The Impact of Moist Corn Grain Preservation on the Ethanol Yield by Simultaneous Saccharification and Fermentation, and on Volatile by-Products

Jacek NOWAK[1], Katarzyna SZAMBELAN[1] and Włodzimierz NOWAK[2]

[1]Institute of Food Technology of Plant Origin, Faculty of Food Science and Nutrition and [2]Department of Animal Nutrition and Feed Management, Faculty of Animal Breeding and Biology, Poznań University of Life Sciences, Poznań, Poland

Abstract

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We assessed the composition of volatile by-products in raw spirits obtained from moist corn fermentation. The average moisture value of the researched samples was 35.4%. A comparative research was conducted applying simultaneous saccharification and fermentation (SSF) process with *Saccharomyces cerevisiae*. The analysis characterised corn grains after three and six months of storage in aerobic and anaerobic conditions. The yield of ethanol fermentation was 42.43 and 39.12 l/100 kg dry matter after three and six months of storage, respectively. The storage of moist grain resulted in the reduction of higher alcohols content in the raw spirits. It was observed that the esters concentration decreased after three, but increased after six months of raw material storage. A significant increase occurred in the quantity of aldehydes detected only after three months of the corn storage. The results show that the application of SSF technology to moist corn, allows the production of bioethanol with quality comparable to that obtained with dried grain.

Keywords: bioethanol; chemical preservation; SSF; volatile compounds

Recently, a great attention has been focused on bioethanol production as a fuel additive. Bioethanol production mainly depends on energy crops containing starch and sugar (wheat, corn, triticale, sugar cane, sweet sorghum, sugar beet, Jerusalem artichoke, cassava etc.) but the researches are also conducted into cellulose material fermentation (Hamelinck *et al*. 2005; Szambelan *et al*. 2005; Gumienna *et al*. 2009; Semencenko *et al*. 2011). The starch and sugar raw materials have the main applicability for bioethanol production. Starch, in starchy materials used for industrial-scale ethanol production, is generally first hydrolysed by adding a liquefying enzyme (α-amylase) and next the liquefied starch is hydrolysed to glucose with a saccharifying enzyme (glucoamylase) (Słomińska *et al.* 2003; Wang *et al.* 2007).

Pressure cooking is still largely used in Poland as a very effective procedure for further fermentation of starchy materials but the production costs are high due to the high energy consumption in the cooking process. An alternative to the classical (pressure) method of starch liquefaction is the non-pressure cooking fermentation system as well as the simultaneous saccharification and fermentation (SSF) method or fermentation with thin part of stillage recycling (Roy *et al.* 2001; Białas *et al*. 2010; Gumienna *et al*. 2011; Nikolić *et al.* 2012). In the SSF technology, the whole process is carried out in a single reactor, which not only allow to reduce the process time but also lowers the costs. An important improvement on SSF method is the application of a new type of amylolytic enzyme (STARGEN 001™; Genencor International, Palo Alto, USA) able to hydrolyse granular non-cooking form of starch. The new enzyme preparation is active at appropriate fermentation pH and temperature, involving gradually the

accessibility of sugar arising due to the the effect of starch simultaneous hydrolysis with preventing the existence of osmotic stress for distillery yeasts. The SSF method may allow for the production of ethanol with a high performance.

Besides wheat and triticale, corn has become a very popular cereal in distillery industry in Eastern Europe (Devantier *et al.* 2005; Kwiatkowski *et al.* 2006). Among cereal crops harvested in Poland, corn has been reported to have a number of advantages as a raw material for bioethanol production. It has a high starch content (over 60%), is characterised by high crop (8.0 t/ha) and ethanol (417 l/t) yields, and is easy to handle as a material for fermentation (Lipski 2002; Belyea *et al.* 2004; Kupczyk 2007).

