# Agricultural Distillates from Polish Varieties of Rye

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#### Abstract

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We evaluated the effect of Polish cultivars of rye on the dynamics of fermentation and the amount and composition of the by-products in agricultural distillates obtained. The scope of research included the analysis of mashes prepared from the following rye cultivars: Dańkowskie Złote, Dańkowskie Diament, Amilo, and Amber. It has been shown that the use of the cv. Dańkowskie Diament rye had a significant effect on the concentration of acetaldehyde in the distillate obtained. Its content in this distillate was 2.5-fold higher than in the spirit obtained from rye cv. Amilo. It is also worth mentioning that in the distillate made from horyńrye cv. Amilo the lowest concentration of higher alcohols was observed.

Keywords: by-products of fermentation; ethanol fermentation; higher alcohols; Secale cereale

According to the definition submitted in the Regulation of the European Parliament and Council Regulation (EC) No 110/2008 of 15 January 2008, vodka is a spirit drink produced from ethyl alcohol of agricultural origin, obtained from potatoes or cereals by fermentation carried out by yeast, which is distilled or rectified in a way providing for selective reduction of the organoleptic properties of the used raw materials and by-products of fermentation. If the manufacturer uses ethanol obtained from other materials than those specified above, then in the description, presentation or labelling of vodka the term manufactured from will have to be shown, supplemented by the name of the raw material that was used to produce ethanol (LUTY 2009).

Over the past few years some significant changes concerning the use of raw materials have occurred in Polish distilling. 20 to 30 years ago the proportion of potato spirits in the total production exceeded 80%, while in 1997 it accounted for only 12.4% (SAMBORSKI 2005). The main criterion for the selection of materials used to prepare the sweet mashes is the starch content. In recent years, agricultural distilleries have generally used grain – mostly rye and maize (JAROSZ 1998). Growing rye in Poland is absolutely justified because of the state of the national soil. More than 30% of the arable soil is light and very light, with a low or very low pH which consequently reduces crop yields; one exception exists i.e. rye (*Secale cereale*) (MARCINIAK 2005). As a distillers' raw material, it is valued for its high efficiency because it contains more than 60% starch, and the stillage of this grain has a much higher nutritional value than the potato stillage (JAROSZ 1957).

The quality of the distillates obtained depends on the raw materials used, as well as on the conditions of fermentation, distillation, and rectification. The by-products formed even in trace amounts can modify and sometimes even adversely affect the quality of ethanol and the spirit drink produced from it. The physical and chemical requirements for ethyl alcohol of agricultural origin are limited to determining the level of basic pollutants, including aldehydes, esters, higher alcohols, acids and methanol (KŁOSOWSKI *et al.* 2003a). The optimisation of the process parameters allows to obtain agricultural distillates which have a high quality, complying with the requirements of the Polish Standards.

The mechanism of the individual by-products formation is a very complex process, which is affected by many factors, including the quality and type of the processed raw material, method of the sweet mashes preparation, density and pH of mashes, yeast strains, inoculum and mineral medium, the presence of microbiological contamination as well as the temperature of the fermentation (KŁOSOWSKI & CZUPRYŃSKI 1994; KOTARSKA *et al.* 2006).

Sometimes sweet mash is prepared with the use of low quality materials. The neglecting of the hygienic and technical parameters leads to increased levels of aldehydes and other by-products in the fermentatiom medium. The resulting spirits, despite careful purification by rectification suitable for the level of the by-products required by the Polish Standards, are characterised by an unfavourable taste and smell (GoJ 1992, 1996). The appropriate hygienic conditions reduce the intense proliferation of foreign micro flora and the appearance of undesirable compounds in the mashes. The sources of infection in fermenting mashes are: the raw material, yeast used in the technological process, the state of hygiene in the distillery (environment, workers).

