



Microbiological Purity and Selected Physicochemical Properties of Cereal Products Stored in Different Packages

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1. Introduction

Cereals and cereal products are particularly susceptible to changes when they are stored at conditions of elevated temperature and humidity. Not only does it cause overheating and moistening but it also leads to the growth of mold which might contaminate stored products with mycotoxins. Permeability of hazardous microorganisms from environment is higher when the barrier properties of packaging are low.

The number of bacteria in raw materials normally ranges from 10² to 10⁵ cfu/g and is the highest while threshing. The bacterial microflora is reduced (the number may drop to only 1000 cells in 1 gram) when grain is stored in granaries or silos and it is substituted for mold developing in favourable environmental conditions [1]. Both the storage process and the packaging have an impact on the quality of raw materials and products. The barrier properties influence the changes in atmospheric conditions of the product and the lower they are, the bigger the risk of unfavourable effects [8].

There is a wide variety of packages used for storing loose food products available on the market and producers have been made to choose packages which do not reduce the quality of food (requirements and procedures necessary to assure food and nutrition safety are laid down by Regulation (EC) No 1935/2004 of the European Parliament and

of the Council of 27 October 2004 and Polish Parliamentary Act of 8 January 2010) [31, 32].

Having in mind the above, the aim of this paper is to determine the influence of the type of packaging on microbiological purity, and also selected physicochemical properties of cereal food after having stored it for 6 months at 20°C.

2. Materials and methods

The research material constitute: wholemeal wheat flour, wholemeal rye flour, wheat flakes and rye flakes (made by company A and B), wheat bran and rye bran coming from conventional agriculture (A) and organic agriculture (B).

The research was carried out on two dates: just after buying the products (i.e. Test 1) and after 6 months of storing them in selected packages (i.e. Test 2). The packages used were: a Twist-off glass jar, a paper bag, a mat polypropylene container with a lid, and original packaging (flours: paper bags with a laminated inner ply, flakes: multiply cardboard boxes, brans: laminated paper bags). The cereal products were stored at 20°C and relative humidity of 61%

The samples for microbiological analysis were taken in accordance with PN-ISO 13690: 2000. Microbiological purity evaluation was carried out in accordance with PN-ISO 6222/2008, PN-ISO 7954:1999. Bacterial cultures were grown on nutrient agar and mold was grown on Sabouraud chloramphenicol agar. The bacteria was identified with BioMerieux Mini API Microbiological Culture Analyzer and using API 50 CHB, ID 32 STAPH, ID 32 GN systems. Molds' identification to the species level was based on macro- and microscopic features of their morphological structures such as the structure of hyphae, sporangia and spores as well as conidiophores or conidia. BioMerieux ID 32C system was used to distinguish yeast grown for the purpose of this study.

Screening tests monitoring the presence of mycotoxins in cereal products were performed with the use of column chromatographic technique (HPLC). Extract separation from analysed material using immunoaffinity columns was carried out with OchraTest and AflaTestu by Vicam, and mycotoxin concentration was measured with The VICAM Series 4 Fluorometer.

The physicochemical analysis comprised of: the determination of acidity of water slurry in accordance with PN-60/A-74007, the determination of moisture content by drying method in accordance with PN-ISO-712, the determination of organic contamination in accordance with PN-74/A-74016 and the determination of the Falling Number in accordance with PN-ISO-3093:2007.

3. Results and discussion

Flour is usually contaminated with microorganisms living on grains and its microbiological condition also depends on the hygiene of mill equipment, packaging and storage accommodation in which flour is kept. The storage room's temperature (temp. permitted 20-30°C), its humidity (cannot exceed 70%) and the period of storage (no longer than 1 year) have an influence on reducing flour's quality while storing [28].

Molds belonging to genera *Aspergillus*, *Penicillium* and *Fusarium*, and also *Cladosporium* i *Alternaria* are especially undesirable among microorganisms contaminating flour and cereal products. They develop rapidly when moisture content of flour is high (more than 15%) and consequently induce changes in its organoleptic properties, increase the acidity and cause the loss of its baking properties due to gluten's quality deterioration [29, 30].