The important problem in ethanol production from corn is the grain storage especially during wet and short summers like those in Poland. The climate conditions in Poland determine the late harvest time of corn which results in fresh grain being sometimes characterised by as much as 40% of moisture and were not applicable for longer storage. Such moisture level results in rapid bacteria and mould growth. The solution of the problem may be drying or chemical preservation of the freshly harvested grain. Dried corn grain is stable and can be stored for a long time in dry conditions, but the drying process is expensive. If we assume that ethanol is produced from wet corn grain directly, instead from dried, the energy efficiency will significantly increase. One of the most appropriate grain preservation methods may be applying biological or chemical preservatives. Most of the chemical preparations used are based on propionic and formic acids. Formic acid is primarily designed to lower the pH and inhibiting the growth of bacteria while propionic acid is highly effective mould inhibitor, commonly used in the food and feed industry. It was shown that it controls the growth of aflatoxigenic fungi and aflatoxin production in high-moisture corn grains (Marin *et al.* 2000; Aksu *et al.* 2004).

Alcoholic fermentation with *S. cerevisiae* results in the production, apart from ethanol, of many volatile compounds passing through to raw spirits during the distillation process. The most important volatile by-products detected in raw distillates are organic acids, higher alcohols, as well as methanol, aldehydes, esters, and sulphur compounds. The use of inferior materials, such as mold-infected, sprouted grain, has a significant impact on the quality of the raw spirits obtained (Miecznikowski & Zielińska 2002; Kłosowski & Mikulski 2010).

The content of volatile by-products, lowering the quality of raw spirits, depends on the process of fermentation, kind and quality of raw the materials used, pH value, temperature, heavy metals content, strain and quantity of yeast as well as the initial density of the sweet mash. The mechanisms of volatile by-products production have already been described (Schmidt *et al.* 1983; Goj 1990; Stanisz *et al.* 2009).

The aim of the present study has been to determine the effects of both chemical corn grain preservation, i.e. aerobic or anaerobic conditions and storage time, on the ethanol yield and formation of volatile by-products when SSF technology has been applied.

MATERIAL AND METHODS

Raw material. The corn grains, kept three and six months at room temperature, were used in the research: control sample A – dried corn grain, sample B (storage in aerobic conditions) – moist corn grain + Stabilizer TMR L (Kemira Chemie, Krems, Austria), sample C (storage in anaerobic conditions) – moist corn grain + KemiSile 2000 plus (Kemira Chemie, Krems, Austria), sample D (storage in aerobic conditions) – moist corn grain without additives, sample E (storage in anaerobic conditions) – moist corn grain without additives. Moist grain was characterised by the initial dry matter at the level of 64.60%. The control sample (A) was a dried sample, characterised by 89.21% of dry matter. The results of moist corn analysis (samples B–E) were compared to those of control sample (A).

Kemira Stabilizer TMR L was a propionic acid based substance while KemiSile 2000 plus was a formic acid based substance. The corn grain samples were stored in micro-silos (aerobic and anaerobic conditions) with a capacity of 3.5 l. To maintain the aerobic conditions, the micro-silos without stoppers were applied. Additionally, a tube with holes was inserted along the silos.

Microorganisms. Distillery yeast *Saccharomyces cerevisiae*, Ethanol Red strain (Lasaffre Company, Marcq-en-Baroeul, France), was used for ethanol production from corn mashes. Dry yeast was hydrated before use and the slurry corresponding to 0.5 g of dried yeast/l was added to the mash.

Enzymes. Bacterial α-amylase Amylex™ BT2 (Genencor International, Palo Alto, USA), produced by fermentation of a non-genetically modified strain *Bacillus stearothermophilus*, was used for corn starch liquefaction. Diazyme SSF, a saccharifying enzyme preparation containing glucoamylase and protease,

both of which had been derived from *Aspergillus niger* (Genencor International), was applied for corn starch hydrolysis. Both enzymes were used according to the producer's recommendations.

Fermentation process. The corn grain samples were ground and mixed with water to obtain the density of the fermented media of 250 g dry solids/l. The fermentation prodecures were carried out in 250 ml Erlenmeyer flasks containing 150 g of medium. The pH-value of the prepared fermentation media was adjusted to 5.0 and Amylex™ BT2 was added. The liquefaction process was carried out at 95°C for 45 minutes. The liquefied mashes were cooled down to 35°C and Diazyme SSF was added. The fermentation media were kept at 35°C for 1 h and than inoculated with distillery yeast Ethanol Red and incubated at 35°C for 72 h under constant stirring at 125 rpm. After 72 h of fermentation the distillation process was applied. The raw spirits obtained were investigated for ethanol and volatile by-products contents. The residual reducing sugars content was controlled in the stillage.

