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Content of microorganisms in cereal straw

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Abstract

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Straw is an important agricultural raw material with application in agriculture, energy sector and other fields of activity. A major impact on reduction of the straw quality is that of an excessive content of microbiological agents caused by improper storage. By simulation of different conditions of storage the development of microorganisms (moulds and yeasts) was determined in the straw of wheat under different water content. Total content of microorganisms varied in a wide range from 10^3 up to 10^5 CFU/g. The excessive amount of these microorganisms results not only in a reduction in the quality of raw material due to the development of biodegradable processes, but also causes an increase of hygienic risks in the nearby area.

Keywords: storage of biomass; microbiology; agricultural constructions; health risks; mould, yeasts

The current development of energy sector in agriculture includes several important aspects from the logistic point of view. The main task is to ensure the quality of raw material in the long-term perspective, which is necessary to a profitable operation. From this view, an important component of the whole process is the proper storage, which ensures long-term availability of raw materials in the required quality.

The issue of storage of agricultural raw materials was described by several authors, especially as regards the technical provision of suitable storage conditions. The current knowledge is based mainly on the principles of storage in the crop and livestock production. An example is the use of the modified algorithm for aeration of bulk feeds in the hayloft (Hutla et al. 1996) in order to reduce effectively the water content. Among foreign working groups, Smith et al. (1977) dealt intensively with the issue of straw storage in the form of briquettes.

Research of mould content and equilibrium water content in relation to the environment was carried out by Sain and Broadbent (1975). This research was focused on rice straw. The authors experimentally found out that the development of fungi in rice straw occurs at the water content over 7.5%, which corresponds to the equilibrium stand with relative humidity of ambient air around 70% at 25°C.

The authors Willcock and Magan (2000) in their work dealt with the classification of mixed microflora nearby the stored wheat straw utilizing the measurement method by means of respirometer. The research was carried out in the temperature range from 10°C to 30°C. On naturally contaminated wheat straw, the maximal fungal activity was recorded at the temperature of 30°C. At the lowest tested temperature of 10°C, a slight decrease in activity was recorded.

The issues of the mould development in wood chips based on the conditions of storage is solved by author (Souček 2014) for three regimes of poplar chips storage with different water content. The obtained results show clearly, that in accordance with theoretical assumptions, the storage of wood chips with high water content is unsuitable in the long-term prospect. On the contrary, for short-term storage (up to 20 days) it is not necessary to dry the material up to very low water content. In

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case of water content around 20%, no important differences were determined in the initial stage of storage in the content of moulds regardless of storage temperature. In the samples of wood chips the moulds of the following genera were identified: *Cladosporium, Mucor, Penicillium, Alternaria, Botrytis, Aspergillus, Rhizopus, Fusarium* (Neděl'ník, Moravcová 2006). Some authors (Coskun et al. 2009) and (Souček et al. 2008) also solved the issue of energy intensity during the reduction of moisture. According to the author, the reduction of excessive moisture is energetically and economically demanding. The water content is sufficient to reduce it to an acceptable value.

MATERIAL AND METHODS

The aim of the realized trials was to determine the development in moulds and yeasts content under different temperatures and at different water content. The real storage conditions were simulated in the laboratory. The material for trials (wheat straw) was obtained at the stand by manual harvest and cutting (by means of sterilized cutting machine) into the particles of average length of 7.53 mm.

All vessels and instruments, which were in contact with the tested material, have been sterilized. The trial was established immediately after harvest and needed modifications. In this way, the risk of contamination by microorganisms from surrounding ambient and their premature reproduction in the tested straw were minimalized.

The prepared samples were placed into sterile vessels with the volume of 0.2 l and closed tightly. The tightness was checked in comparison of sample weight during the establishment of the trial and after termination of storage time and before microbiological analysis.