The research has shown (Tab. 1) that already in Test 1 the number of molds in wheat flour was significant though still within threshold limit values (4.0×10^3 cfu/g) and in case of wholemeal rye flour the limit was exceeded ($1,1 \times 10^4$ cfu/g). Molds such as *Penicillium* sp., *Aspergillus* sp. and *Botrytis* sp. and *Saccharomyces cerevisiae* yeast were isolated from both wheat and rye flour. Test 2 proved that glass and polypropylene packages are unsuitable for storing flours as the general number of molds increased exceeding limit values [1, 15].

Czerwiecki [3] claims that the most frequent reason for the presence of mycotoxins in a product is its contamination with fungi originating from environment e.g. soil whose growth is favoured by a rise in humidity and temperature of the surroundings [22,23].

Current European Union legislation allows for a maximum level of 2 µg/kg for aflatoxin B1 and 4 µg/kg for aflatoxin total (B1, B2, G1, G2) in all cereals and cereal products [24]. The number of mycotoxins in

tested flours (Tab. 1) did not exceed acceptable limits [2, 7].

According to requirements set to cereals and food concentrates such as instant products, mold contamination should not exceed $1,0 \times 10^3$ cfu/g [30]. The following study has shown (Tab. 1) that the general number of molds surpassed the acceptable level in wheat flakes produced by company A and B in the first test, and in wheat and rye flakes produced by company B in the second test when they were stored in a glass container.

Penicillium sp., *Aspergillus* sp. and *Rhizopus* sp. molds were detected. The level of aflatoxin and ochratoxin A in tested flakes did not go over acceptable limits [24,33].

In the first test the number of molds in wheat and rye brans made by company A was higher than in the rest of products. The second test proved that the number of molds in rye bran stored in polypropylene packaging (Tab. 1) exceeded the limit values [18]. The highest number of molds was present in wheat rye coming from organic farming, especially when they were stored in polypropylene and glass containers, which favoured their growth. *Penicillium* sp. and *Rhizopus* sp. molds were identified in the tested material. Together with *Aspergillus* sp. they are regarded as dominating in domestic environment. The presence of *Saccharomyces cerevisiae* and *Rhodotorula glutinis* yeasts was also discovered. Rooms such as kitchens and cellars, which are damp and where ventilation is limited, are particularly favourable to them [5, 6, 9].

The presence of aflatoxins and ochratoxin A in stored brans was insignificant and did not surpass acceptable limits (Tab. 1). It turned out that the highest level of ochratoxin (but still below the acceptable limit) was detected in rye bran originating from conventional agriculture, which confirms the theory that rye grains are the most susceptible to molds producing ochratoxin [2].

Many bacteria, belonging to genera *Achromobacter*, *Flavobacterium*, *Sarcina*, *Micrococcus*, *Alcaligenes* and *Serratia*, may be present in flour. However, *Bacillus* sp. bacilli and endospores are the most frequent and their number should not be higher than 100 spores in 1 g. Yet the amount of spores is not constant and the longer the storage period the more spores there are [4, 26].

Table 1. Quantitative and qualitative analysis of molds**Tabela 1.** Analiza ilościowa i jakościowa grzybów

