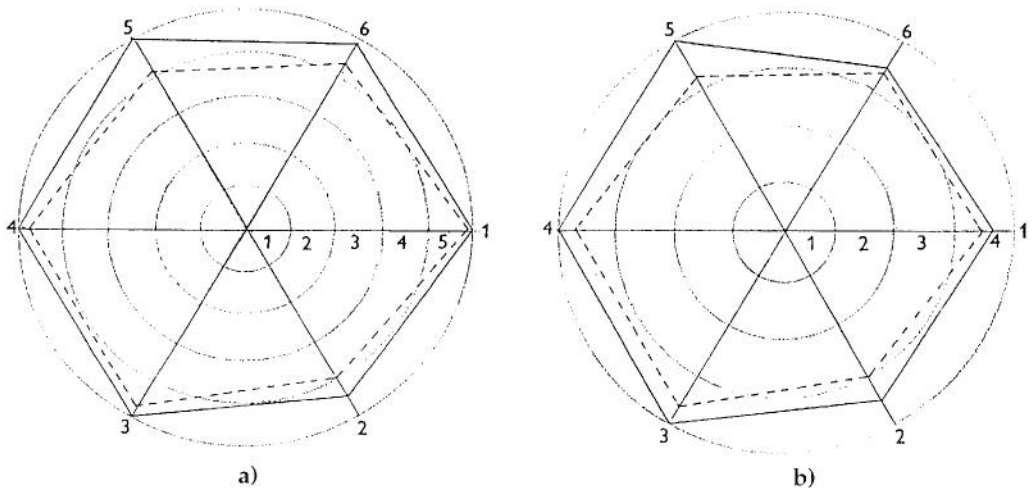

Fig. 4. Quality diagram of snacks (a) and crackers (b) during the six months storage.
Sensory properties: 1 – shape, 2 – surface appearance; 3 – colour; 4 – consistency; 5 – odour; 6 – taste.
———— before storage; - - - - - - - - after storage

Slika 4. Dijagram kakvoće proizvoda »snack« (a) i krekera (b) tijekom šestomjesečnog skladištenja.
Senzorna svojstva: 1 – oblik, 2 – vanjski izgled; 3 – boja; 4 – konzistencija; 5 – miris; 6 – okus.
———— prije skladištenja; - - - - - - - - nakon skladištenja

By comparing the results of the sensory evaluation (Fig. 4) it was found that during the storage all qualitative parameters would decrease in both snacks and crackers. The maximum drop was registered in evaluating the odour (0.9 points for snacks and 0.6 points for crackers) of both products and the taste of snacks (0.6 points). The least change was observed in the colour of products (0.4 points for snacks, 0.2 points for crackers). The products were of the requested shapes which did not change over the whole time of storage, nor did their surfaces. The consistency was given a better evaluation for crackers (3.9 of 4 points) than for snacks (4.6 of 5 points) which lost their crispness and became friable. In general, all these products were evaluated by 8 panel members as highly harmonic and tasty. They did not display the amaranth flavour. However, in some snacks it was possible to observe the flavour of the baking powder. Notwithstanding, both kinds of amaranth products are recommended to enrich and expand the assortment of biscuits in healthy nutrition.

Conclusion

Since the microbiological and sensory evaluation of the newly-developed cereal products complies with all criteria, they were highly evaluated. Therefore it is likely that they will find the application in the market and will expand the assortment of shelf-life products designed not only for a gluten-free diet but also for other consumers.

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