The trials were carried out at four different water contents. The different water content was obtained by drying straw in the drying oven (at 60°C). The samples were prepared for 4 different water contents (55%, 19%, 12%, 0%). Temperatures during the storage of samples have been set to –15°C (freezer), +12°C (refrigerator), +25°C (thermostat 1) and +50°C (thermostat 2). The samples for microbiological analysis were taken 4× during the experiment (at establishment, 7th, 14th, and 28th day). Water content in samples was determined according to the standard ČSN 44 1377:1980.

Initial suspension for microbiological analysis was prepared by shaking out the straw sample of 10 g weight into 100 ml 0.1% pepton water. Another tenfold dilution was chosen so that the resulting number of colonies grown on Petri dishes was not higher than 150 (dilution 10^{-4}, 10^{-5} and 10^{-6}). Inoculation was carried out by pipette transferring 0.1 ml of inoculum on a solid nutrient medium and by its titration on agar surface with curved glass rod. The prepared plates were incubated aerobically with lids pointing upwards in thermostat at 25°C. The number of plates was read after 3–5 days of incubation. Cultivation was carried out on a selective nutrient medium with addition of antibiotics. For the preparation of culture medium dehydrated complete culture medium, chloramphenicol agar containing dichloran and red bengal dye were used.

The main indicator of microbiological analysis was total number of microorganisms (CFU – colony-forming units) related to 1 g of sample and was calculated by the formula:

$$N = \frac{\sum C}{\left(n_1 + n_2 \times d\right)} \text{ (CFU/g)} \quad (1)$$

where: ΣC – sum of all colonies counted on selected plates; n_1 – number of plates used for the calculation from the first dilution; n_2 – number of plates used for the calculation from the second dilution; d – factor of the first dilution for calculation of the second dilution

Because the samples had different water contents, the results were based on the amount of dry matter. It enabled mutual comparison of individual samples. The resulting value of the number of microorganisms based on the amount of dry matter was calculated as:

$$P = N \times \frac{100}{s} \text{ (CFU/g}_{\text{dry mater}}\text{)} \quad (2)$$

where: N – sum of all colony-forming units (CFU/g); s – share of dry matter in the sample (%)

The average number of microorganisms in straw before experiments establishment was 1.1×10^3 CFU/g.

RESULTS AND DISCUSSION

At the water contents of 55%, as it is obvious from the Fig. 1, the number of microorganisms in the course of storage period have been changed. In