Product	Test 1		Mycotoxins		Package	Test 2	
	Total number of molds in I time limit/term					Total number of molds after 6 months of storage	
	Result [cfu/g]	Type/Species	Aflatoxin [µg/kg]	Ochratoxin A [µg/kg]		Result [cfu/g]	Type/Species
Wholemeal rye flour	$1,1 \times 10^4$	<i>Saccharomyces cerevisiae</i> <i>Penicillium</i> sp. <i>Aspergillus</i> sp. <i>Botrytis</i> sp.	0,012	0,01	polypropylene	$7,5 \times 10^5$	<i>Saccharomyces cerevisiae</i> <i>Penicillium</i> sp.
Wholemeal wheat flour	$4,0 \times 10^3$	<i>Saccharomyces cerevisiae</i> <i>Penicillium</i> sp. <i>Aspergillus</i> sp.	0,01	0.053	glass	$6,0 \times 10^4$	<i>Saccharomyces cerevisiae</i> <i>Penicillium</i> sp.
Wheat flakes A	$2,8 \times 10^4$	<i>Penicillium</i> sp. <i>Rhizopus</i> sp.	0,023	0,097	glass	$1,8 \times 10^2$	<i>Penicillium</i> sp.
Rye flakes A	$2,8 \times 10^2$	<i>Penicillium</i> sp.	0,045	0.145	polypropylene	$2,2 \times 10^2$	<i>Penicillium</i> sp.
Wheat flakes B	$1,8 \times 10^4$	<i>Penicillium</i> sp. <i>Aspergillus</i> sp.	0,11	0,12	glass	$3,0 \times 10^5$	<i>Penicillium</i> <i>Aspergillus</i>
Rye flakes B	$1,8 \times 10^2$	<i>Penicillium</i> sp.	0,01	0,097	glass	$1,8 \times 10^5$	<i>Penicillium</i>
Wheat bran A	$1,5 \times 10^4$	<i>Penicillium</i> sp. <i>Rhizopus</i> sp.	0,028	0,11	polypropylene	$2,4 \times 10^5$	<i>Saccharomyces cerevisiae</i> <i>Rhizopus</i> sp. <i>Rhodotorula glutinis</i>
Wheat bran B	$1,5 \times 10^3$	<i>Saccharomyces cerevisiae</i> <i>Penicillium</i> sp.	0,066	0.11	polypropylene	$3,0 \times 10^4$	<i>Saccharomyces cerevisiae</i> <i>Rhodotorula glutinis</i>
Rye bran A	$3,6 \times 10^4$	<i>Saccharomyces cerevisiae</i> <i>Rhodotorula glutinis</i> <i>Penicillium</i> sp.	0,012	0,16	polypropylene	$2,1 \times 10^6$	<i>Saccharomyces cerevisiae</i> <i>Rhodotorula glutinis</i> <i>Penicillium</i> sp.
Rye bran B	$1,8 \times 10^2$	<i>Saccharomyces cerevisiae</i> <i>Penicillium</i> sp.	0,024	0,29	polypropylene	$3,8 \times 10^5$	<i>Saccharomyces cerevisiae</i>

The study has shown (Tab. 2) that the quantity of bacteria did not go over the limit values [16, 17] and in both tests it was at comparable level (10^4 cfu/g). The highest content of the bacteria was present in flours stored in glass containers. *Bacillus* sp., *Pantoea* sp., and also *Micrococcus luteus* were identified.

The results of bacterial contamination of cereal flakes were compared to standards for cereal grains [19, 20]. The total amount of mesophilic aerobic bacteria in flakes should not be bigger than 3.0×10^4 cfu/g. The presence of *Salmonella* sp. and pathogenic staphylococci is unacceptable, whereas coli group bacteria should not be present in 0.1 g of a product [15, 19, 20, 30]. Rye flakes B contamination with bacteria (Tab. 2) exceeded the acceptable limit and was 1.6×10^5 cfu/g. The second test proved that the most bacteria were present in wheat flakes stored in paper bags. Low barrier properties of the packaging most probably contributed to permeability of microorganisms from environment. The presence of aerobic spore forming bacteria of the genus *Bacillus* was noticed.