Analytical methods. The dry matter content of corn grain samples was estimated directly by drying at 130°C for 90 minutes. The content of reducing sugars was determined using 3.5-dinitrosalicylic acid method (Miller 1959). The starch content was analysed according to Holm *et al*. (1986). The pH-value was determined in aqueous solutions of the samples, allocated in a further step to the process of fermentation. The ethanol concentration was assayed after distillation using areometric method.

The composition and purity of the raw spirits obtained were checked on a Hewlett Packard HP 6890 gas chromatograph (Hewlett Packard, Waldbronn, Germany), using a Supelcowax-10 (60 m × 0.53 mm × 1.0 μm) column and a FID detector. Hydrogen was used as a carrier gas. The by-products in the spirits were determined using the retention times of the peaks and normalised using the retention time of the internal standard 2-heptanol.

The microbiological analyses for the presence of *Clostridium*, yeast and molds, lactic acid bacteria, and *Enterobacteriaceae* in the material after three and six months of storage were performed on Willis-Hobbs medium, on the synthetic medium for the determination of yeast and molds, on MRS agar medium, and on agar medium with kanamycin, respectively. The concentrations of lactic, acetic, propionic, and butyric acids were determined by HPLC method using Waters Alliance, HPX-87H BIO-RAD column with a RI detector, 30°C, flow speed 0.6 cm^3/minute.

All experiments were carried out in triplicate. The significance and standard deviations were calculated by the analysis of variance using Statistica 6.0 (StatSoft, Tulsa, USA) ($\alpha = 0.05$).

RESULTS AND DISCUSSION

The corn samples used in this study were characterised by significantly low ($P < 0.05$) dry matter contents (52.44–62.26%) after three months of storage (Table 1). Prolonging the storage time up to six

Table 1. Corn material analysis after three and six months of storage

Corn sample	pH	Dry matter (%)	Reducing sugars		Starch	
			(mg/g)	(% DM)	(mg/g)	(% DM)
Three months storage						
A	6.52 ± 0.02^{d}	89.27 ± 0.05^{d}	9.25 ± 0.06	1.04 ± 0.01	589.74 ± 4.12^{d}	66.06 ± 0.46
B	5.69 ± 0.97^{c}	59.92 ± 0.39^{b}	18.12 ± 6.64	3.03 ± 1.12	363.57 ± 7.97^{b}	60.66 ± 2.64
C	4.16 ± 0.08^{a}	62.26 ± 0.30^{c}	13.47 ± 0.69	2.17 ± 0.10	407.39 ± 4.43^{c}	65.43 ± 0.40
D	5.13 ± 0.08^{bc}	52.44 ± 1.54^{a}	10.25 ± 0.53	1.96 ± 0.13	290.13 ± 10.69^{a}	55.34 ± 0.82
E	4.71 ± 0.03^{ab}	61.41 ± 0.13^{c}	6.02 ± 0.15	0.98 ± 0.02	397.64 ± 3.61^{c}	64.76 ± 0.54
Six months storage						
A	6.40 ± 0.37^{c}	88.41 ± 0.06^{c}	9.73 ± 0.28	1.10 ± 0.04	584.02 ± 3.17^{d}	64.93 ± 0.27
B	7.58 ± 0.36^{d}	58.11 ± 8.06^{b}	18.05 ± 1.37	3.09 ± 0.41	375.16 ± 7.35^{b}	64.28 ± 3.74
C	4.18 ± 0.08^{a}	63.11 ± 0.81^{b}	14.54 ± 0.66	2.31 ± 0.10	414.90 ± 3.61^{c}	65.75 ± 1.05
D	6.46 ± 0.26^{c}	46.99 ± 2.51^{a}	10.96 ± 1.26	2.33 ± 0.18	295.95 ± 14.56^{a}	62.99 ± 1.39
E	4.77 ± 0.43^{b}	62.47 ± 0.42^{b}	4.04 ± 0.02	0.65 ± 0.01	422.79 ± 4.84^{c}	67.68 ± 1.02