## MATERIAL AND METHODS

*Rye cultivars*. Dańkowskie Złote, Dańkowskie Diament, Amilo and Amber, derived from plant breeding Danko Sp. z o.o. (Choryń, Poland).

Microbiologically derived enzymatic preparations (obtained from Novozymes, Bagsvaerd, Denmark). Termamyl S.C. ( $\alpha$ -amylase), SAN Extra (glukoamylase), Termamyl SC preparation, including thermostable, bacterial  $\alpha$ -amylase was used to starch liquefaction, at a dose recommended by the manufacturer. The activation of this enzyme takes place at high temperatures, i.e. 85–90°C. The recommended mash pH for this enzyme preparation is 6–6.5.

San Extra preparation was used for the saccharification of dextrins formed during liquefaction. The primary component is amyloglucosidase, hydrolysing the  $\alpha$ -1,4,  $\alpha$ -1,6 linkages in the liquefied starch and dextrins and acid  $\alpha$ -amylase which hydrolyses  $\alpha$ -1,4 linkages in amylose and amylopectin (KOTARSKA *et al.* 2005).

**Dried distillates yeast Saccharomyces cerevisiae**. In the experiments, a commercial preparation of dried distillers yeast strain As-4 was used. These yeasts are characterised by resistance to the high temperature of fermentation (38–39°C) and osmotic pressure, indicating fermentation activity at a concentration of ethanol in the mash up to 12% (CZUPRYŃSKI & KŁOSOWSKI 1994; KŁOSOWSKI *et al.* 2006).

*Sweet mash preparation*. Sweet mashes were prepared from four varieties of rye. The destruction

of the cellular structure of rye grain which enables the release of the starch took place in the steamer (Henze method), under water vapour pressure 0.4 MPa (150°C) with the duration of one hour. Whole grains were transferred to the steamer and then water was added (3.5 dm<sup>3</sup> per each kg of raw material). This stage is called pressure-cooking. In the next step the mashing process was carried out and the steamed mass was cooled in a mash tub to temperature 90°C, and then enzymatic preparation Termamyl SC was added (recommended dose 0.13 ml/kg of starch) for liquefaction of starch, followed by continuous mixing. The process conditions were maintained for 30 minutes. Subsequently the mash was cooled to 65°C and the enzymatic preparation Extra SAN (in dose 0.10 ml/kg of starch) was added. The sweet mash obtained was cooled to temperature 30°C (optimal to inoculate yeast). Mashes were acidified with 25% sulphuric acid solution to pH 4.8, which is optimal for the action of yeast.

*Yeast preparation*. Before the addition to mash, yeasts were subjected to rehydration and disinfection. For this purpose, to the weighed portion of the yeast suspension a small portion of water was added subsequently the obtained inoculum was acidified with 25% sulphuric acid to pH value 2.5. These conditions were held for 15–20 minutes.

*Fermentation*. The study was conducted on a laboratory scale. A 8 l of sweet mash was transferred into the fermentation vessels with a capacity of 10 litres. In order to improve the efficiency of fermentation, the sweet mash was supplemented with a nutrient solution  $(NH_4)_2HPO_4$  in an amount of 0.2 g/l mash. A weighed portion of the mineral medium was dissolved in water and then added to the sweet mash. After the acidification to an appropriate pH and inoculation with the yeast, the vessel was closed up thoroughly allowing the withdrawal of the sample, and thermostated at 28–30°C for 72 hours.

*Alcoholic distillation from fermented mash*. After 72 h of the process, the fermented mash was subjected to distillation to separate ethanol. The process was carried out in a simple distillation apparatus and controlled refractometrically. The moment at which there was no alcohol in the distillate was considered as the end of the process. The raw distillates obtained were concentrated to the power of 40% vol. in order to conduct the of analyses of them in accordance with current standards.