Table 2. Quantitative and qualitative analysis of bacteria

Tabela 2. Analiza ilościowa i jakościowa bakterii

Product	Test 1 Total number of bacteria in 1 time limit/term [cfu/g]	Identified microorganisms	Package	Test 2 Total number of bacteria after 6 months of storage	
				Result [cfu/g]	Type/Species
Wholemeal rye flour	$1,8 \times 10^4$	<i>Bacillus</i> sp.	glass	$4,1 \times 10^4$	<i>Bacillus</i> sp. <i>Micrococcus luteus</i>
Wholemeal wheat flour	$5,2 \times 10^4$	<i>Bacillus</i> sp.	glass	$4,6 \times 10^4$	<i>Pantoea</i> sp.
Wheat flakes A	$4,6 \times 10^3$	<i>Bacillus</i> sp.	paper bag	$1,4 \times 10^5$	<i>Bacillus</i> , <i>Pantoea</i> sp.
Rye flakes A	$7,6 \times 10^4$	<i>Bacillus</i> sp.	paper bag	$1,7 \times 10^5$	<i>Bacillus</i> sp. <i>Pantoea</i> sp.
Wheat flakes B	$1,6 \times 10^2$	<i>Bacillus</i> sp.	paper bag	$6,0 \times 10^4$	<i>Bacillus</i> sp.
Rye flakes B	$1,6 \times 10^5$	<i>Bacillus</i> sp.	paper bag	$2,4 \times 10^5$	<i>Bacillus</i> sp.
Wheat bran A	$2,0 \times 10^4$	<i>Bacillus</i> sp.	paper bag	$3,1 \times 10^4$	<i>Bacillus</i> sp. <i>Micrococcus luteus</i>
Wheat bran B	$3,6 \times 10^3$	<i>Bacillus</i> sp.	paper bag	$3,7 \times 10^3$	<i>Bacillus</i> sp.
Rye bran A	$3,1 \times 10^4$	<i>Staphylococcus lentus</i> <i>Bacillus</i> sp.	paper bag	$3,5 \times 10^4$	<i>Bacillus</i> sp. <i>Pseudomonas fluorescens</i> <i>Micrococcus luteus</i> <i>Pantoea</i>
Rye bran B	$3,1 \times 10^3$	<i>Bacillus</i> sp.	paper bag	$4,9 \times 10^3$	<i>Bacillus</i> sp.

The overall number of bacteria (Tab. 2) proved to be higher in rye bran made by company A which used material from organic agriculture. Additionally, after 6 months of storing it in paper bags, this product turned out to be the most contaminated with microorganisms. Higher bacteriological contamination of products coming from organic agriculture could have been brought about by the rejection of chemical methods of protecting plants [9]. Products kept in paper packages should maintain recommended microbiological purity and appropriate sensory properties i.e. liquidity of powder [27], but the porosity of the material, which guarantees a specific air circulation, could have led to product's contamination with bacteria coming from environment.

Physicochemical properties of examined flours (Tab. 3) did not surpass the threshold limit values [25] in both Test 1 and Test 2.

Table 3. Physicochemical properties of flours

Tabela 3. Właściwości fizykochemiczne mąk

Product	Test 1 I TERM			Test 2 II TERM			
	Humidity %	Acidity °SH	Falling number sek.	Package	Humidity %	Acidity °SH	Falling number sek.
Wholemeal rye flour	10,5	5,3	291	glass	11,6	7,3	337
Wholemeal wheat flour	9,1	3,1	303	glass	12,0	7,6	351

However, an increase in all tested areas was observed after 6 months of storage in glass containers. The Falling Number was the highest in rye flour – 337 sec. Physicochemical requirements for wheat and rye flakes determine acceptable moisture content of 14.5% and the acidity level of 3 to 8% [19,20].

These values were not exceeded in the tested materials but there was a considerable increase of moisture content and the acidity level of the flakes stored in glass containers (Tab. 4).

The acidity of bran (Tab. 5) did not surpass the acceptable maximum value, whereas moisture content in all types of bran stored in glass and polypropylene containers was higher [18].

Table 4. Physicochemical properties of flakes**Tabela 4.** Właściwości fizykochemiczne płatków

Product	Test 1 I TERM		Test 2 II TERM		
	Humidity %	Acidity ⁰ SH	Package	Humidity %	Acidity ⁰ SH
Wheat flakes A	10,1	3,6	glass	12,9	7,5
Rye flakes A	10,8	4,3	glass	13,7	7,2
Wheat flakes B	9,7	4,3	glass	12,5	7,8
Rye flakes B	9,3	3,9	glass	13,9	7,3

Similarly, the content of organic contamination of wheat bran made by company A reached the threshold limit value of 0.3%, which might have been a result of microorganisms' presence.

