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Effect of various activators on the course of alcoholic fermentation

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Abstract

The aim of the paper was to study the effect of various activators, i.e. vitamins, mineral compounds as well as products of vegetable and microbiological origin, on the efficiency, dynamics and other biotechnological indices of rye mash fermentation and on the quality of the high wines obtained. Studies were carried out on the sweet rye mash (density 19.5 °Blg) brought from the agricultural distillery.

It was found that the addition of calcium pentothenate to the rye mash resulted in the improvement of the high wines quality by limiting the amount of the formed aldehydes and higher alcohols and simultaneously, it had no effect on the fermentation process. Soybean flour turned out to be a good activator of fermentation and it improved the majority of biotechnological indices of the process. The only negative aspect while applying that activator was the formation of higher amounts of higher alcohols in high wines. A comparative analysis of all the variants allowed us to state that, although some activators limited the level of higher alcohols, they did not contribute to the reduction of the formed aldehydes and even caused that their amount permissible by Polish Standard was exceeded; it was observed when biotin, biotin with thiamine, magnesium sulphate and higher amounts of thiamine (above 0.1 g/dm^3 of mash) were applied.

Keywords: Process of alcoholic fermentation; Activators of fermentation; Efficiency of ethanol from starch

1. Introduction

During fermentation of mashes, the difficulties concerning a preliminary fermentation and complete secondary fermentation of pitches are encountered. The reason of disturbances is the increasing concentration of alcohol which inhibits the growth of yeasts and has an effect on the amount of fermentation metabolites, which in turn, has an effect on the quality of the final product of fermentation, i.e. high wines.

The addition of the mineral nutrient media to mash has a stimulating effect on the yeasts and contributes to an increase in the efficiency of fermentation (Jarosz & Łączyński, 1985) and the improvement of the high wines quality (Then & Radler, 1970). In the distillery technology, most often applied additives which activate fermentation are for example: superphosphate, ammonium sulphate, ammonium phosphate, urea, ammonia water, potassium phosphate, magnesium sulphate. The doses of the individual mineral compounds depend on the raw material used which sometimes can be characterized by too poor chemical composition as a substrate for yeasts during fermentation. The mineral compounds take part in metabolism of yeasts as the activators of enzymes or elements of compounds being components of a cell. In the case when the amount of the above mentioned compounds is insufficient, the yeast reproduce and lead fermentation slowly or even their reproduction is impossible.

The most favourable fermentation conditions imposed in order to improve the efficiency of ethanol from the raw material used and to obtain the final product with the reduced amount of impurities are anaerobic conditions

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with exogenous addition of unsaturated sterols, unsaturated fatty acids, vitamins, cell membranes of yeasts and mineral compounds (Bučko, 1986; Chmiel, 1994; Grajek & Pernak, 1996).

Viegas, Sa-Correira, and Novias (1985) applied the addition of soybean flour (2–4%) which resulted in the increase in the final content of ethanol and fermentation rate. Similar effects were obtained in the case when the water solution of soybean flour was applied.

Bugajewska and Wzorek (1995) applied thiamine as an activator of fermentation. Thiamine (vitamin B_1) caused improvement in attenuation since it had an effect on the growth of yeast cells, whereas, according to Kączkowski (1993), its concentration above 200 mg/l caused deceleration of the process.

Very good results were obtained by applying calcium pantothenate whose addition caused an increase by 1.5–1.9% v/v in the concentration of alcohol in comparison with the control sample. Pantothenic acid increases the yeast tolerance to alcohol since it stimulates synthesis of lipids and moreover, it acts similarly to preparations from cell membranes of yeasts (Casey & Ingledew, 1986).

The aim of the work was to study the effect of different activators, i.e. vitamins, mineral compounds and products of vegetable origin, on the rate and efficiency of fermentation of the sweet rye mash as well as on the quality of the high wines obtained.