*Analytical methods*. During the fermentation (at times 0, 17, 21, 25, 41, 45, 49, 65, and 72 h), the samples were taken to determine:

- the actual and apparent density (areometric method using termo-saccharimeter (°Blg);
- ethanol content (method of analysis in accordance with Polish Standard PN-A-79528-3: 2007) (% v/v);
- content of reducing sugars in sweet and fermented mashes (method Schoorl-Regenbogen) (g glucose/100 ml mash).

The results of the analyses were used to assess the correctness of the fermentation duration and to calculate the biotechnological indicators of the fermentation process, such as:

- ethanol yield (expressed as % of theoretical yield, calculated from the total sugars in the sweet mash);
- intake of total sugar (the use of sugars during fermentation) (in %).

Chromatographic analysis. The samples of agricultural distillates were analysed by gas chromatography, to determine the qualitative and quantitative composition of the fermentation by-products. The contents of the compounds from the groups of esters, aldehydes, and higher alcohols were assessed. The analysis was performed using a gas chromatograph Agilent 6890 N (Agilent Technologies, Inc., Santa Clara, USA) equipped with a flame-ionisation detector (FID), a sample injector SSL (split/splitless) and Agilent Chemstation software. The good separation of volatile components of agricultural distillates resulted in the possibility of using the HP-Innowax capillary column (Agilent) with dimensions of 60 m × 0.32 mm  $\times$  0.5 µm with the stationary phase in the form of polyethylene glycol. The temperature of the detector was 250°C, and the samples were dispensed by the dispenser (injector temperature 250°C) of the gas stream splitting (split) 1:45. Helium was used as carrier gas - flow rate of 2 ml/minute. The programmed temperature conditions were as follows: 40°C (6 min), 83°C (2°C/min), and 190°C (5°C/min) (2 min). The injected volume of the sample was 2.5 ml.

*Statistical analysis*. The results were statistically analysed using the STATISTICA 7.0 PL, in order to determine the differences between the contents of the by-products (esters, aldehydes, fusel oil, methanol)

in mashes prepared from the four varieties of rye. One-way analysis of variance (ANOVA) was used followed by Tukey's post-hoc multiple comparison test. Differences were considered significant at a P-value of  $\leq 0.05$ .

### **RESULTS AND DISCUSSIONS**

*Effects of rye varieties on the dynamics of fer-mentation*. Sweet mashes were prepared from four varieties of rye. In order to assess the quality of the raw materials, a basic analysis was performed, indicating the contents of starch, reducing sugars, proteins, ash, and moisture. The results of the analysis are shown in Table 1.

The high starch content in the tested rye varieties qualified them for the process of fermentation.

As part of the planned research, fermentation of the mashes prepared from the four Polish rye varieties was conducted. The process was carried out using commercial preparation of dried distillers yeast As-4. Based on the results of the ethanol content measurement in the subsequent hours of fermentation, the growth curves were constructed (Figure 1). The values obtained in the process are summarised in Table 2. The mash prepared with the use of the rye cv. Dańkowskie Złote was fermented dynamically with a short period of the initial phase of fermentation. In the 17<sup>th</sup> h of the process, the ethanol concentration was found to be 1.57% vol., which corresponded to 25.61% of its theoretical yield. However, in the 72<sup>nd</sup> h of the process, the lowest concentration of ethanol was noted in this trial. The longest period of the initial phase of the process was observed in the variant where the sweet mash was prepared from the rye cv. Amber. In this cultivart, the highest alcohol content was achieved in the 72<sup>nd</sup> h of the process (5.77% vol.) and a high intake of total sugars was reached (91.89%).

*Volatile by-products of fermentation in distillates.* Chromatographic analysis of the distillates allowed the determination of the quantitative composition of the most representative ones (i.e.