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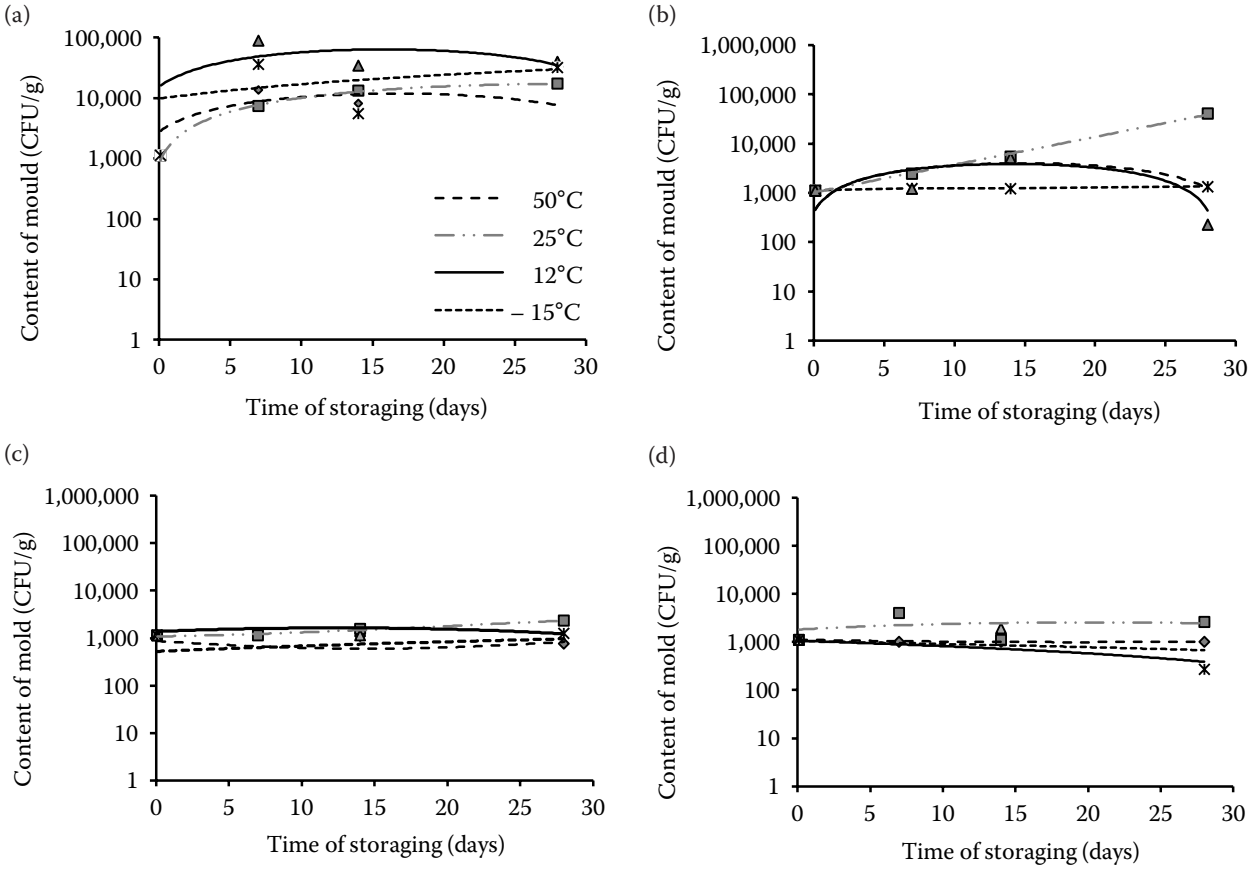

Fig. 1. Development of mould number in wheat straw at total water content of (a) 55%, (b) 19%, (c) 12% and (d) 0%

the initial stage, an increase of their number was recorded (from 3×10^3 CFU/g to 5×10^4 CFU/g). At the temperature of 25°C the number of microorganisms was still growing. At the higher temperatures (50°C) and lower temperatures (12°C) after a peak (around 7th day of storage) an increase in the number of microorganisms stagnated (Fig. 1). From the 15th storage day the number of microorganisms have been reducing.

In case of samples with lower water content (12% and 0%) no significant change was recorded in the number of moulds and yeasts in the course of storage, less important changes had rather a decreasing trend. The slight increase was recorded in case of material with water content of 19% under storage temperature of 25°C (from 1.1×10^3 CFU/g to 4.2×10^4 CFU/g).

In case of storage under temperature of –15°C, the microbiological contamination of material was the same regardless of the storage period. At the temperature of 25°C a gradual increase of the number of microorganisms was recorded during the whole period of experiment, regardless of the water content. The main reason is generic composition of these microorganisms. The same reason contributes to the fact that at the temperatures of 50°C and 12°C, a decrease of total quantity of microorganisms was recorded in the second half of the experiment, for whose development these temperatures are not suitable.

The highest concentration of microorganisms was detected at the highest water content in material (55%, 12°C) after 7th storage day (Fig. 1a). The values approached to 3.4×10^5 CFU/g. The highest concentration of microorganisms at the end of the experiment were 4×10^4 CFU/g (55%, 12°C) and 4.1×10^4 CFU/g.

The statistical values (median, standard deviation and combined standard uncertainty) of individual variants are given in Table 1.