2. Materials

2.1. Raw materials

Studies were carried out on a sweet distillery mash prepared in an agricultural distillery in Pińsko in Poland. The mash was prepared from a good quality grain according to Henze's technology: steaming of raw material in a steamer under the pressure of 4 atm, at a temp. of 151 °C. The mash, after liquefaction (enzymatic preparation Termamyl 120 L, Novo-Nordisk) and saccharification (enzymatic preparation San Super 240 L, Novo-Nordisk), was transported to a laboratory in hermetic plastic canisters. Then, tyndallization was performed under laboratory conditions in order to protect the whole lot of mash during investigations.

The distillery rye mash was characterized by density of 19.5 °Blg and pH 5.4.

2.2. Microorganisms

The D-2 strain of distillery yeasts adapted for fermentation of starchy media, commonly used in Polish agricultural distilleries and originated from the collection of pure cultures of the Institute of Biotechnology of the Agricultural and Food Industry in Bydgoszcz (Sałek, 1989) was applied in our investigations. The strain isolated by "alcoholic shock" method is tetraploidal, thermophilic (to 39 °C) and alcohol-resistant (over 12% v/v).

3. Methods

3.1. Microbiological methods

3.1.1. Methods of yeast cream preparation for mash inoculation

Dry yeasts (5 g) were rehydrated and disinfected in 50 cm^3 of water solution of concentrated sulphuric acid in a proportion of 1:200 at a temperature of 30 °C. The yeast cream obtained was stirred with a magnetic stirrer for 10 min and then added to the ready mash at a dose in amount of 2.4 cm^3/0.3 dm^3.

Rehydratation and acidic disinfection caused the atrophy of 10–20% of the weakest cells (test with methylene blue). The other of cells were characterized by a good vitality and fermentation activity and practically were free from the undesirable microflora.

3.1.2. Determination of microbiological purity

When fermentation of each pitch had been finished, the control of microbiological purity was performed by microscopic method according to standard methods applied in agricultural distilleries (Boettger, 1996; Salek & Arnold, 1994).

3.2. Technological methods

3.2.1. Preparation of fermentation samples

Studies were carried out in laboratory scale by applying glass flasks (0.5 cm^3) closed with fermentation bungs containing glycerine.

For each variant of experimental fermentation, two series of pitches were prepared and simultaneously, the control fermentations without activators were led for each series. All fermentation samples were pitched in two parallel repetitions for each day of fermentation. They were applied to control the process an to evaluate correctness of its course during the successive days.

Each pitch contained additionally two flasks of 2.0 dm^3 (filled to 1.5 dm^3) destined for distillation after fermentation had been completed (after 72 h) in order to determine by-products.

The mash was cooled to a temperature of 38 °C, poured into the sterile fermentation flasks, the individual activators were added and subsequently, it was inoculated with yeast cream. After inoculation with yeast cream, the content of flasks was fermented for 72 h in an incubator at 38 °C.

3.2.2. Additives applied for modification of fermentation substrate

Studies on the effect of additives enriching the fermentation medium (distillery rye mash) were carried out with the application of various activator at doses (per 1 dm^3 of mash) mentioned below

Variant I thiamine—0.1 g (Roche, France);
Variant II thiamine—0.5 g (Roche, France);

Variant III "Fibosel" preparation—0.3 g (Lallemand & France);
Variant IV "Fibosel" preparation—0.3 g + thiamine—0.1 g, (Lallemand & Roche, France);
Variant V soybean flour—2 g (Wholesalers of Natural Food "Buda", Bydgoszcz);
Variant VI calcium pantothenate—1 g (Roche, France);
Variant VII calcium pantothenate—1 g + thiamine—0.1 g (Roche, France);
Variant VIII soybean oil—3.4 cm^3 (Salvadori, Italy);
Variant IX magnesium sulphate—2 g (Polish Chemical Reagents, Gliwice);
Variant X zinc sulphate—0.3 g (Grentag, Kędzierzów-Koźle);
Variant XI mineral mix: ammonium sulphate—1 g + dipotassium phosphate—1 g + magnesium sulphate—2 g (Polish Chemical Reagents, Gliwice);
Variant XII biotin—0.001 g (Roche, France);
Variant XIII biotin—0.001 g + thiamine—0.1 g (Roche, France).