A – control; B – storage in aerobic conditions + Stabilizer TMR L; C – storage in anaerobic conditions + KemiSile 2000 plus; D – storage in aerobic conditions without additives; E – storage in anaerobic conditions without additives; DM – dry matter; $^{a–d}$values with different letters within the same column are significantly different ($P < 0.05$)

Table 2. Ethanol yield from corn material after three and six months of storage

Corn sample	pH after fermentation	Ethanol					Reducing sugars in spent wash (mg/g)
		(% v/v)	(l/100 kg starch)	theoretical value (%)	(l/100 kg material)	(l/100 kg DM)	
Three months storage							
A	3.94 ± 0.01	9.34 ± 0.19	56.52 ± 1.14	78.61 ± 1.58	33.34 ± 0.67	37.34 ± 0.75^{a}	8.95 ± 0.11^{ab}
B	5.02 ± 0.32	9.89 ± 0.49	65.46 ± 0.57	91.04 ± 0.79	23.80 ± 1.36	39.71 ± 2.04^{bc}	7.58 ± 1.69^{a}
C	5.36 ± 0.14	10.57 ± 0.17	64.86 ± 1.59	90.20 ± 2.21	26.42 ± 0.43	42.43 ± 0.85^{d}	6.73 ± 0.58^{a}
D	5.20 ± 0.08	9.55 ± 0.26	68.89 ± 0.80	95.82 ± 1.11	20.00 ± 0.96	38.12 ± 0.91^{ab}	10.94 ± 2.83^{b}
E	5.25 ± 0.07	10.37 ± 0.01	64.11 ± 0.59	89.16 ± 0.81	25.49 ± 0.01	41.47 ± 0.10^{cd}	26.53 ± 0.40^{c}
Six month storage							
A	4.41 ± 0.08	9.20 ± 0.18	57.24 ± 1.17	79.61 ± 1.62	32.86 ± 0.67	37.16 ± 0.76^{b}	7.68 ± 0.11^{a}
B	5.33 ± 0.13	9.18 ± 0.57	57.14 ± 1.05	79.47 ± 1.38	21.46 ± 1.83	36.71 ± 1.28^{b}	13.40 ± 1.14^{b}
C	5.39 ± 0.35	9.27 ± 0.12	56.38 ± 0.24	78.41 ± 0.34	23.39 ± 0.22	37.07 ± 0.46^{b}	16.14 ± 2.88^{b}
D	5.37 ± 0.06	8.31 ± 0.62	52.71 ± 1.09	73.31 ± 1.25	15.63 ± 0.93	33.17 ± 1.40^{a}	8.10 ± 0.79^{a}
E	5.27 ± 0.61	9.78 ± 0.12	57.82 ± 1.01	80.42 ± 1.41	24.44 ± 0.18	39.12 ± 0.47^{b}	14.15 ± 1.76^{b}

A – control; B – storage in aerobic conditions + Stabilizer TMR L; C – storage in anaerobic conditions + KemiSile 2000 plus; D – storage in aerobic conditions without additives; E – storage in anaerobic conditions without additives; DM – dry matter; $^{a–d}$values with different letters within the same column are significantly different ($P < 0.05$)

months did not significantly ($P > 0.05$) influence the dry matter level for particular trials, except for sample D (Table 1).

In order to assess the suitability of the raw material for bioethanol production, starch content was determined. The samples tested differed significantly in their contents of starch. The samples stored under anaerobic conditions were characterised by the highest starch content ranging from 397.64 mg/g to 422.79 mg/g, both after three and six months of storage. The storage time did not significantly ($P > 0.05$) affect the changes in the starch content in the analysed samples (Table 1).

The samples differed in pH but only with samples B and D the pH increased importantly ($P < 0.05$) after six months of storage.

