EFFECT OF ALKYLRESORCINOLS DERIVATIVES ON THE ACTIVITY OF ENZYME PREPARATIONS

Target setting. Rye is known to be a stubborn raw material for the industrial production of alcohol because of alkylresorcinols contained in grain of the said crop these effecting the enzymes of amylase complex in the negative way. Thus it is important to find and scientifically substantiate proper deactivating substances for batches preparation stabilizing enzyme activity without increase of normative consumption rates of enzyme preparations.

Actual scientific research and issues analysis. Outer cuticle shell of rye grain contains alkylresorcinols – compounds of resorcin and alkyls of different chain length and odd number of carbon atoms. The presence of alkylresorcinols containing carbon atoms is characteristic for rye only. While grinding grain, the most part of alkylresorcinols remains in bran. When effected by high temperatures alkylresorcinols partly disintegrate, and in alcohol production alkylresorcinol retain their native form and effect the activity of enzyme preparations in negative way.

Defining of uninvestigated parts of general matter. Objects of research were: rye, fractions obtained while grinding; batches and wort made of rye mill under the conditions of low temperature digestion with the use of enzyme preparations of alpha- and glucoamylase action and auxiliary cytolytic enzyme preparations, mature mash. To fulfill the research, media were prepared of rye mill and water, rye bran was added as a source of alkylresorcinol; fermentative hydrolysis of starch was fulfilled with the use of «Novozymes» enzyme preparations.

The research objective. The aim of the research was to determine the effect of alkylresorcinol derivatives upon the saccharifying ability of wort and to choose substances to neutralize alkylresorcinols.

The statement of basic materials. The efficiency of catalytic activity of enzyme preparation during wort fermentation is effected by a number of factors, the index of saccharifying ability of wort being an index of activity of enzyme preparations. The research of deactivating alkylresorcinol derivatives with different substances was fulfilled to confirm or to reject the said assumption. For the research, food grade sodium bicarbonate, ammonia water and sodium hydroxide solution were used as deactivators.

Conclusions and proposals. The alkylresorcinols contained in rye effect enzyme preparations of alpha amylitic action in the negative way. The neutralization of alkylresorcinol derivatives promotes retention of enzyme preparations activity along the entire fermentation period of the media. The use of ammonia water and lactic acid at the stage of batches preparation positively influences the fermentation activity of yeast owing to the media enrichment with a source of nitrogen and increases alcohol content in mature mash.

Key words: alcohol; alkylresorcinol derivatives; enzyme preparations; bran; fermentation; mash; rye; saccharifying ability; starch; wort.

Fig.: 1. Tabl.: 3. Bibl.: 8.

Target setting. Rye is known as stubborn raw material for the industrial production of alcohol because of alkylresorcinols contained in grain of the said crop these effecting the enzymes of amylase complex in the negative way. Thus it is important to find and scientifically substantiate proper deactivating substances for batches preparation stabilizing enzyme activity without increase of normative consumption rates of enzyme preparations

Actual scientific research and issues analysis. Outer cuticle shell of rye grain is known [1] to contain alkylresorcinols – compounds of resorcin and alkyls of different chain length and odd number of carbon atoms ranging from 15 to 23. Their content in rye grains amounts as much as 40 % of cuticle volume. The presence of alkylresorcinols containing 15 carbon atoms is characteristic for rye only this being the principal negative factor effecting the enzymes of amylase complex [2].

The negative effect of rye grain under the conditions of uniform feeding of agricultural animals was recorded it being connected with the presence of 5-alkylresorcinols in grain, these being known as toxic substances for live organisms [3, 4].

While grinding grain, the most part of alkylresorcinols remains in bran. When effected by temperatures higher than 100 °C alkylresorcinols partly disintegrate [5], and in alcohol production alkylresorcinol retain their native form and effect the activity of enzyme preparations in negative way.

Defining of uninvestigated parts of general matter. We considered it necessary to study such objects of research as: rye (containing 57.1 % of starch), fractions obtained while grinding (mill of the sizing corresponding to 95 % passage through the sieve of 1 mm mesh, bran); batches and wort made of rye mill under the conditions of low temperature digestion with the
use of enzyme preparations of alpha- and glucoamylase action and auxiliary cytolytic enzyme preparations, mature mash.