	Dańkowskie Złote	Dańkowskie Diament	Amilo	Amber
Moisture (%)	$10.60 \pm 0.07^{a}$	$10.50 \pm 0.06^{a}$	$10.32\pm0.02^{\text{a}}$	$10.50 \pm 0.02^{a}$
Protein (% s.s.)	$9.03 \pm 0.05^{a}$	$9.30 \pm 0.07^{a}$	$11.58\pm0.10^b$	$9.35 \pm 0.05^{a}$
Reducing sugars (g/100 ml mash)	$2.20\pm0.01^{b}$	$3.50\pm0.02^{\circ}$	$1.80\pm0.02a$	$4.35 \pm 0.01^{d}$
Starch (%)	$63.5 \pm 0.2^{ab}$	$66.5 \pm 0.2^{b}$	$62.6 \pm 0.1^{a}$	$67.8\pm0.3^{\rm b}$
Ash (% s.s.)	$1.66 \pm 0.02^{a}$	$1.67 \pm 0.05^{a}$	$1.72\pm0.04$	$1.74 \pm 0.02^{a}$

Table 1. Characteristics of raw materials

<sup>a-d</sup>difference between the average values marked with different letters in the same line are statistically significant (P < 0.05)

	Mash after fermentation						
Varietes of rye	extract (°Blg)		reducing sugars	ethanol (%)	intake of total	ethanol yield (%	
	apparent	real	(g/100 ml mash)	ethanoi (%)	sugars (%)	of theoretical)	
Dańkowskie Złote	$0.62\pm0.02^{\rm b}$	$2.67 \pm 0.03^{a}$	$0.16 \pm 0.01^{a}$	$4.79 \pm 0.03^{a}$	$88.8\pm1.1^{\rm b}$	$78.1\pm0.8^{\text{a}}$	
Dańkowskie Diament	$1.20\pm0.02^{\rm a}$	$1.90\pm0.02^{\rm b}$	$0.17 \pm 0.01^{a}$	$5.00 \pm 0.02^{a}$	$84.1\pm0.9^{\rm a}$	$77.5 \pm 1.8^{a}$	
Amilo	$1.10 \pm 0.03^{a}$	$2.80\pm0.01^{a}$	$0.18\pm0.01^{\text{a}}$	$5.20\pm0.03^{\rm a}$	$88.0\pm0.1^{ab}$	$78.6\pm0.5^a$	
Amber	$1.16 \pm 0.01^{a}$	$3.25 \pm 0.02^{c}$	$0.17 \pm 0.02a$	$5.77 \pm 0.05^{b}$	$91.9 \pm 0.2^{c}$	$81.0\pm0.8^{\rm a}$	

Table 2. Results of the fermentation of the mashes

 $^{a-d}$ difference between the average values marked with different letters in the same column are statistically significant (P < 0.05)

present in the largest quantity) from the particular groups of fermentation by-products: higher alcohols (*n*-propanol, 2-methyl-1-propanol, *n*-butanol, 2-methyl-1-butanol, and 3-methyl-1-butanol), carbonyl compounds (acetaldehyde, propion aldehyde, isobutyl, 2-methyl-butyric aldehyde, isovaleric aldehyde), esters (ethyl acetate, isoamyl acetate, ethyl butyrate). Table 3 shows the concentrations of the tested groups of by-products. The results show great differences in the contents of by-products in the obtained distillates, depending on the rye cultivar.

The qualitative and quantitative composition of fusel oil in the fermentation medium is affected by several factors such as the type of material used to prepare the mash, yeast strain and yeast inoculum size, the type of mineral medium, as well as the fermentation process and distillation. Higher alcohols are present in the amounts from 0.1% to 0.7% in relation to the produced ethanol (SINGH & KUNKEE 1976; LACHEN-MEIER *et al.* 2007). The research has shown that among the compounds which belong to this group