The study investigated the effects of the preservative agent addition, conditions (aerobic and anaerobic), and storage time on the efficiency of corn bioethanol production. To compare the efficiency of ethanol production from corn dried and moist, it was decided to refer to the yield based on the dry matter content.

Table 2 shows the yield of ethanol from the samples tested after three months of storage. It has been noted that the use of the KemiSile 2000 plus preparation and storage in anaerobic conditions allowed to obtain the highest ethanol yield (42.43 l/100 kg dry matter – DM), higher by 13.6% than that from the control sample. Previous research (Nowak *et al*. 2008) confirmed that the preserved corn grain with the addition of KemiSile 2000 plus (at different doses) was a very good material for bioethanol production (94% of theoretical ethanol yield when low-temperature-cooking and high-pressure-cooking fermentation method was used).

The extension of the storage time up to 6 months resulted in ethanol yield from the stored samples at the level comparable to those from dried corn, and significantly lower ($P < 0.05$) for D sample (Table 2).

It should be also noted that only in the case of the sample with the Stabilizer TMR L addition (B), extending storage time did not affect significantly ($P > 0.05$) the changes in ethanol production (Table 2). Samples C, D, and E were characterised with significantly ($P < 0.05$) lower ethanol yields after six months of storage than after three months.

The raw spirits obtained from the fermentation of stored moist corn grains were analysed by gas chromatography for the contents of major volatile compounds. All the results were compared to the control sample (A) (Tables 3 and 4).

Table 3 shows the results of raw spirits obtained from corn material after three months storage. It was noted that in the raw spirits obtained from the stored moist grain, the content of aldehydes increased ($P < 0.05$). The amount of aldehydes constituted 0.12–0.19% of all volatile compounds detected, while in the control sample it was 0.09%. Acetaldehyde had a small share in the total concentration of aldehydes and ketones.

Table 3. Ethanol and by-products content (g/l 100% EtOH) in raw spirits produced from corn material after three months of storage

Volatile compound	A	B	C	D	E	Polish standards for raw alcohol
Σ Aldehydes, ketones	0.712 ± 0.081^{a}	1.215 ± 0.106^{b}	1.536 ± 0.228^{c}	0.941 ± 0.201^{b}	1.079 ± 0.056^{b}	
Acetaldehyde	0.548 ± 0.037	0.765 ± 0.108	1.050 ± 0.049	0.520 ± 0.030	0.632 ± 0.091	aldehydes < 0.1
Acetone	0.005 ± 0.001	0.056 ± 0.004	0.008 ± 0.002	0.022 ± 0.002	0.004 ± 0.000	
Acetaldehyde diethylacetal	0.158 ± 0.017	0.394 ± 0.068	0.478 ± 0.038	0.399 ± 0.029	0.433 ± 0.046	
Σ Esters	0.128 ± 0.006^{c}	0.028 ± 0.011^{a}	0.069 ± 0.008^{ab}	0.077 ± 0.011^{b}	0.036 ± 0.003^{a}	esters not normalised
Ethyl acetate	0.128 ± 0.006	0.028 ± 0.011	0.069 ± 0.008	0.060 ± 0.002	0.015 ± 0.002	
Isoamyl acetate	nd	nd	nd	0.017 ± 0.008	0.022 ± 0.000	
Ethyl caprylate	nd	nd	nd	nd	nd	
Σ Higher alcohols	6.141 ± 0.238^{c}	2.408 ± 0.291^{ab}	2.971 ± 0.147^{b}	1.799 ± 0.133^{a}	2.522 ± 0.046^{ab}	higher alcohols < 3.5
2-Butanol	nd	0.007 ± 0.001	0.005 ± 0.001	0.078 ± 0.001	0.250 ± 0.007	
Propanol	0.395 ± 0.020	0.374 ± 0.017	0.632 ± 0.073	0.648 ± 0.043	0.753 ± 0.027	
Isobutanol	1.417 ± 0.032	0.655 ± 0.096	0.509 ± 0.063	0.249 ± 0.015	0.373 ± 0.009	
1-Butanol	0.014 ± 0.000	0.034 ± 0.008	0.011 ± 0.004	0.033 ± 0.009	0.285 ± 0.015	
2-Methyl-1-butanol	1.253 ± 0.035	0.382 ± 0.035	0.404 ± 0.047	0.178 ± 0.008	0.245 ± 0.011	
3-Methyl-1-butanol	3.061 ± 0.149	0.710 ± 0.016	1.408 ± 0.055	0.607 ± 0.033	0.614 ± 0.046	
1-Pentanol	0.001 ± 0.000	0.002 ± 0.000	0.002 ± 0.000	0.004 ± 0.001	0.003 ± 0.000	
Methanol	0.035 ± 0.004^{a}	0.051 ± 0.006^{b}	0.086 ± 0.004^{d}	0.058 ± 0.005^{b}	0.075 ± 0.002^{c}	methanol < 0.08
Ethanol, % of total volatile compounds	99.12 ± 0.02^{a}	99.52 ± 0.05^{b}	99.41 ± 0.21^{b}	99.63 ± 0.18^{b}	99.53 ± 0.01^{b}	–