The following features were taken into consideration while selecting the activators of fermentation: harmlessness for health, effectiveness in small doses and beneficial effect on the course of fermentation.

3.3. Analytical methods

3.3.1. Measurements and determination of biotechnological indices of fermentation

Each time, after 24, 48 and 72 h of fermentation the following measurements and determinations were performed according to the methods applied in the distillery industry in order to evaluate the correctness of the fermentation course (Jarosz & Łączyński, 1985; Sałek, 1989):

- CO_2 decrement—by gravimetric method (g),
- apparent and real density of fermenting and attenuated mash—by aerometric method (after filtration) (°Blg),
- concentration of alcohol—using immersion refractometer and alcoholometric tables (after distillation of 100 cm^3 of filtrated mash) (% v/v),
- pH of fermenting and attenuated mash—by digital pH-meter (Hanna Instruments HI 9318),
- degree of starch liquefaction and saccharification in mashes—by microscopic method (iodine test), after 72 h,
- residue of the reducing sugars in mashes during and after completion of fermentation—by Lane–Eynon's method (%).

The mean results of measurements and determinations were applied to calculate the biotechnological indices of fermentation as follows: efficiency of alcohol from starch (dm^3 A_{100}/100 kg of starch); real rate of fermentation (cm^3 A_{100}/kg of glucose × h); productivity of process (cm^3 A_{100}/dm^3 of mash × h); energy of fermentation (% of CO_2 decrement); and efficiency of fermentation (% of alcohol theoretical yield/100 kg of starch).

Both the efficiency of ethanol from a unit of starch and biotechnological indices were the criteria which enabled the evaluation of alcoholic fermentations with the addition of activators in comparison with control fermentations.

Variance analysis was performed using ANOVA–MANOVA packet in STATISTICA program.

3.3.2. Laboratory methods for analysis of the fermentation by-products

The attenuated mashes were distilled in glass assembly with a glass column equipped with 26 bubble-cap plates. Temperature of steams supplied to condenser was kept at a constant level, i.e. 80–81 °C, in order to assure the sufficient repeatability of the distillation conditions and to obtain distillates of 88–92% v/v after a single distillation.

Analysis of the spirit impurities was carried out and involved determination of the following components:

- Acids and esters (Kłosowski, Czupryński, Sieliwanowicz, Kotarska, & Wolska, 2001).
- Aldehydes (total amount counted over to acetic aldehyde), methanol, higher alcohols, acrolein, acetals, furfural—by capillary gas chromatography (Kłosowski, Czupryński, Sieliwanowicz, & Kotarska, 2000).

4. Results

The improvement of parameters and biotechnological indices of the fermentation process as well as the quality of high wines in comparison with the control sample was the most apparent in variants: V, VIII and XI.

The highest efficiencies of ethanol from starch in comparison with the control sample (65.56 dm^3A_{100}/100 kg of starch) were obtained in variant V (soybean flour—its starch content was taken into consideration while calculating biotechnological indices of fermentation)—65.92 dm^3 A_{100}/100 kg of starch, in variant VIII (soybean oil)—65.82 dm^3A_{100}/100 kg of starch and in variant XI (mineral mix)—65.95 dm^3A_{100}/100 kg of starch, (Fig. 1).

An increase in the efficiency of ethanol from starch was accompanied by the high efficiency of fermentation: 91.7% (variant V); 91.6% (variant VIII); 91.8% (variant XI), with respect to control sample (91.2%). These variants were characterized by high dynamics and energy of fermentation—after 48 h, it was: 87.62% (variant V); 88.62% (variant VIII); 91.69% (variant XI) in comparison to a control sample (87.29%).