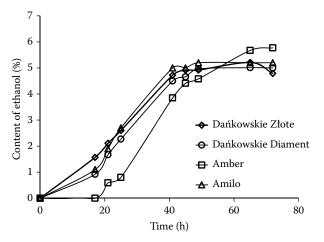


Figure 1. Effect of rye cultivars on the dynamics of ethanol synthesis

of fermentation by-products, the largest proportion is that of isoamyl alcohols (2-methyl-1-butanol and 3-methyl-1-butanol) in the distillate obtained from the rye cv. Amilo it constituted as much as 71.4%. Much lower concentrations were determined for the other compounds, i.e. isobutanol - from 21.7% (cv. Dańkowskie Diament) to 27.4% (cv. Dańkowskie Złote) and *n*-propanol – from 4.1% (cv. Amber) to 6.9% (cv. Dańkowskie Diament) of the total content of fusel oil. The lowest concentrations were recorded with *n*-butanol, whose percentage in total tested higher alcohols was 0.09-0.25%. The results confirmed that the rye variety affects the quantity of fusel oils. The highest content of fusel oil in the distillate was recorded in the variant where the rye cv. Amber (6125.01 mg/l sp. 100% vol.) had been used. This variant was characterised by a high intake of total sugars and by a high ethanol yield. In the distillate prepared from rye cv. Amilo a reduction by 53.2% was observed (Table 3).

The content of aldehydes is an important characteristic of spirits, as it allows one to pre-judge the quality of the raw material and manufacturing process, as well as the sanitary and hygienic conditions of the production (GoJ 1996). According to CZUPRYŃSKI and KŁOSOWSKI (1994), the presence of abnormal levels of by-products during fermentation (including aldehydes) can be the result of carrying out the pressure-cooking treatment improperly. The increase in the concentration of aldehydes in fermented mashes is caused by exceeding the limit of steaming temperature for the raw material or prolonging this process. Under these conditions, the formation of alcohol dehydrogenase inhibitors was noted which results in the inhibition of the process of the acetaldehyde to ethanol reduction. Aldehydes content in spirits is given in terms of acetaldehyde, which is the dominant carbonyl compound, but has a relatively high odour threshold of sensory perception (Czupryński et al. 1997). In the distillates

	Dańkowskie Złote	Dańkowskie Diament	Amilo	Amber
Alcohols				
<i>n</i> -Propanol	$245.9 \pm 5.5^{b}$	$249.4 \pm 2.2^{b}$	$163.0 \pm 1.8^{a}$	$248.2\pm2.6^{\rm b}$
2-Methyl-1-propanol	$1143.5 \pm 8.4^{\circ}$	$783.0 \pm 3.9^{b}$	$667.7 \pm 2.1^{a}$	$1598.0 \pm 5.7^{d}$
<i>n</i> -Butanol	$6.00 \pm 0.08^{a}$	$8.9 \pm 0.10^{\circ}$	$5.3 \pm 0.05^{b}$	$5.90 \pm 0.10^{a}$
2-Methyl-1-butanol	$597.5 \pm 2.0^{\circ}$	$449.4 \pm 5.83^{b}$	$396.8 \pm 4.8^{a}$	$1023.7 \pm 7.1^{d}$
3-Methyl-1-butanol	$2183.9 \pm 12.8^{a}$	$2109.9 \pm 6.7^{a}$	$1693.3 \pm 9.8^{b}$	$3249.5 \pm 24.7^{\circ}$
Aldehydes				
Acetaldehyde	$190.8 \pm 2.5^{\circ}$	$355.9 \pm 3.9^{d}$	$98.9 \pm 0.9^{a}$	$122.9\pm2.8^{\rm b}$
Propion aldehyde	$1.23 \pm 0.01^{\circ}$	$1.60 \pm 0.03^{d}$	$0.00^{a}$	$0.89 \pm 0.02^{b}$
Isobutyl aldehyde	$2.71 \pm 0.04^{a}$	$3.63 \pm 0.07^{b}$	$2.51\pm0.05^{\rm b}$	$2.71 \pm 0.07^{a}$
Isovaleric aldehyde	$2.55 \pm 0.06^{\circ}$	$3.64\pm0.05^d$	$1.40\pm0.01^{\rm b}$	0.00 <sup>a</sup>
2-methyl-butanal	$2.64\pm0.04^d$	$2.41 \pm 0.05^{\circ}$	$1.22\pm0.01^{\rm b}$	0.00 <sup>a</sup>
Esters				
Eethyl acetate	$114.1 \pm 1.4^{c}$	$130.3 \pm 0.9^{d}$	$55.7 \pm 0.9^{a}$	$88.73 \pm 1.2^{b}$
Ethyl butyrate	$0.00^{b}$	$7.32\pm0.17^{a}$	$7.50 \pm 0.17^{a}$	$0.72 \pm 0.02^{\circ}$
Isoamyl acetate	$7.61 \pm 0.10^{a}$	$7.55 \pm 0.15^{a}$	$6.30 \pm 0.10^{\circ}$	$5.98 \pm 0.17^{b}$

