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EFFECT OF RAW MATERIAL QUALITY ON FERMENTATION ACTIVITY OF DISTILLERY YEAST

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Key words: maize, ethanol, fermentation, yeast

The aim of the work was determination of the effect of raw material quality on the course and final effect of ethanol fermentation of maize mash using cultured and rehydrated distillery yeast *Saccharomyces cerevisia* Safethanol 3035

The material for investigation was maize grain of Kosmo 230 cultivar: fresh (immediately after harvest), dried or stored without preservation (8 and 19 months, 20°C). Maize grain stored for 8 months was infected with *Fusarium* fungi in 100%. After 19 months of grain storage the amount of *Fusarium spp.* isolates decreased but the amount of *Penicillium spp.* increased. The process of fermentation of maize dried or stored for 19 months was over after *ca*. 2 days. The mashes of fresh corn or stored for 8 months need *ca*. 3 days to complete the fermentation. The degree of carbohydrates consumption was very high, over 99% in all samples. Physiological condition of yeast after fermentation of lower quality material was significantly worse than in control samples. The yield of ethanol fermentation of poor quality grain were slightly worse than in case of fresh or dried grain, but the differences were not significant, which confirms the suitability of this raw material for fuel production purposes.

INTRODUCTION

Reducing the costs of raw material used for ethanol production, which currently range 50–60% contribution of ethanol price, is a considerable problem to be solved. Numerous properties decide about usefulness of particular raw material in ethanol production: the yield of ethanol from one unit of matter, easiness of processing, cost of raw material counted over the units of ethanol produced, possibility of long–term storing and waste utilization belong to the most important ones. Taking into account the factors presented above, maize seems to be the kind of raw material worth paying attention to [Borktun, 2003; Lipski, 2002, 2003; Michalski, 2005]. Its advantage is high yielding (8 ton/ha) and high content of starch: 55–65%. The fact that lower quality grain can be used as raw material (microbe – contaminated, cracked, unripe), yet still of high distillery quality, is also not the least importance. Application of such a raw material allows, on one hand, to diminish the cost of ethanol production as that kind of maize is purchased by distilleries at lower price, on the other one to eliminate that raw material from trade turnover. The use of low quality grain, not suitable for consumption or fodder purposes, is connected with the risk of infecting raw material, among the others with mycotoxins, as well as with the presence of bacterial microflora originating from the grain [Bothas *et al*., 1992; Newsome, 2006].

The aim of the work was determination of the effect of raw material quality on the course and final effect of ethanol fermentation of maize mash using cultured and rehydrated distillery yeast.

MATERIALS AND METHODS

The material for investigation was maize grain of Kosmo 230 cultivar coming from plant production station "Nasiona Kobierzyc" LTD Company. The following kind of grain was subjected to investigations: fresh – immediately after harvesting, stored without preservation at the temperature of 20°C for 8 and 19 month and the dried one. Industrial strain of distillery yeast *Saccharomyces cerevisiae* Safethanol 3035, coming from Lasaffre Company was biological material for this investigation. In the examined raw material there were assessed dry matter concentration (according to the dryer method) and starch concentration (Lintner's method). The results obtained were the basis for calculating fermentation yield. Mycological analysis of the grain was conducted at the Department of Plant Protection – Institute of Phytopathology of the Wrocław University of Environmental and Life Sciences. Determination of microlfora existing on moist maize grain was done according to Polish Standard [PN–R 65950:1994]. Mycological analysis of the stored grain followed de Tempe method [1970]; there were analysed 100 randomly collected seeds out of each samples. All kinds of fungi isolated from the grain were identified, regarding their species, basing on the following monographies [Raper *et al*., 1965, 1968; Ellis *et al*., 1971; Nelson *et al*., 1983].