In the above-mentioned variants, dynamics of the increase in alcohol content was better starting from the first day of fermentation and the higher concentration of alcohol was obtained after the process was completed, i.e.: 10.37% v/v (variant V); 10.34% v/v (variant VIII) and 10.36% v/v (variant XI) in comparison with other variants

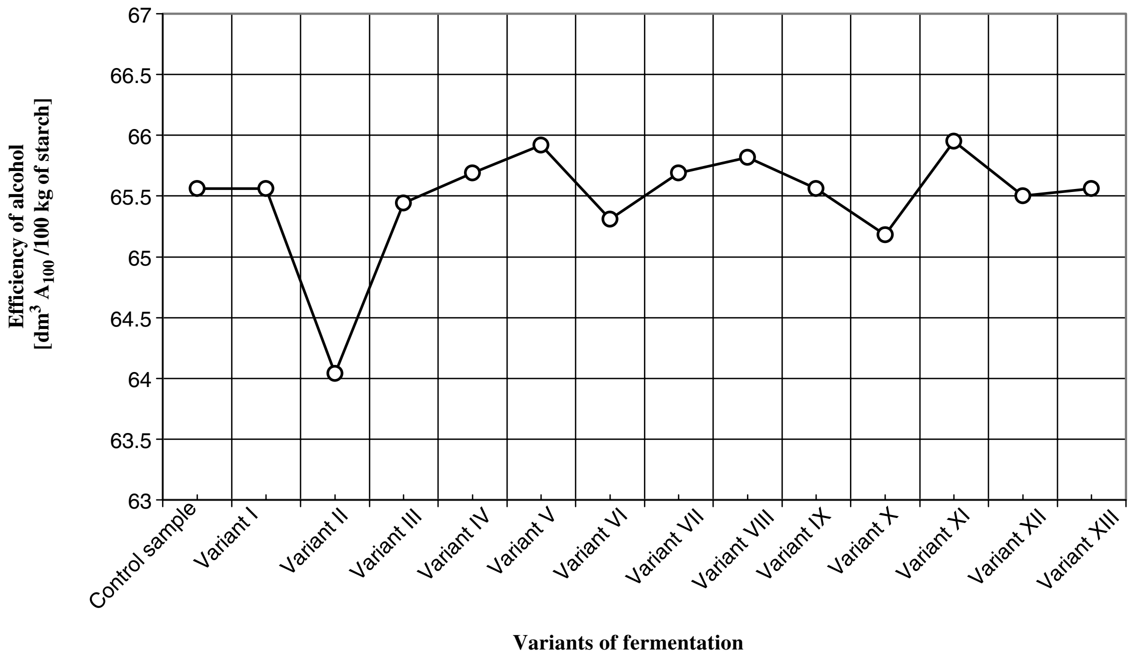

Fig. 1. Final efficiencies of alcohol from starch in the individual variants of rye mash (19.5 °Blg) fermentation with the addition of activators.

and the control sample—10.30% v/v (Table 1). Utilization of reducing saccharides was also better in these variants—in 48th hour of the process it was: 0.42% (variant V); 0.55% (variant VIII) and 0.52% (variant XI)—in control sample 0.63% (Table 1). An increase in alcohol content and reduction of fermenting saccharides in fermentation medium, starting from the first phase of the process to the completion of fermentation after 72 h, proceeded significantly quicker than in other variants of fermentation (Table 1).

In order to confirm significance of results diversification, the statistical calculations were performed on the basis of LSD Fisher's test (ANOVA–MANOVA packet in STATISTICA program). Analysis of variance confirmed the significant diversification of ethanol yield from the starch and alcohol concentration between variant XI (mineral mix) and control sample as well as between variants I, II (thiamine), III ("Fibosel" preparation), VI (calcium pantothenate) and X (zinc sulfate) (Table 1). Calculations showed significant differences between variant II (higher thiamine dose) and variants V (soya-bean flour), VIII (soya-bean oil) and XIII (biotin with thiamine). Moreover, statistical analysis pointed out the differences between variants VI (calcium pantothenate), VIII (soya-been oil) and XIII (biotin with thiamine).