Table 3. The content of higher alcohols, aldehydes, and esters in agricultural distillates (mg/l sp. 100%)

 $a^{-d}$ difference between the average values marked with different letters in the same line are statistically significant (P < 0.05)

tested, the content of this compound ranged from 95.0% to 97.2% of the total amount of aldehydes. The presence of acetaldehyde is observed throughout the whole fermentation. In the first stage of the process, its concentration in the fermenting mash increases rapidly, then it returns to the level which is observed in the rest of fermentation. In the trial, in which the rye cv. Amilo was used to prepare the sweet mash, the lowest concentration of these by-products was observed (104 mg/l sp. 100%). In comparison with the variant where the rye cv. Dańkowskie Diament was used, the concentration of aldehydes in the distillate was lower by 71.7% (Table 3).

Esters play an important role in shaping the sensory attributes in spirits. Most of them are compounds of organic acids and ethanol. Ethyl acetate - an undesirable, colourless liquid with ester-floral odour, is the dominant compound in this group of fermentation by-products. In the tested samples, its proportion of the total ester content ranged from 80.1% (cv. Amilo) to 93.7% (cv. Dańkowskie Złote) (Table 3). Most of esters are formed during the turbulent fermentation and then their synthesis is reduced. In the process of the formation of this group of compounds, several factors play an important role: the type of the raw material, the yeast strain, the pH of the mash and its microbiological purity (KŁOSOWSKI et al. 2003b). Similarly, as in the case of other by-products discussed, the lowest concentration of esters was registered in

the distillate obtained from the rye cv. Amilo. In the variant in which the rye cv. Dańkowskie Złote was used, the concentration was higher by 57.2%.

During the alcoholic fermentation of agricultural products (cereals, fruits) with the participation of yeast, apart from the main product-ethanol, some chemical compounds are also generated. Some of them, including methanol and hydrogen cyanide, are harmful to the human body. The problem of alcohol contamination with methanol requires special attention, due to the extremely high toxicity of this compound. The presence of methanol in spirits is inevitable. It can be formed during alcoholic fermentation and as a result of hydrolysis of pectin contained in the raw materials (DZIĘCIOŁ 2003). The use of the steaming process increases the content of methanol because the high temperature in the steamer affects the relaxation of the grain structure and thus the disintegration of pectin, which is associated with the release of methanol molecules. In the tested variants of agricultural distillates, only a very low concentration of this compound was observed.

### CONCLUSIONS

The rye varieties, which were used in the process dealt with here, have a significant impact on the dynamic fermentation and concentration of byproducts. The mash prepared from the rye cv. Amber was characterised by a longer period of the initial phase of fermentation. The highest concentration of ethanol was observed in the 72<sup>nd</sup> h of the process with the highest efficiency of fermentation (89.9% of yield theoretical) in the mash prepared from rye cv. Amber. The lowest concentrations of the by-products tested were noted with the mash prepared from the rye cv. Amilo.

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