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Influence of storage conditions on microbial quality of rapeseed cake and middlings

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A b s t r a c t. The effect of storage moisture and temperature on the microbiological quality of rapeseed cake and middlings was determined. Samples of rapeseed cake and middlings, with moisture levels of 9 and 11%, were stored in pressure chambers at temperature of 20 and 30°C for a period of 120 days. The design of the pressure chambers ensured simulation of storage conditions such as those in industrial silos. Changes in the microbiological parameters of the rapeseed cake and middlings *ie* total numbers of fungi, and numbers of lipolytic and proteolytic fungi, were assayed prior to storage and after 120 days of storage. The study showed that both storage temperature and moisture of the materials under study significantly affected the values of microbiological parameters of rapeseed cake and middlings.

K e y w o r d s: storage, rapeseed cake, rapeseed middlings, microbial quality

INTRODUCTION

Recently, rape seeds and the products of their processing, such as rapeseed cake and middlings, are more and more frequently used in animal feeding, mainly as a source of proteins and energy. This is due to the elimination of meat meals, among other things, from animal feeding (Directive (EC) No. 1774/2002 of European Parliament and Council dated 3rd October 2002). With relation to the spread of the BSE disease, stringent restriction were introduced for the application of animal meals in the feeding of farm animals. The production of animal feeds cannot involve the application of materials that do not meet the requirements of the referenced Directive specifying the requirements for by products of animal origin that are not meant for consumption. The elimination of meat meals from farm animal feeding caused an increased demand for vegetable protein fodders, including rapeseed cake. At the same time, the introduction in EU of regulations limiting the permissible levels of nitrogen and phosphorus excreted by animals enforced greater attention to the quality of rapeseed cake and middlings as a source of protein and energy (Pastuszewska and Raj, 2003). Rapeseed and its products (cake and middlings) are a source of protein with relatively high nutritive value and well-balanced amino acid composition, and with a certain content of minerals and vitamins. High protein is characterized by very well-balanced amino acid composition. High content of sulfur amino acids is the reason for which the components of rapeseed are used widely in feed rations for all animals, mostly as feed additives or fodder (Yumiko *et al*., 2008). With its over a dozen percent content of oil, rapeseed cake improves also the concentration of energy in feeding doses (Brand *et al*., 2001; Ghodsvali *et al*., 2005; Jensen *et al*., 1995).

Those rapeseed products are perishable by nature, which causes that they are subject to rapid decay. Long-term or even short-time storage of rapeseed products is burdened with notable risk. The reason for this is the considerable content of reserve substances (carbohydrates, protein, oil) that are also an excellent substrate for microorganisms. Improper storage of rapeseed cake and middlings causes rapid biochemical transformations that lead to intensification of processes of oxidation, going rancid, and hydrolysis, and to activation of enzymatic processes (lipolytic, proteolytic). Therefore, safe storage of the material requires that the time of storage be adequate to the storage conditions and to the quality of the initial material, so as to avoid spontaneous heating and lumping of the material, and rapid

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growth of microorganisms (Skiba *et al*., 2005). The most common fungi in the storage are *Penicillium* spp., *Aspergillus* spp. and *Fusarium* spp. In research conducted by the Pronyk *et al*., initial fungal counts showed that the canola seeds were infected with high levels of preharvest fungi *Alternaria alternata* and *Cladosporium* spp. and low levels of storage fungi *Eurotium* spp., *Aspergillus candidus*, and *Penicillium* spp. (Pronyk *et al*., 2006).