The addition of the mineral mix (variant XI) contributed to the improvement of quality of the high wines obtained; it limited the level of higher alcohols by

Table 1
The course of 3-day fermentation of rye mash in the individual variants

Variant of fermentation	Apparent extract after h (°Blg)			Concentration of alcohol after h (% v/v)			Real extract after h (°Blg)			Reducing sugars in slops (%)			pH
	24	48	72	24	48	72	24	48	72	24	48	72	72
Control sample	7.4	1.7	1.3	6.92	9.94	10.30	8.8	4.3	3.8	5.37	0.63	0.38	4.5
Variant I	8.2	1.6	1.3	6.96	10.17	10.30	9.2	4.3	3.8	5.88	0.59	0.38	4.5
Variant II	8.9	2.5	1.4	6.80	9.71	10.06	9.4	4.7	3.9	5.96	1.11	0.40	4.5
Variant III	7.7	2.2	1.4	6.89	9.85	10.28	9.0	4.5	3.9	5.59	0.79	0.40	4.5
Variant IV	7.4	1.8	1.3	7.51	9.98	10.32	8.8	4.4	3.8	5.43	0.71	0.38	4.5
Variant V	6.5	1.6	1.2	7.57	10.14	10.37	8.3	4.2	3.7	4.99	0.42	0.37	4.6
Variant VI	8.5	1.8	1.3	6.61	9.84	10.26	9.8	5.3	3.8	6.48	1.39	0.39	4.6
Variant VII	7.3	1.5	1.3	7.29	10.08	10.32	8.9	4.3	3.8	5.52	0.65	0.38	4.5
Variant VIII	6.4	1.5	1.2	7.81	10.17	10.34	8.5	4.3	3.7	4.78	0.55	0.37	4.6
Variant IX	6.5	1.8	1.3	7.58	10.05	10.30	8.7	4.3	3.8	5.14	0.58	0.39	4.5
Variant X	7.8	2.3	1.4	7.05	9.92	10.24	8.9	4.7	3.9	5.54	1.01	0.40	4.6
Variant XI	5.6	1.5	1.2	8.52	10.11	10.36	7.4	4.2	3.7	3.58	0.52	0.37	4.5
Variant XII	7.6	2.2	1.4	7.38	9.97	10.29	8.9	4.7	3.9	5.42	0.86	0.40	4.5
Variant XIII	5.8	1.9	1.3	8.08	10.06	10.30	7.8	4.3	3.8	4.26	0.61	0.39	4.6

Note: Each result is the mean from three pitches (fermentations).