Fermentation tests were conducted using the method of periodical fermentation in starch mashes prepared from maize of different quality; A – moist, B – stored for 8 months, C – stored for 19 months, D – dried. Maize mashes of 200 g/kg raw mate-

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rial concentration were prepared by non-pressure starch release method in ZL1 type E laboratory mashing device. Starch was liquidized and saccharified with the use of commercial enzymatic preparations Termamyl 120L, San Ultra L, basing the doses on the instructions by the producer [Solarek, 2004]. The fermentation process was carried out at temperature of 37°C using distillery yeast *Saccharomyces cerevisia* Safethanol 3035. Yeast inoculum was obtained through rehydration of dried yeast (Sr) and through yeast cultivation (Sc) on YM medium and was dosed into mashes in the amount of 2 g yeast dry matter/kg of the sample.

The amount of CO_2 (g) released in the course of the process was assumed as a criterion assessing fermentation dynamics. The samples were weighed every 3 h until the difference in mass measurements was less than 0.05 g. There was compared percentage contribution of CO_2 released after 3, 18, 24, 42 and 67 h of fermentation in relation to total amount of released CO_2 (g).

After fermentation had been completed, the samples twice underwent direct distillation. After first distillation samples were neutralized by using 30% NaOH. They were determined for reducing sugars content, according to the method by Nizowkin & Jemielianowa, modified by Soczyński, in the decoctions previously clarified and subjected to hydrolysis [Lisińska *et al*., 2002]. The degree of sugar consumption (%) was calculated from the difference in starch content before and after fermentation.

Ethanol content (g/L) was assayed pycnometrically in distillates. Ethanol yield was determined as well.

The following parameters were assumed as a criterion of fermentation activity assessment: dynamics of fermentation expressed by the quantity of CO_2 released during process, ethanol content (g/L), practical yield of ethanol relation to theoretical yield, yield of ethanol counted over 100 g of dry matter Y (g ethanol/100 g dry matter), the degree of starch consumption (%) and the physiological condition of yeast cells after fermentation, expressed as percentage of budding cells and ones stained with methylene blue.

RESULTS

Our own investigation aimed at the assessment of usefulness of low–technological quality maize grain for ethanol production. To this end fresh grain and the stored one was subjected to microbiological analysis. Percentage contribution of a moist maize grain contaminated by fungi was shown in Table 1.

On the basis of the results obtained it was possible to state that the percentage quantity of grain contaminated by *Ascochyta* mould was the lowest. Twice that quantity of grain occurred in the presence of *Fusarium* species. Both of those fungi species were found on 9% grain. The highest amount of grain was covered with mould coating of *Botrytis cinerea* species – five times higher than that of other species.

TABLE 1. Percentage contribution of maize grain contaminated by fungi.

Maize	Fungi species		
	Ascochyta spp.	*Fusarium* spp.	*Botrytis cinerea*
A- moist grain	3%	6%	34%

There was isolated mainly one species of *Fusarim subglutinans* from maize grain stored for 8 months (Table 2). All the examined grain showed external symptoms of contamination with that fungus–white – *Pinkish mycelium.* Moreover, grain was also characterized fungi agglomeration represented by other species *Fusarium* (*F. avenaceum, F. culmorum*) and *Penicillium* (*P. notatum, P. purpurogenum, P. vermiculatum*), yet those fungi contribution was 15% of all the isolates (*Fusarium* – 4%, *Penicylium* – 11%). The grain stored for 8 months did not show any external signs of decay and the symptoms of contamination became visible only after the 19 month of storing. In the case of 19–month stored grain the number of isolated fungi of *Fusarium* species decreased, while that of *Penicylium spp.* increased. *Fusarium spp*. range up to 55% of all isolates. The most numerous proved to be isolated *Fusarium subglutinans* (100% of all *Fusarium spp*.). Among *Penicylium spp. Penicylium notatum* was the dominant one. A few isolates belonging to *Penicylium melagrinum*, *Aspergilus niger* (4%) and *Alternaria alternate* (3%) were also isolated from Kosmo maize cultivar.