Most moulds produce natural compounds, which are unnecessary for a mould that is a producer. Mycotoxins are secondary metabolites of fungi. The most common mycotoxins are aflatoxins, ochratoxins A and zearalenon. Aflatoxins are a group of toxins produced mainly by Aspergillus in particular by *Aspergillus flavus* and *A. parasiticus* and *A. nominus.* They are a group of mycotoxins with the greatest significance in food and feed. There are four distinguished groups of aflatoxins: B1, B2, G1, G2, M1 and M2, of which B1 is a mycotoxin frequently encountered attacking feed and food products. Aflatoxin is called lethal toxin. Ochratoxin A (OTA) is the major mycotoxin produced by fungi shreds during improper storage in all climatic zones. It is produced mainly by Aspergillus fungi, including *Aspergillus ochraceus, A. niger* and A. sclerotonium as well as by *Penicillium verrucosu* and *P. nordicum*. The possibility of contamination of cereals and foodstuffs by ochratoxin A is conditioned by ground, water activity, moisture, temperature, incubation time and most of all by the species of fungi. (Batista *et al*., 2009; Sweeney end Dobson, 1998).

The rate of transformations taking place during the storage of rapeseed cake and middlings depends on a number of factors, and in particular on the initial condition of the material, resulting from the harvest technology applied and from the post-harvest treatment, and on the storage conditions (Gawrysiak-Witulska *et al*., 2005; Tańska and Rotkiewicz, 2003). This pertains in particular to storage temperature, access of light and oxygen and the moisture of the material, duration of storage, and also to the value of loads acting on the material during storage (Bilght, 2004; Jayas and White, 2003; Tys and Rybacki, 2001).

Inadequate management of raw materials during storage can result in excessive moisture or dryness, condensation, heating, leakage of rainwater and insect infestation, leading to undesirable growth of fungi (Cavaglieri *et al*., 2009).

Knowledge of those conditions permits to predict the quality of stored materials at the final phase of storage, and to determine the limit time of their storage with relation to the storage conditions (Osek, 2000; Tys *et al.*, 2007).

MATERIALS AND METHODS

The experimental material consisted of rapeseed cake from the oil-producing company. The adopted method for the determination of rapeseed cake storage conditions is based on research-tested special pressure chambers (Tys and Szwed, 1997). The design and equipment of the chambers, as well as the test and measurement apparatus, permit the simulation of storage conditions such as prevail in industrial grain silos during the storage of granular material. In the course of the experiments, monitoring was conducted of the basic factors that affect the process of storage *ie* moisture and temperature.

The studied factors modifying the processes taking place in the rapeseed cake in storage included the following:

- storage conditions:
  - varied temperature (20, 30°C);
  - loading of samples (50 kPa);
  - duration of storage (120 days);
- initial parameters of the products:
  - winter rapeseed cake;
  - winter rapeseed middlings;
  - varied moisture level (9, 11%).

The methods included performance of microbiological analyses in accordance with the Polish Standards PN-ISO 6887-1, PN-R-64791, PN-ISO 7698. Due to the high content of protein – 32.6% and oil – 12% in rapeseed cake, the analyses consisted in assaying the total numbers of fungi, and numbers of proteolytic and lipolytic fungi in the experimental material.

In order to gain full spectrum of changes in tested material, microbiological analyses compatible with Polish Norms PN-ISO 6887-1, PN-R-64791, PN-ISO 7698 were made. Weighted portion of 10 g was inserted into the bottle with 90 ml of one percentage solution of sodium pyrophosphate, obtaining dilution No. 1 (10^{-1}). After thorough shaking in rotational shaker, 10 cm of suspension was uptaken with a sterile pipette from first suspension and inserted into 90 ml of distilled, barren water. Dilution No. 2 (10^{-2}) was obtained. Series of following ten-times dilutions were obtained in the same way, till the 10^{-6} dilution was made. Three last dilutions (10^{-4}, 10^{-5}, 10^{-6}) constituted the material for inoculation. 1 ml of suspension was collected from every dilution and inoculated into 4 parallel and barren Petri plates (every plate constituted one repetition). Next, liquefied and chilled to 40°C, selective agar mediums for individual groups of fungi was poured into plates (Pochon and Tardieux, 1962; Parkinson, 1994; Rodina, 1968). Incubation of cultures proceeded in temperature of 25 ± 1°C for 5-7 days in aerobic conditions. After incubation period, the number of grown fungi (mould) colonies were counted on every plates from particular dilution. The number of colonies on plates was stated, according to Malicki (1980), in conversion on the gram of dry mass of material. In order to obtain tribes for further identification from fungi grown in colonies on plates, the material from every plate was immaculately moved into test tubes with slants of peptide-glucose-agar medium. Incubation of cultures proceeded in temperature of 25 ± 1°C for 5-7 days in aerobic conditions. Microscope preparations of fungi were made by inoculation of mycelium obtained from agar slants on barren disc of medium with potato extract on glass slide. Identification of isolated fungi species was carried out