The obtained conclusions are in accordance with those of other authors (Willcock, Magan 2000), who, of course, carried out the experiments in strongly limited extent and supported the claim of authors (Sain, Broadbent 1975), that the development of microorganisms is practically stopped, when the water content in straw is minimal.

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Table 1. Storage conditions, median, standard deviation and combined standard uncertainty of the mould content of individual variants (CFU– colony-forming units)

Variant	Water content (%)	Temperature (°C)	Median (CFU/g)	Standard deviation (CFU/g)	Combined standard uncertainty (CFU/g)
55/50	55.31	50.0	9,900	747.9	773.7
55/25	55.31	24.6	12,448	1,212.4	1,237.7
55/12	55.31	11.6	53,737	900.8	1,402.3
55/–15	55.31	–15.3	24,343	610.2	780.6
19/50	19.17	50.0	2,512	33.7	60.5
19/25	19.17	24.6	16,533	455.5	562.8
19/12	19.17	11.6	2,212	199.1	203.9
19/–15	19.17	–15.3	1,275	15.0	30.0
12/50	11.84	50.0	925	52.5	55.6
12/25	11.84	24.6	1.671	149.0	152.7
12/12	11.84	11.6	1.571	149.6	152.9
12/–15	11.84	–15.3	877	108.3	109.7
0/50	0.45	50.0	1.019	91.7	93.9
0/25	0.45	24.6	2.624	363.2	367.0
0/12	0.45	11.6	1.945	175.1	179.3
0/–15	0.45	–15.3	1204	277.9	278.9

In comparison with the results achieved during the experiments with wood chips (Souček 2014) it is obvious, that the straw is a bit more resistant against occurrence of microbiological agents. From the technological point of view, the advantage of straw is an easier way to reduce the moisture by its drying up on the field with subsequent collection (eventually by pressing) and with possibility of transport directly on the site of storage.

In the samples of straw the same moulds as in the wood chips were recorded, except the genus *Alternaria* and the genus *Botrytis*; it means the following genera: *Cladosporium, Mucor, Penicillium, Aspergillus, Rhizopus, Fusarium*. The generic composition of moulds is practically identical with the results determined by Souček (2014) and Nedělník and Moravcová (2006). However, due to the similar composition of microflora, the effect of environmental conditions on its development is analogous.

CONCLUSION

Occurrence of microorganisms in straw mainly depends on the water content. A significant influence of temperature was observed at higher water content (19% and 50%).

In case of higher water content, the greatest increase during the first days of storage was recorded at all monitored temperatures with the exception of –15°C.

In case of samples with lower water content (12% and 0%) no significant change in number of moulds and yeasts was recorded in the course of storage, less important changes had rather a decreasing trend.

In comparison with wood chips, wheat straw is a bit more resistant against occurrence of microbiological agents.

In the samples of straw, the moulds of the following genera were identified: *Cladosporium, Mucor, Penicillium, Aspergillus, Rhizopus, Fusarium*. From the yeasts, those of the *Rhodotorula* genus were identified in the examined samples. The occurrence of the genera *Saccharomyces* and *Candida* was not recorded.

From a practical point of view, it is an important finding that due to the generic composition of microorganisms their best development occurred at the temperatures around 25°C. During the outdoor storage in the warmer period it is therefore necessary to store the straw in the dry state (less than 19%) and prevent it from getting wet.

The second important finding is that the straw with higher water content may not contain a high

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amount of moulds. However, it may include their metabolites emerging during the dynamic growth in the initial stage of storage.

Maintaining a low level of microorganisms in biomass is also important in terms of minimizing the health risks associated with exposure of service staff to them and pollutant emissions into the environment. From the health point of view, not only moulds and yeasts are dangerous, but also their spores and mainly metabolites emerging as a result of an increased exposure.

For this reason it is common practice to store the straw with water content up to 20%. At the storage temperature around 25°C the moisture content of the stored material should not exceed 12–15%, or the storage time should be shorter than 20 days.

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