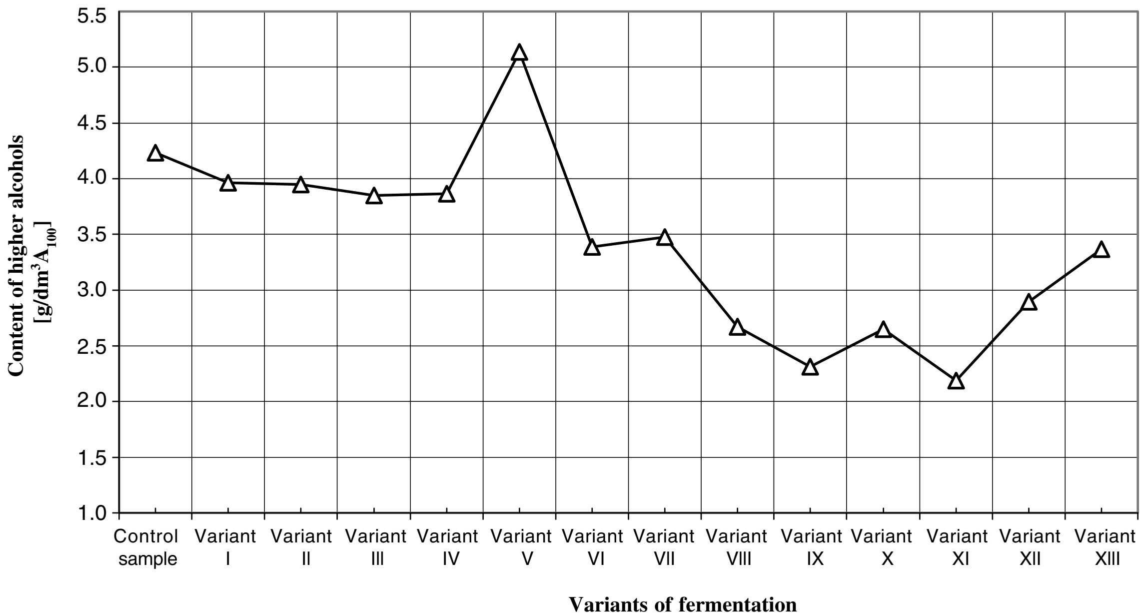

Fig. 2. Content of higher alcohols in high wines obtained in the individual variants of rye mash (19.5 °Blg) fermentation with the addition of activators.

about 48% in comparison with the control sample (Fig. 2). In the case of soybean oil, the amount of higher alcohols was reduced by about 37%, whereas the addition of soybean flour caused their increase by about 21% (mainly by increase in amyl alcohols level). The contribution of several components, i.e. ammonium sulphate, dipotassium phosphate and magnesium sulphate, had the advantageous effect on fermentation in the case of variant XI.

A slight reduction in aldehyde content was observed in variants with mineral mix and soybean oil—by 0.002–0.001 g/dm^3 A_{100}. The reduction of carbonyl compounds was higher at the addition of soybean flour (variant V)—by 0.01 g/dm^3 A_{100} (Fig. 3).

The addition of calcium panthothenate (variant VI and VII—with thiamine), resulted in the limited formation of carbonyl compounds. The reduction in the amount of aldehydes in variant VI accounted for 39%, compared to the control sample (0.083 g/dm^3 A_{100}) and in variant VII—for 31% (Fig. 3).

Calcium pantothenate was also observed to affect the level of higher alcohols; it caused their reduction by 20% (variant VI) and by 18% (variant VII) in comparison with the control sample (4.231 g/dm^3 A_{100}) (Fig. 2).