In the further part of this work the grain contaminated by different groups of microorganisms was assessed regarding its usability for ethanol production. The course and final effect of fermentation involving low technological quality grain was compared with the data obtained for fresh maize after harvesting and preserved by drying. The first criterion of contaminated raw material usability for fermentation was observed dynamics of the process conducted with the use of *Saccharomyces cerevisiae* Safethanol 3035 (Figure 1).

Cultured yeast (Sc) featured higher dynamics of carbon dioxide release after 3h of fermentation in comparison to rehydrated yeast (Sr), regardless the quality of raw material used. Different dynamics of fermentation could be recorded only until the 18th h of the process and after the first 24 h those differences became insignificant. The highest dynamics was recorded for fermentation of maize grain stored without preservation for 19 months (alternative experiment C). After 18 h of fermentation the amount of CO_2 released ranged 92% for rehydrated yeast, while for the cultured ones this parameter was 99%. Fermentation process of poor quality and dried

TABLE 2. Fungi isolated from maize grain stored in laboratory conditions for 8 and 19 months.

Fungi species	Maize grain stored for 8 months (B)	Maize grain stored for 19 months (C)
Alternaria alternata (Fr.) Keissl.	-	3
Aspergillus niger van Tieghem	-	5
Fusarium avenaceum (Corda ex Fr.) Sacc.	2	-
Fusarium culmorum (W.G. Smith) Sacc.	3	-
Fusarium subglutinans (Wollenw. Et Reinking)	100	60
Nelson, Toussoun et Marasas		
Penicillium melagrinum Biourge	-	3
Penicillium notatum Westling	7	42
Penicillium purpurogenum Stoll	2	-
Penicillium vermiculatum Dangeard	4	-

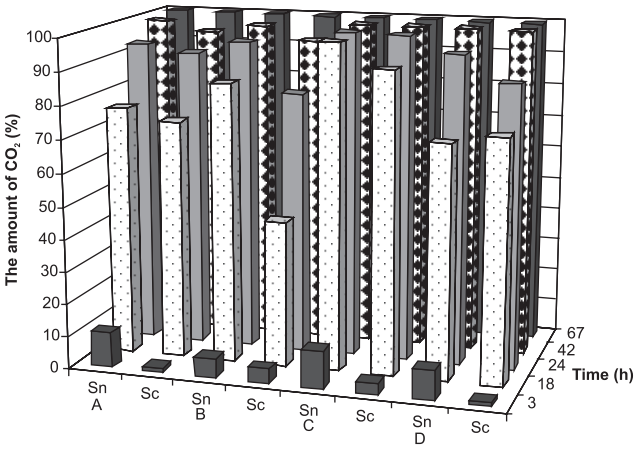

FIGURE 1. Dynamics of CO_2 release during fermentation of maize mashes using cultured (Sc) and rehdrated (Sr) yeast. (A – moist grain, B – grain stored for 8 months, C – grain stored for 19 months, D – dried grain).

maize grain (alternative experiment C and D) was over after about 2 days (about 41 h), while less than three days it took mashes made of bad grain (alternative experiment B) and moist maize grain (alternative experiment A) to complete fermentation. Final effects of ethanol fermentation were shown in Table 3.

The lowest practical ethanol yield in relation to theoretical one was observed for fermentation of the substrate prepared from maize grain 100% contaminated with *Fusarium* species (alternative experiment C). Ethanol yield was lower average by 3% for cultured yeast and about 5% for rehydrated yeast in comparison to the remaining samples, whose value of the parameter in question was more than 85%. Ethanol yield from dry matter of raw material was similar for fresh and dried maize grain (about 36 g ethanol/100 g dry matter). Less ethanol from each 100 g of dry matter of raw material was obtained when mashes were prepared from 8-month-stored grain (average by 1-2 g) and 19-month-stored grain (average by 2-3 g). The degree of starch utilization was very high and it ranged more than 99% in all the samples.

A considerable parameter regarding the assessment of yeast fermentation activity is their physiological condition after completed fermentation process.