A – control; B – storage in aerobic conditions + Stabilizer TMR L; C – storage in anaerobic conditions + KemiSile 2000 plus; D – storage in aerobic conditions without additives; E – storage in anaerobic conditions without additives; $^{a–d}$values with different letters within the same column are significantly different ($P < 0.05$); nd – not detected

An increase of methanol content was also observed, by 46% for sample B to 146% for sample C, as compared to the control. However, when aerobic conditions were applied, the increase of methanol was lower than that under anaerobic conditions (Table 3).

Higher alcohols and esters concentrations showed opposite tendencies to those of aldehydes and methanol (Table 3). The methods of preserving and conditions of storing resulted in a significant ($P < 0.05$) decrease in higher alcohols content which constituted 0.23–0.38% of all volatile compounds in comparison with 0.77% for control sample. In the spirits from moist samples fermentation, additionally 2-butanol was detected, but this had a small share in the total higher alcohols concentrations. The gas chromatography analysis showed that 3-methyl-1-butanol in samples B and C as well as propanol in samples D and E were the predominant higher alcohols produced by the yeast from the moist corn stored for three months. The next group of compounds, affecting the quality of raw spirits, was esters. The use of moist corn after three months of storage for ethanol fermentation decreased the esters concentration in raw spirits by 22–60%. It is worth noting that samples B and C (with chemical substances additions) were characterised, as the control one, only by the content of ethyl acetate. Additionally isoamyl acetate was detected in the samples preserved without chemical additives. Statistical analysis of volatile compounds showed that the raw spirits from moist corn used after three months of storage for bioethanol production were characterised by fewer ($P < 0.05$) fermentation by-products than those from dried grain.

The next stage of experiments concerned the analysis of raw spirits obtained from the corn fermentation after six months of storage (Table 4). Despite the long-term storage similar trends as with three months storage were observed in the contents of raw spirits, aldehydes, higher alcohols, and methanol. As compared to the control, significantly ($P < 0.05$) higher amounts (by 19–73%) of aldehydes and ketones were observed, similarly with E, while in the case of methanol a significant increase was observed only for samples C

Table 4. Ethanol and by-products content (g/l 100% EtOH) in raw spirits produced from corn material after six months of storage