To fulfill the research, media were prepared of rye mill and water (hydromodule 1:3), rye bran was added as a source of alkylresorcinols; fermentative hydrolysis of starch was fulfilled with the use of «Novozymes» enzyme preparations: thinning enzyme preparation was added calculated as 1 unit of activity per 1 g of starch, saccharifying enzyme preparation calculated as 6 units of activity per 1 g of starch and enzyme preparation of cytolytic action calculated as 0,015 cm³ per 100 g of mill; batches were digested by the temperature of 65 °C for 2,5 h, the batches were saccharified by the temperature of 58 °C for 1 h. Alcohol fermentation was held by XII-T yeast race by the temperature of 30-32 °C for 72-80 h.

The analyses of mature mash and the fermentation process were fulfilled according to the methods typical for alcohol production [6,7].

The research objective. The aim of the research was to determine the effect of alkylresorcinols derivatives (ARD) upon the saccharifying ability of wort and to choose substances to neutralize alkylresorcinols.

The statement of basic materials. The digested batch (under optimal temperature conditions) for the period of 1 h can be saccharified for 70-75 % only that is why the additional saccharification is important to occur also during wort fermentation. The efficiency of catalytic activity of enzyme preparation during wort fermentation is effected by a number of factors, the index of saccharifying ability (SA) of wort being an index of activity of enzyme preparations.

The results of research aiming in determination of the effect of alkylresorcinols derivatives upon the activity of enzyme preparations by the parameters of SA are shown in Table 1.

It can be seen from the data adduced in Table 1 that for the control, rye bran having been added to, the parameter of saccharifying ability was 1,2 units/100 cm³ of brot, this being 2,75 less than the value of saccharifying ability of control without any rye bran added.

After first 24 h of fermentation the parameter of saccharifying ability of brot could not be detected and one of the probable reasons for decrease in enzyme preparations activity is the presence of alkylresorcinols in rye bran, these acting as inhibitors of enzyme preparations activity.

| Duration of fermentation | Parameters of saccharification ability, units/100 cm³ of wort |
|-------------------------|---------------------------------------------------------------|
|                         | control (wheat starch) | experiment (wheat starch + rye bran) |
| Brot after saccharification | 3,3                        | 1,2                            |
| 24 h                    | 2,4                        | none                           |
| 48 h                    | 1,3                        | none                           |
| 72 h                    | 0,8                        | none                           |

The research of deactivating alkylresorcinols derivatives with different substances was fulfilled to confirm or to reject the said assumption.

It should be mentioned that alkylresorcinols derivatives can be deactivated in the media their pH value ranging from 10 to 11 units [5].

For the research, food grade sodium bicarbonate (variant I), ammonia water (variant II) and sodium hydroxide solution (variant III) were used as deactivators, these having been added to rye batches before addition of enzyme preparations providing that pH values of batches are within the range from 10 to 11 units.

After holding for 25-30 min under such conditions to renew pH values of batches, these were acidulated with N-solution of lactic acid to the values to be in the range from 6,0 to 6,3 units. The batches obtained were digested, saccharified and used for fermentation. The results for the mature mashes obtained are given in Table 2.
The analysis of the results obtained makes it possible to conclude that the neutralization of ARD with ammonia water (variant II) allowed to get the most significant alcohol content amounting to 7,4 vol.%, this exceeding the control by 0,15 vol.%, while the content values of the water soluble carbohydrates not fermented and of starch not fermented were the lowest amounting to 0,38 % (by 40,6 % less) and 0,03 % (by 70 % less) correspondingly, when comparing to the values for the control variant. The amount of reducing substances can be assumed to increase due to fuller starch hydrolysis while using ammonia water at the stage of batch preparation, this resulting in higher alcohol content.

A peculiarity of the chemical composition of rye shall be mentioned, this including a significant amount of the polysaccharides non-starch and slime, these determining rye as raw material difficult for processing comparing to other crops. According to literature data [8], the increase in enzyme preparations consumption compared to normative consumption rates is allowed: up to 25 % for alpha-amylose and up to 15 % for glucoamylase.

One of the reasons to increase normative consumption rates of enzyme preparations is the negative effect of the alkylresorcinols derivatives on the activity of the sad preparations. For the reason the research of rye wort fermentation with preliminary neutralization of ARD was fulfilled. There were the following variants of wort preparation for the research: control – consumption rate of enzyme preparation – 1,15 units per 1 g of starch; variant I – consumption rate of enzyme preparation – 1 unit per 1 g of starch; variant II – consumption rate of enzyme preparation – 1 unit per 1 g of starch providing that ammonia water is used. The results obtained during the research are given in Table 3.