In Figure 2 there was shown contribution of dead and budding yeast cells of *Saccharomyces cerevisiae* Safethanol 3035 after fermentation of maize mash. It was recorded that yeast *Saccharomyces cerevisiae* Safethanol 3035 (both cultured and rehydrated) after fermentation of mashes made of dried maize grain was characterized by the lowest number of inactive cells and high percentage of budding cells. Considerable worsening of the physiological condition of the examined yeast was observed after fermentation of mashes prepared from bad grain contaminated with different groups of microorganisms. As far as rehydrated yeast were concerned, the highest contribution of inactive cells was recorded for fermentation of mashes made of maize grain stored for 8 months, 100% contaminated with mould of *Fusarium* species. High percentage of dead cells featured both cultured and rehydrated yeast when fermentation involved the longest – stored grain.

TABLE 3. Final effects of ethanol fermentation by using rehydrated (Sr) and cultured (Sc) *Saccharomyces cerevisiae* Safethanol 3035 yeast.

Maize mashes	Yeast	Final effects of ethanol fermentation		
		Ethanol yield % theoretical	Ethanol yield $Y_{(g\ ethanol\ /\ 100\ g\ dry\ matter)}$	The degree of starch consumption (%)
A	Sc	85.2	35.9	99.48
	Sr	85.4	36.0	99.58
B	Sc	82.7	34.9	99.67
	Sr	81.0	34.2	99.63
C	Sc	85.4	33.2	99.61
	Sr	84.9	33.4	99.59
D	Sc	84.5	35.5	99.37
	Sr	86.3	36.5	99.56

Maize grain: A – moist, B – stored for 8 months, C – stored for 19 months, D – dried.

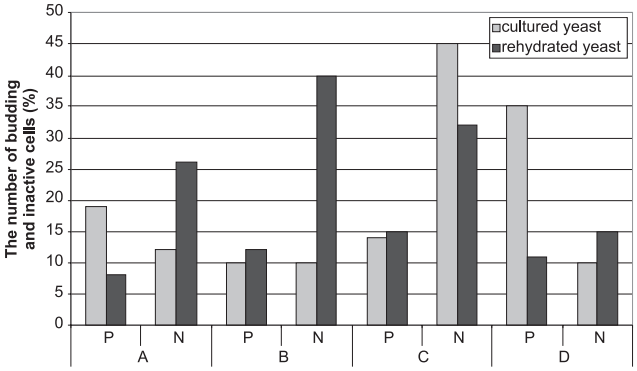

FIGURE 2. The physiological condition of cultured (Sc) and rehydrated (Sr) yeast cells after maize mash fermentation (A – moist grain, B – grain stored for 8 months, C – grain stored for 19 months, D – dried grain; P – budding yeast cells, N- inactive yeast cells).

DISCUSSION

Benefits and advantages of maize utilisation as raw material for bioethanol production were presented in anumber of works [Gulami *et al*., 1996; Krishnan *et al*., 2000; Latif & Rajoka, 2001; Lee & Yoon, 2000; Soto *et al*., 2005] and they have also been referred to in the introductory part of this work. Most investigation dealt with comparison of maize to other distillery raw materials traditional in our country, like rye [Kłosowski *et al*., 2001], or to unconventional one, *i.e.* amaranthus [Dobrzeniecka *et al*.1996]. Other authors assessed silage maize usefulness for ethanol production [Zielińska, 2003] and energy saving method of cold mashing in preparation of maize mash [Shigetchi *et al*., 2004; Stecka *et al*., 1996].