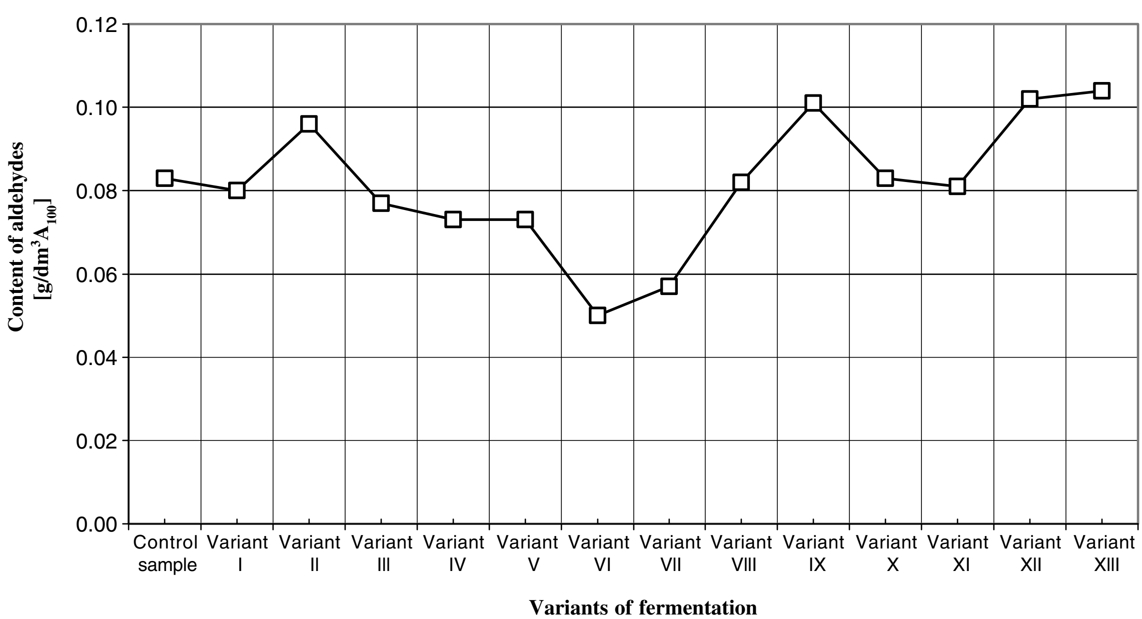

Fig. 3. Content of aldehydes in high wines obtained in the individual variants of rye mash (19.5 °Blg) fermentation with the addition of activators.

The addition of "Fibosel" preparation (variant III) had no effect on the increase in the efficiency ("Fibosel with thiamine in variant IV caused an increase in yield), however, among others, it contributed to the reduction of both aldehydes formed in high wines from 0.083 g/dm^3 A_{100} (control sample) to 0.077 g/dm^3 A_{100} (Fig. 3) and the content of higher alcohols by about 0.379 g/dm^3 A_{100} (Fig. 2).

While comparing all the variants, it should be noticed that some activators cause the reduction of higher alcohols, however, unfortunately, they do not limit the amount of carbonyl compounds, but even more, they cause that their permissible amount according to Polish Standard is exceeded; which was observed in variant II (thiamine), IX (magnesium sulphate), XII (biotin) and XIII (biotin with thiamine).

It was found that the increased addition of thiamine to the fermentation medium had an inhibiting effect on the fermentation. Thiamine added at a dose of 0.5 g per 1 dm^3 of mash caused a decrease in ethanol efficiency from starch from 65.56 dm^3 A_{100}/100 kg (control sample) to 64.04 dm^3 A_{100}/100 kg (Fig. 1), whereas, a lower amount of thiamine added, i.e. 0.1 g/dm^3 of mash (variant I), did not result in the decrease in efficiency; it was at the level of the control sample (Fig. 1). Moreover, the level of carbonyl compounds was lower by 0.016 g/dm^3 A_{100} in samples containing a lower amount of thiamine (variant I) (Fig. 3).

The addition of biotin (variant XII) and biotin with thiamine (variant XIII) had no significant effect on the fermentation process and its dynamics. However, it limited the formation of higher alcohols in the high wines obtained (by about 1.336 g/dm^3 A_{100} in variant XII and by about 0.0864 g/dm^3 A_{100} in variant XIII) in comparison with the control sample (Fig. 2). The addition of biotin favoured the formation of aldehydes, since their permissible amounts, i.e. above 0.1 g/dm^3 A_{100}, were exceeded in those samples (Fig. 3).

The application of magnesium sulphate (variant IX) and zinc sulphate (variant X) for the fermentation had no significant effect on the improvement of fermentation course and biotechnological indices but in the case of zinc sulphate, it even reduced ethanol efficiency from the introduced starch by about 0.6%. However, the addition of the above-mentioned activators to fermentation medium limited the formation of higher alcohols in high wines (by about 45%) in the case of magnesium sulphate (variant IX) and by about 37% when zinc sulphate was used (variant X) (Fig. 2). The amount of aldehydes in high wines obtained from samples containing zinc sulphate (variant X) was analogous to that of the control sample. However, in the case of magnesium sulphate (variant IX), their content increased by 0.018 g/dm^3 A_{100} with respect to control variant (Fig. 3).

The best results concerning the course of three-day fermentations (Table 1) and biotechnological indices of the process—with simultaneous limitation of impurities formation in high wines—were obtained in variants containing the following additives: "Fibosel" preparation with thiamine (variant IV), soybean flour (variant V), calcium pantothenate with thiamine (variant VII), soybean flour (variant VIII), and mineral mix (variant XI).

Acrolein was absent in all the variants and control sample. The content of furfural was very low in all the samples tested, reaching 0.0008–0.0030 g/dm^3 A_{100}.