### Table 2

| Parameter | Variants |
|-----------|----------|
|           | I  | II | III | Control |
| Alcohol content, vol. % | 6,8 | 7,4 | 7,0 | 7,25 |
| pH of medium, units | 4,5 | 4,55 | 4,6 | 4,4 |
| Acidity, grad. | 0,56 | 0,54 | 0,4 | 0,6 |
| Carbohydrates not fermented, g/100cm³: | | | | |
| - total | 0,65 | 0,38 | 0,58 | 0,64 |
| - water soluble | 0,52 | 0,35 | 0,56 | 0,53 |
| - starch not dissolved | 0,12 | 0,03 | 0,11 | 0,1 |

### Table 3

| Parameter | Variants |
|-----------|----------|
|           | Control | I  | II |
| Alcohol content, vol. % | 7,25 | 7,0 | 7,4 |
| pH of medium, units | 4,3 | 4,1 | 4,5 |
| Acidity, grad. | 0,65 | 0,71 | 0,56 |
| Carbohydrates not fermented, g/100cm³: | | | |
| - total | 0,62 | 0,86 | 0,48 |
| - water soluble | 0,51 | 0,63 | 0,45 |
| - starch not dissolved | 0,1 | 0,21 | 0,03 |

According to the data of Table 3, during the use of enzyme preparation Termamyl SC DS in the amount of 1 unit per 1 g of starch almost twofold increase of undissolved starch content is recorded for the variant I comparing to the control. As for the variant II, preliminary use of ammonia water at the stage of batch preparation makes it possible to reduce the content of undissolved starch down to 0,03 % without increase in the consumption of thinning enzyme preparation Termamyl SC DS by 15 %. The maximal hydrolysis of starch and increase in the content of reducing sugars in digested and saccharified rye wort let increase the alcohol content in mature mash.
Thus comparing to the control with 7.25 vol.% alcohol content, the alcohol content of the variant I (without additional neutralization of ARD) increased by 3.5 vol.% to reach 7.0 vol.%, when for the variant II (with additional neutralization of ARD) alcohol content increased by 2 vol.% to reach 7.4 vol. %.

According to results of the research principal technological scheme of wort preparation for fermentation with the use of ammonia water aiming in ARD neutralization was developed (fig.1).

**Conclusions and proposals.** The alkylresorcinols contained in rye effect enzyme preparations of alpha-amylitic action in the negative way.

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**Fig. 1. Principal technological scheme of rye wort preparation for fermentation with the use of ammonia water**

It is determined that the best deactivating substance for batches preparation is ammonia water, its use making stabilization of enzyme preparations activity possible during the preparation of enzyme preparations for fermentation without any simultaneous increase of normative consumption rates of enzyme preparations.

ADR neutralization promotes retention of enzyme preparations activity within the entire fermentation period of the media.

The use of ammonia water and lactic acid at the stage of batches preparation positively influences the fermentation activity of yeast owing to the media enrichment with a source of nitrogen and increases alcohol content in mature mash.

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*Список використаних джерел*

1. Конюхов В. Г. Образцы ржи с характеристикой содержания 5-алкил-резорцинолов в зерне / В. Г. Конюхов. – Л.: ВИР, 1977. – Вып. 197. – С. 50.
Фенольні соединения растительного происхождения / А. Блажный, Л. Щутый. – М. : Мир, 1977. – 239 с.
3. Сысуев В. А. Использование озимой ржи в кормлении животных / В. А. Сысуев, Л. И. Кедрова // Сельскохозяйственные вести. – 2006. – № 2. – С. 28.
4. Алладердин И. Зерно озимой ржи в комбикормах для молодняка КРС / И. Алладердин // Животноводство России. – 2006. – № 11. – С. 52–53.
5. Физер Л. Органическая химия / Л. Физер, М. Физер ; под. ред. Н. С. Вульфсона. – М. : Химия, 1970. – С. 278-319.
6. ГСТУ 46.045-2003. Галузевий стандарт України. «Зерно. Методи визначення умовної крохмалестійності».— Вид. офіц. – К., 2004. – 20 с.
7. СТУ 15.9-37-242:2005 Стандарт МінАгрополітики України «Сировина крохмалевмісна зброджена для виробництва етилового спирту. Методи визначення об’ємної частки етилового спирту». – Вид. офіц. – К., 2005. – 14 с.
8. Техноло́гічний регламент виробництва етилового спирту з крохмалевмісної сировини. ТРУ 18.8049-2000. – 33 с.