Volatile compound	A)	B	C	D	E
Σ Aldehydes, ketones	0.644 ± 0.072[a]	0.768 ± 0.055[bc]	0.969 ± 0.108[cd]	1.117 ± 0.182[d]	0.599 ± 0.105[ab]
Acetaldehyde	0.514 ± 0.019	0.598 ± 0.048	0.632 ± 0.091	0.780 ± 0.086	0.356 ± 0.085
Acetone	0.005 ± 0.001	nd	nd	nd	nd
Acetaldehyde diethylacetal	0.125 ± 0.021	0.170 ± 0.014	0.337 ± 0.077	0.336 ± 0.067	0.243 ± 0.037
Σ Esters	0.140 ± 0.027[a]	0.624 ± 0.069[b]	0.929 ± 0.106[c]	0.471 ± 0.041[b]	0.454 ± 0.031[b]
Ethyl acetate	0.140 ± 0.027	0.028 ± 0.002	0.081 ± 0.005	0.036 ± 0.003	0.012 ± 0.009
Isoamyl acetate	nd	0.594 ± 0.061	0.848 ± 0.104	0.433 ± 0.043	0.439 ± 0.026
Ethyl caprylate	nd	0.002 ± 0.001	0.001 ± 0.000	0.001 ± 0.000	0.002 ± 0.001
Σ Higher alcohols	6.109 ± 0.148[d]	1.649 ± 0.085[a]	3.436 ± 0.126[c]	1.831 ± 0.030[a]	2.477 ± 0.455[b]
2-butanol	nd	0.025 ± 0.006	0.003 ± 0.001	0.032 ± 0.002	0.078 ± 0.016
Propanol	0.378 ± 0.039	0.300 ± 0.028	0.686 ± 0.093	0.611 ± 0.025	0.936 ± 0.018
Isobutanol	1.397 ± 0.052	nd	nd	nd	ndD
1-butanol	0.019 ± 0.000	0.014 ± 0.007	0.011 ± 0.002	0.052 ± 0.007	0.250 ± 0.015
2-methyl-1-butanol	1.213 ± 0.076	1.057 ± 0.071	2.256 ± 0.240	0.879 ± 0.015	0.973 ± 0.083
3-methyl-1-butanol	3.101 ± 0.122	0.253 ± 0.056	0.478 ± 0.013	0.256 ± 0.018	0.239 ± 0.087
1-pentanol	0.001 ± 0.000	0.002 ± 0.001	0.002 ± 0.000	0.001 ± 0.000	0.001 ± 0.000
Methanol	0.040 ± 0.002[a]	0.059 ± 0.011[a]	1.137 ± 0.033[c]	0.074 ± 0.009[a]	0.632 ± 0.089[b]
Ethanol, % of total volatile compounds	99.13 ± 0.03[a]	99.61 ± 0.10[b]	99.19 ± 0.07[a]	99.56 ± 0.05[b]	99.48 ± 0.07[b]

A – control; B – storage in aerobic conditions + Stabilizer TMR L; C – storage in anaerobic conditions + KemiSile 2000 plus; D – storage in aerobic conditions without additives; E – storage in anaerobic conditions without additives; [a–d]values with different letters within the same column are significantly different ($P < 0.05$); nd – not detected

and E. Samples B and D were characterised by the methanol content similar to that in the control. Still, the investigated spirits showed a lower content (by 43–70%) of higher alcohols but the profile of these compounds was changed. 2-methyl-1-butanol turned out to be the predominant compound constituting 40–65% of total higher alcohols, whereas isobutanol was not detected in the tested samples.

It is worth noticing that, unlike after three months of storage, an increase was observed in the amount of esters after six months of storage. Except for ethyl acetate and isoamyl acetate, also trace amounts of ethyl caprylate were identified. Despite the prolonged storage time, the percentage of volatile by-products was still significantly ($P < 0.05$) lower for samples B, D, and E, and similarly for C, compared to the control.

Taking into account the fact that microflora present in the material can affect not only ethanol yield but also the composition of the fermentation volatile by-products in raw spirits, microbiological analysis was carried out after three and six months of raw material storage. The applied additives, as well as storage in anaerobic conditions without additives, resulted in lower levels of designated microorganisms after three months of storage (Table 5). The lowest ($P < 0.05$) level of corn contamination used as raw material after six months of storage was determined in the samples treated with KemiSile 2000 plus. The lower content of undesirable microflora resulted in a higher ethanol yield, however, when KemiSile 2000 plus was applied, higher amounts of higher alcohols and methanol were detected after both storage periods (Tables 2–4). The corn sample storage in aerobic conditions without additives resulted in the occurrence of the highest number of undesirable microflora and caused the decrease of ethanol yield, both after three and six months of storage (Tables 2–5). The highest concentration of lactic and acetic acids was determined in the samples stored in aerobic conditions without additives, the lowest with the addition of both Stabilizer TMR L and KemiSile 2000 plus and under anaerobic storage without additives (Table 5). The applied additives also caused butyric acid reduction whereas the highest content of this acid was found in the samples stored in anaerobic conditions without additives (Table 5).