In spite of numerous advantages of using maize as distiller raw material, in practice there do occur some problems with is processing. The basic disadvantage is high moisture of grain after harvesting. Most maize grain can be stored without loses of its energetic and utilizing value after preservation according to one of the following methods: drying, preservation with chemical compounds or silaging. Generally, these treatments are highly energy-consuming and expensive and

therefore, to diminish the costs of ethanol production moist grain is applied. Climatic condition of our country cause that maize grain at the moment of harvesting features up to 40% moisture. When stored that kind of grain characterizes high activity of enzymes and intensive breathing, as well as there does place starch decomposition to simple sugars and easily soluble proteins which become a nutrient medium for microorganisms. In fresh, appropriately stored corn grain fungi occur in smaller amount. Field fungi of *Alternaria*, *Clodosporium* and *Fusarium* species attack ripening grain before its harvesting and therefore, they are dominant fungi. In optimum storage conditions (moisture less than 18%) they die within a few weeks. After 6 months they are not capable of development and they die. After the harvest the fungi of *Penicillium* and *Aspergillus* species occur in their place, so-called store fungi, which can bear drier conditions (13-18%). They are able to survive several–month-lasting storage, especially *Aspergillus niger.* They are characterized by not very intensive growth at the temperature of 20°C, while alternations of fungi attacked material become visible as long as 6 month to 12 months storing [Kluczek & Kojder, 2000]. High microbiological activity can lead to complete decay of grain and contamination with growing mould, as well as to mycotoxins occurrence (aflatoxin, ochratoxins and zearalenon), [Newsome, 2006]. The presence of fungi metabolites and loses of nutrients can negatively affect the course of technological process and the quality of final products

CONCLUSIONS

Maize grain stored for 8 months was infected with *Fusarium* fungi in 100%. After 19 months of grain storage the amount of *Fusarium* isolates decreased but the amount of *Penicillium spp.* increased. It was observed that both the quality of material used for fermentation substrate and the method used for the preparation of inoculum influenced the results of ethanol fermentation. Rehydrated yeasts needed more time to adapt to fermentation medium and were more sensitive to environment conditions. The rehydrated yeast were characterised by the lowest activity during fermentation of maize infected by 100% of *Fusarium* fungi. The process of fermentation of maize dried or stored for 19 months was completed after *ca*. 2 days (*ca*. 41 h). The mashes of fresh corn or stored for 8 months need *ca.* 3 days to complete the fermentation. The degree of carbohydrates consumption was very high, over 99% in all samples. The physiological condition of yeasts after the fermentation of lower quality material was significantly worse than in control samples. The yield of ethanol fermentation of poor quality grain were slightly worse than in the case of fresh or dried grain, but the differences were not significant, which confirms the suitability of this raw material for fuel production purposes.

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WPŁYW JAKOŚCI SUROWCA NA AKTYWNOŚĆ FERMENTACYJNĄ DROŻDŻY GORZELNICZYCH

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Celem pracy było określenie wpływu jakości surowca na przebieg i efekty końcowe fermentacji etanolowej zacierów kukurydzianych z udziałem namnażanych i rehydratowanych drożdży gorzelniczych *Saccharomyces cerevisia* Safethanol 3035.

Materiał badawczy stanowiło ziarno kukurydzy odmiany Kosmo 230: świeże (bezpośrednio po zbiorze), suszone oraz przechowywane bez utrwalenia (w temperaturze 20°C przez 8 i 19 miesięcy).

Ziarno kukurydzy przechowywane przez 8 miesięcy w 100% było porażone przez grzyby z rodzaju *Fusarium*. W ziarnie przechowywanym przez 19 miesięcy liczba izolatów grzybów z rodzaju *Fusarium* spadła, a wzrosła *Penicillium spp.*

Proces fermentacji kukurydzy suszonej i przechowywanej przez 19 miesięcy zakończył się po ok. 2 dobach. Natomiast fermentacji niespełna trzydobowej wymagały zaciery przygotowane z kukurydzy wilgotnej i przechowywanej przez 8 miesięcy. Stopień wykorzystania skrobi był bardzo wysoki i we wszystkich próbach wynosił ponad 99%. Po fermentacji surowców gorszej jakości obserwowano znaczne pogorszenie stanu fizjologicznego drożdży. Wydajności fermentacji etanolowej ziarna zepsutego (B i C) były nieco niższe w porównaniu z ziarnem wilgotnym (A) czy suszonym (D) ale różnice nie były duże, co potwierdza przydatność takiego surowca na cele paliwowe.