after 4-7 days of incubation in temperature of 21-22°C. It was made using keys (Barnett, 1962; Domsch and Gams, 1972; Marcinkowska, 2003) enabling to ascertain the attachment to different rank of taxons. Identification of colonies was made using optical microscope technique of observation of fungi morphological features. Considering the characteristic features of thallus and sprification, it is sufficient in most cases.

Estimation of microbiological quality of rapeseed cake and middlings before storage and after 120 days of storage permitted determination of changes in the studied quality indices with relation to storage time and temperature. Additionally, for rapeseed cake determination was made of the content of mycotoxins (aflatoxin B1, G1, B2, G2, ochratoxin A). The determinations were made by means of high-pressure liquid chromatograph with LC-MS/MS detector.

The experimental material required moistening of some batches of rapeseed cake and middlings to the level of 11% (ASAE Standards, 1997).

RESULTS AND DISCUSSION

Rape seeds, as well as the products of their processing (cake, middlings), are prone to decay in storage due to their chemical composition. Hence long-term storage of such a delicate material always involves a deterioration of its quality parameters. Effective production consists in ensuring safety and microbiological quality of products of the agricultural-food industry and observance of the relevant international standards (Trojanowska, 2002).

Microbiological estimation of the experimental materials consisted in quantitative determination of total fungi and of lipolytic and proteolytic fungi. Table 1 presents the microbiological characterization of the tested samples prior to their storage. Data indicate that rapeseed middlings, compared to rapeseed cake, were characterized by the strongest infestation with microorganisms in the case of proteolytic fungi. For rapeseed cake, the recorded values for total fungi, lipolytic fungi and proteolytic fungi were 22, 17 and 39 cfu 10^3 g^{-1} d.m., respectively.

The microbiological analyses performed after 120 days of storage under controlled conditions, covering quantitative assays of total fungi, lipolytic fungi and proteolytic fungi, demonstrated that storage conditions had a significant effect on the numbers of fungi. The microbiological characterization of the experimental material after storage is given in Fig. 1. It was observed that both rapeseed cake and middlings with moisture content of 11% were characterized by the greatest microbial infestation in all the variants of the experiment. Also, the highest numbers of total fungi, as well as of proteolytic and lipolytic fungi, were recorded for rapeseed cake. Undoubtedly this is related with its rich chemical composition, as it contains considerable amounts of protein (31-35% d.m.) and fats (10-22% d.m.) (Osek, 2000; Skiba *et al*., 2007; Sobota, 2004).

T a b l e 1. Microbiological characterization of experimental material in conversion to dry matter

Rapeseed material	Populations of fungi (cfu 10^3 g^{-1} d.m.)		
	Total	Lipolytic	Proteolytic
Cake	20	24	26
Middlings	22	17	39