The amounts of higher alcohols detected in raw spirits obtained after fermentation of the stored moist

Table 5. Effect of chemical preparation addition and time of storage on corn microflora and acids concentration

Corn sample	Acids (% DM)				(log CFU)			
	lactic	acetic	propionic	butyric	*Clostridium*	yeast, molds	lactic acid bacteria	*Enterobacteriaceae*
Three months of storage								
B	1.33^{b}	2.32^{a}	0.28^{a}	0.11^{c}	6.71^{b}	6.58^{c}	6.92^{b}	5.98^{b}
C	1.17^{b}	0.93^{bc}	0.27^{a}	0.00^{c}	5.50^{b}	6.60^{c}	5.69^{b}	3.28^{c}
D	2.42^{a}	1.82^{ab}	0.07^{b}	0.34^{b}	8.69^{a}	7.86^{b}	8.81^{a}	7.89^{a}
E	0.79^{b}	0.44^{c}	0.08^{b}	0.60^{a}	5.11^{b}	7.13^{b}	6.68^{b}	2.84^{c}
Six months of storage								
B	1.48^{ab}	0.36^{c}	0.58^{c}	0.19^{b}	7.76^{a}	7.25^{b}	6.99^{b}	4.32^{c}
C	1.17^{b}	0.60^{b}	0.23^{a}	0.12^{b}	5.83^{b}	6.40^{c}	5.80^{b}	3.01^{c}
D	1.83^{a}	1.56^{a}	0.03^{b}	0.23^{b}	8.50^{a}	8.35^{a}	7.69^{a}	7.76^{a}
E	1.01^{b}	0.30^{c}	0.07^{b}	0.42^{a}	6.31^{b}	6.90^{bc}	7.97^{a}	5.53^{b}

A – control; B – storage in aerobic conditions + Stabilizer TMR L; C – storage in anaerobic conditions + KemiSile 2000 plus; D – storage in aerobic conditions without additives; E – storage in anaerobic conditions without additives; $^{a–c}$values with different letters within the same column are significantly different ($P < 0.05$) ; DM– dry matter

corn grains did not exceed the values limited for raw cereal spirits as stated by Polish Standard (PN-A-79523:2002). Only methanol amounts in samples C and E, as well as aldehydes contents in the spirits obtained from samples B, C, D, E and control, exceeded these values. However, while the high levels of higher alcohols make no problem in view of their exclusion in the rectification process, aldehydes may be undesirable in the commercial consumable spirit production as well as methanol in some samples. The quality of the obtained spirits is sufficient to be successfully utilised in the fermentation industry for bioethanol production.

CONCLUSIONS

This study has shown that wet corn can be successfully stored up to six months for bioethanol production, thus improving the process effectiveness.

The highest ethanol yield (as calculated from 100 kg DM) was obtained from raw material after three months of storage with KemiSile 2000 plus addition. However, it is to be stated that the addition of Stabilizer TMR L allowed to obtain ethanol yield at the same level, regardless of the storage time applied. The tested preparations (KemiSile 2000 plus and Stabilizer TMR L) allowed to keep the raw material in better microbiological quality during storage. The lower content of undesirable microflora as well as of lactic and butyric acids resulted in higher ethanol yield.

The raw spirits obtained by stored moist corn fermentation were characterised by lower amounts of volatile by-products, in comparison to dried control. Moist corn grain showed the potential as suitable raw material for bioethanol production giving the possibility to reduce the fermentation process costs by omitting the drying step.

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Corresponding author:

Dr Katarzyna Szambelan, Poznań University of Life Sciences, Faculty of Food Science and Nutrition, Institute of Food Technology of Plant Origin, Wojska Polskiego 31, 60-624 Poznań, Poland; E-mail: kasiasz@up.poznan.pl