Table 5. Physicochemical properties of bran**Tabela 5.** Właściwości fizykochemiczne otrąb

Product	Test 1 I TERM				Test 2 II TERM		
	Humidity %	Acidity ⁰ SH	Organic pollutants %	Package	Humidity %	Acidity ⁰ SH	Organic pollutants %
Wheat bran A	9,5	3,9	0,1	glass	10,7	6,5	0,3
				polypropylene	10,9	7,3	0,1
Wheat bran B	5,8	4,7	0,1	glass	10,6	7,0	0,1
Rye bran A	9,5	4,3	0,1	glass	11,1	7,0	0,1
				polypropylene	10,5	7,4	0,1
Wheat bran B	7,7	5,4	0,1	glass	10,5	7,3	0,1
				polypropylene	10,7	7,5	0,1

The results of the study show that the type of packaging is one of the factors which determine the quality of cereal products. Storing them in polypropylene and glass containers particularly influences the changes in microbiological and physicochemical parameters. This in consequence may lead to deterioration of food properties .

Grains obtained from producers are stored in cloth sacks and then the finished product is repacked into paper bags with inner layers of bonded paper known for its barrier properties [8]. It is supposed to protect the grains from humidity. The original packaging is recommended for storing cereal products but the research has shown that even that does not protect it from microbiological contamination, which was detected in tested products just after the purchase. It may be a result of the contamination that took place during the growth of plants or in the grain store. In such situation, using packaging with high barrier properties to store cereal products is connected to their quality deterioration what has been proven in this research [8].

Not only do producers have the responsibility to bear in mind Good Manufacturing Practice in relation to materials and products having contact with food, but they should also obey regulations whose aim is to limit the risks of health hazards [27]. Still, it is not always possible to maintain expected purity of food even though production procedures are observed. Consumers should protect purchased products from physical and biological components of environment. The products should not be stored longer than the expiry date recommended by the producer.

4. Conclusion

1. Commercial samples of grain and flour products, despite their distribution in appropriate packaging, may contain bacterial and fungal microflora.
2. Storing such products for 6 months in tightly closed containers or inappropriate paper bags influence negatively their physicochemical properties and microbiological purity.
3. In such cases it may be advisable to shorten the shelf life recommended by producers, especially under conditions of higher temperature.

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Czystość mikrobiologiczna i wybrane cechy fizykochemiczne produktów zbożowych przechowywanych w różnych opakowaniach

Streszczenie

Właściwości organoleptyczne przechowywanych produktów zbożowych mogą ulegać zmianie m.in. w zależności od rodzaju opakowania, w którym się je przetrzymuje. Łatwość przenikania ze środowiska zewnętrznego składników stanowiących zagrożenie dla bezpieczeństwa żywności, zbyt wysoka temperatura i przegrzanie produktów oraz duża wilgotność wywołująca zaparowanie, to najczęstsze przyczyny, które obniżają wartość konsumpcyjną przechowywanej żywności. Barirowość opakowania wpływa bowiem na zmianę warunków atmosferycznych środowiska produktu i im jest niższa, tym większe prawdopodobieństwo w/w skutków. Zgodnie z ustawą (wymagania i procedury niezbędne do zapewnienia bezpieczeństwa żywności i żywienia zgodnie z przepisami rozporządzenia (WE) nr 1935/2004 Parlamentu Europejskiego i Rady z 27 października 2004 r. reguluje znowelizowana ustawa z dnia 8 stycznia 2010 r.) materiały przeznaczone do kontaktu z żywnością nie obniżają jej jakości i są bezpieczne dla zdrowia człowieka. Sytuacja ulega jednak zmianie, gdy w warunkach domowych oryginalne opakowania zastępuje się takimi, które nie gwarantują, że przechowywana w nich żywność jest zabezpieczona przed negatywnym oddziaływaniem czynników środowiska zewnętrznego, np. przed skażeniem przez mikroorganizmy.

Celem badań było określenie wpływu jaki wywierał rodzaj zastosowanego opakowania na czystość mikrobiologiczną i wybrane cechy fizykochemiczne produktów zbożowych przechowywanych przez okres 6 miesięcy w temperaturze 20°C.

Słowa kluczowe: produkty zbożowe, stan mikrobiologiczny, cechy fizykochemiczne, opakowania

Key words: cereal products, microbiological status, physical and chemical characteristics, package