The interference in fermentation process by applying various activators (vitamins, mineral compounds and vegetable products) would not change radically the technology commonly applied in distillery industry (thus, costs of spirit production) but it would only improve the fermentative abilities of yeasts by stimulation of biological processes occurring in cells and biochemical processes proceeding in the mash under the influence of their biotic processes.

The development works described by many authors prove that the above-mentioned components can play a very important role both in the course of fermentation and vitality of yeasts. Grajek and Pernak (1996) described the effect of protective action of soya-bean oil which increased with the increase of the environmental stress resulting from the increase in ethanol concentration. In their studies on the effect of fatty acids (soya-bean) introduced into the alginate carrier on the vitality and growth of yeast cells as well as on the efficiency of ethanol production, they proved that this additive resulted in increase in the final concentration of ethanol by 0.4–1.1% and increase in ethanol yield by 2.5–4.4%. At higher sugar concentrations, the differences in ethanol yield between the fermentations with participation of immobilized cells with addition of oil and without oil added were more considerable, what confirmed the protective action of soya-bean on the yeast cells.

Pogorzelski, Koch, and Fajkowski (2000) studied the effect of autolysate (centrifuged fraction of cell membranes fixed by lyophilization, sterilization and spray-drying) on fermentation process and they proved that the autolysate addition had an effect on decrease in concentration of higher alcohols in comparison with control sample and it contributed to shortening of the fermentation time.

The knowledge of mechanism of higher alcohols formation is important due to the fact that, their amount in high wines production is not normalized up to now, otherwise than in the case of bioethanol production. The amount of these compounds is precisely determined and it can not exceed 2% (v/v) (requirement due to the fact that higher alcohols deactivate catalysts during production of ethyl–tertbutyl ethers).

Authors do not present the detailed economic calculations connected with the costs of activators applied and the differences in prices of high wines with respect to their quality. The simulations make sense only under the local conditions respecting the actual level of the prices of activators and distillate.

5. Summary

A good solution of problems occurring in agricultural distilleries can be the application of additives which modify the fermentation medium, contribute to an increase in the efficiency of ethanol from starch and reduce the formation of the undesired by-products during the production of high wines.

The application of calcium pantothenate with thiamine in technological process could prevent the problems connected with the occurrence of excessive amounts of aldehydes in the high wines obtained. It can be achieved also by the addition of soybean flour to fermentation medium, however, it should be remembered that the addition of that activator can result in an increase in higher alcohol content in spirits obtained. Thus, soybean flour can be applied when the level of fusels is ignored.

The addition of soybean oil to rye mash results in limited formation of higher alcohols and aldehydes (to a lesser degree) but it has a significant effect on the improvement of parameters and biotechnological indices of fermentation. However, the application of oil directly in technology can pose problems concerning the maintenance of cleanness in processing line or the need to use the special cleaning agents.

The application of activators in the form of several mineral compounds i.e. ammonium sulphate, dipotassium phosphate and magnesium sulphate, is the cheapest mean to reduce the amount of the formed higher alcohols without reducing of the level of carbonyl compounds in high wines.

6. Conclusions

1. The application of activators such as: mineral mix and soybean oil in fermentation process contributes to the increase in the efficiency of ethanol from starch and to the improvement of other biotechnological indices of fermentation and, simultaneously, the level of fusels in high wines is decreased.
2. The addition of calcium pantothenate to fermentation medium improves the quality of high wines through reduction of the amount of impurities formed (mainly aldehydes and higher alcohols) without significant effect on the fermentation process.
3. Soybean flour applied to fermentation medium has a stimulating effect on the course of process by increasing, among others, the efficiency of ethanol from starch and it contributes to the limitation of the amount of aldehydes, however, the unfavourable increase in higher alcohols in the obtained high wines occurs.
4. It was found that the increase in thiamine dose to 0.5 g/dm^3 of mash had an inhibiting effect on the fermentation process; it decreased the concentration of alcohol and thus, the efficiency of ethanol from starch and furthermore, it did not reduce the amount of impurities formed in high wines.

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