References

1. Konarev, V.G. (1977). Obraztsy rzhi s kharakteristikoj soderzhaniya 5-alkil-rezorcinolov v zerne [Specimens of rye with characteristic of 5-alkylresorcinols in rye]. VIR, issue 197, p. 50 (in Russian).
2. Blazhej, A., Shuty, L. (1977). Fenolnye soyedineniya rastitelnogo proiskhodjenija [Phenol compounds of plant origin]. Moscow: Mir (in Russian).
3. Sysuev, V.A., Kedrova, L.I. (2006). Ispolzovanie rzhi v kormlenii zhivotnykh [Use of rye for animal feeding]. Selskokhozyajstvennye vesti – Agricultural news, no. 2, p. 28 (in Russian).
4. Allaberdin, I. (2006). Zerno oziomoi rzhi v kombikormakh dlia molodniaka KRS [Grain of autumn rye in feed staffs for young stock cattle]. Zhyivotnovodstvo Rossi – Livestock breeding of Russia, no. 11, pp. 52–53 (in Russian).
5. Fizer, L., Fizer, M. (1970). Organicheskaia khimiia [Organic chemistry]. Moscow: Khimiia (in Russian).
6. GSTU 46.045-2003 (2003). Galuzeyvi standart Ukrainy «Zerno. Metody vyznachennia umovnoyi krokhmalystosti» [Branch standard of Ukraine. Methods to determine apparent starch content]. Official edition. Kyiv: Minagropolityky Ukrainy – Ministry of agrarian policy of Ukraine (in Ukrainian).
7. SOU 15.9-37-242:2005 (2005). Standart Minagropolityky Ukrainy «Syrovyna krokhmalievmytina zbrozhena dla vyrobnytstva etylovogo spyrtu. Metody vyznachennia ob`emnoyi chastki etylovogo spyrtu» [Standard of Ministry of agrarian policy of Ukraine «Fermented starch containing raw materials for production of ethanol. Method to determine content of ethanol»]. Kyiv (in Ukrainian).
8. TRU 18.8049-2000 (2000). Tekhnologichnyy reglament vyrobnytstva etylovogo spyrtu z krokhmalievmytinoi syrovyny [Technological regulation for production of ethanol from starch containing raw materials](in Ukrainian).

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ВПЛИВ ПОХІДНИХ АЛКІЛРЕЗОРЦІНОЛІВ НА АКТИВНІСТЬ ФЕРМЕНТНИХ ПРЕПАРАТІВ

Постановка проблеми. Відомо, що жито є проблемною сировиною для виготовлення етилового спирту через наявність у зернах цього злаку алкілрезорціної, що негативно впливають на ферменти амілазного комплексу. Отже, важливо знайти та науково обґрунтувати ефективні дезактивувальні речовини, які під час приготування замісів стабілізують ферментну активність без збільшення витрат ферментних препаратів. Важливо знайти та науково обґрунтувати ефективні дезактивувальні речовини, які під час приготування замісів стабілізують ферментну активність без збільшення витрат ферментних препаратів.

Аналіз останніх досліджень і публікацій. Зовнішня оболонка кутікули зернівки жита містить алкілрезорциноли – сполуки резорцина та алкілів з різною довжиною замінних ланцюгів та різною кількістю атомів карбону. Наявність алкілрезорцинолів в атомами карбону є характерною відомої для жита. При подрібненні зерна більша частина алкілрезорцинолів залишається у нативному вигляді та негативно впливає на ферменти амілазного комплексу.

Виявлені частини загальної проблеми. Об’єктом досліджень були: жито, фракції, отримані під час подрібнення; заміси та сусло з помелу жита, отримані в умовах розварювання за низьких температур з використанням ферментних препаратів альфа- та елюокамелозної дії та допоміжних ферментних препа-
Вплив производных алкилрезорцинолов на активность ферментных препаратов

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Введение производных алкилрезорцинолов на активность ферментных препаратов

Определено негативное влияние производных алкилрезорцинолов на активность ферментных препаратов а-амилолитической активности. Подобрано вещество — аммиачную воду — для дезактивации производных алкилрезорцинолов, разработана принципиальная технологическая схема приготовления сусла для сбраживания с использованием аммиачной воды с целью нейтрализации производных алкилрезорцинолов и стабилизации активности ферментных препаратов во время приготовления среды для сбраживания, при нормативных затратах ферментных препаратов. Ключевые слова: бражка; отруби; рожь; сбраживание; крахмал; осахаривающая способность, производные алкилрезорцинолов, спирт, сусло, ферментные препараты.

Рис.: 1. Табл.: 3. Библ.: 8.

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Protsan N., Oliynichuk S., Verbytskyi S. (2017). Effect of alkylresorcinols derivatives on the activity of enzyme preparations. Technical sciences and technologies, no. 4 (10), pp. 235-240.

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