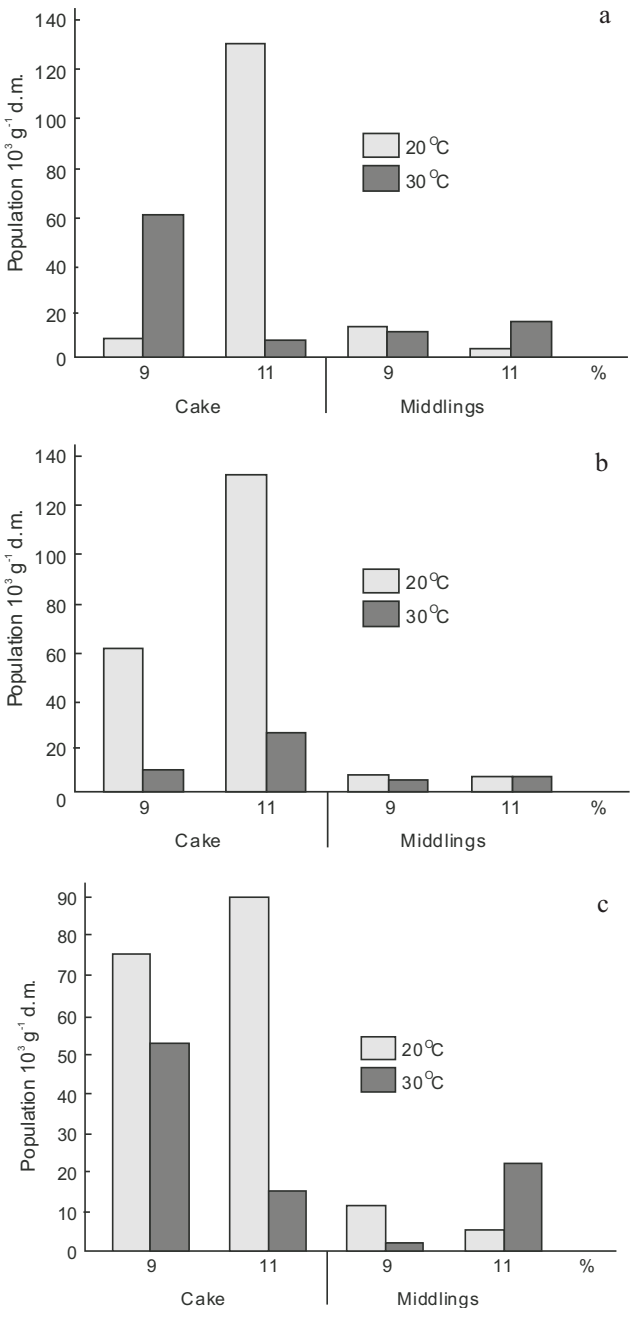

Fig. 1. Population of fungi: a – total, b – lipolytic, c – proteolytic (cfu $10^3 g^{-1}$ d.m.) in analyzed material.

Studies conducted by Rybacki (2003) showed that both the variety features of rapeseed and the storage conditions had a significant effect on the numbers of microorganisms on whole seeds. That author determined that rapeseed cv. Lisek was less infested with fungi, with the recorded number of colonies varying within the range from 18.2 to 75.4 10^5 g^{-1}, compared to rapeseed cv. Kana (26.2-95.8 $10^5 g^{-1}$). Based on the analyses he performed, that author concluded, however, that it was the storage conditions that determined primarily the level of that infestation. Seeds with the lowest moisture, 7% even though stored at temperature of 30°C, proved to be the most suitable for longer storage. Higher rapeseed moisture – 11%, had a negative effect on microbiological purity, even when the seeds were stored at 7°C.

Additionally, for rapeseed middlings determinations of the content of mycotoxins was made – aflatoxins B1, G1, B2, G2 and ochratoxin A. In none of the tested samples the content of mycotoxins was below the lower threshold of detection (LTD) that for aflatoxins has been set at the level of 1 g kg^{-1} , while for ochratoxin A at the level of 2 g kg^{-1}.

CONCLUSIONS

1. The study showed that proper storage conditions protect agricultural products from deterioration of their quality, as well as from microbiological infestation. They guarantee maintenance of high technological value of products destined for food or fodder purposes. In this respect it is very important to ensure suitable microclimate parameters in the storage silo (moisture, temperature).

2. The study showed that fungal growth depended primarily on storage temperature, moisture and duration. Unfavourable conditions of storage (high moisture and temperature) lead to strong infestation with microorganisms. Moisture content of experimental material at the level of 11% was the most favourable to the growth of mildews.

3. Rapeseed cake was characterized by significantly higher degree of infestation with microorganisms than rapeseed middlings.

4. In spite of the observable infestation of rapeseed cake and middlings with filamentous fungi, no occurrence of mycotoxins was found.

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