Journal of Chemistry and Technologies, 2023, *31*(3), 627-634

Journal of Chemistry and Technologies

pISSN 2663-2934 (Print), ISSN 2663-2942 (Online).

journal homepage: http://chemistry.dnu.dp.ua

UDC 663.533

DEXTRINIZATION OF STARCH WITH ALPHA-AMILASE IN THE CONDITIONS OF ETHYL ALCOHOL PRODUCTION

Sergiy T. Oliynichuk, Kateryna O. Danilova*

Institute of Food Recourses of National Academy of Agrarian Sinces of Ukraine, 4A, E. Sverstiyka str., Kyiv, 03190, Ukraine

Received 2 June 2023; accepted 12 September 2023; available online 25 October 2023

Abstract

Fermentation of starch-containing raw materials in alcohol production is limited by the rate of enzymatic hydrolysis of dextrins, which depends on the productivity of alcohol per ton of raw materials. We conducted a study of the kinetics of hydrolysis of corn starch by enzymes of the amylase complex depending on their dosage in the conditions of a one-stage and two-stage dilution process. Research methods generally accepted in the alcohol industry were used in the work. Conventional starchiness was determined polarimetrically according to the Evers method. Enzymatic activity was determined by the photocolorimetric method. If the enzymatic hydrolysis of starch was carried out at a temperature of 90 °C, the loss of activity of the enzyme preparation "Amilex 4T was 73 % already after the first hour of exposure, and after three hours of exposure, the activity was only 15.4 % of the initial level. The dilution regime at temperatures of 50–70 °C for one hour and raising it to 90 °C and holding for two hours ensured the preservation of the enzyme activity, which was 62.9 % of the initial one. As a result of dilution of the wort with different dosages of the alpha-amylase enzyme, it was determined that a dose of the enzyme of 1.5 units of activity per 1 g of starch provided the necessary and sufficient level of formation of fermentable sugars. It was established that the dissolution of starch under the conditions of stepwise two-stage heating of the wort was better in comparison with the one-stage regime. The content of undissolved starch in the matured brew was 0.03 against 0.13 g/100 cm^3 in the control, the indicators of unfermented sugars in the brew corresponded to the regulated ones, and the alcohol content increased by 0.2 %.

Keywords: corn; starch; hydrolysis; enzyme preparation; alpha-amylase; enzyme activity; sugars

ДЕКСТРИНІЗАЦІЯ КРОХМАЛЮ АЛЬФА-АМІЛАЗОЮ В УМОВАХ ВИРОБНИЦТВА ЕТИЛОВОГО СПИРТУ

Сергій Т. Олійнічук, Катерина О. Данілова*

Інститут продовольчих ресурсів Національної академії аграрних наук України, вул. Є.Сверстюка, 4А, Київ, 03190, Україна

Анотація

Зброджування крохмалевмісної сировини в спиртовому виробництві обмежене швидкістю ферментативного гідролізу декстринів, від чого залежить продуктивність спирту з тонни сировини. Нами проведено дослідження кінетики гідролізу крохмалю кукурудзи ферментами амілазного комплексу в залежності від їх дозування в умовах одностадійного та двостадійного процесу розрідження. В роботі використовували загальноприйняті в спиртовій промисловості методи досліджень. Умовну крохмалистість визначали поляриметрично за методом Еверса. Ферментативну активність визначали фотоколориметричним методом. За умовами здійснення ферментативного гідролізу крохмалю за температури 90 °С втрата активності ферментного препарату «Амілекс 4Т» становила 73 % вже після першої години витримування, а після трьох годин витримки активність його складала лише 15.4 % від початкової. Режим розрідження за температури 50–70 °С впродовж однієї години та підвищення до 90 °С і витримки дві години забезпечив збереження активності ферменту, яка становила 62.9 % від початкової. У результаті розрідження сусла за різним дозуванням ферменту альфа-амілази визначено, що доза ферменту 1.5 одиниці активності на 1 г крохмалю забезпечує необхідний і достатній рівень утворення зброджуваних цукрів. Встановлено, що розчинення крохмалю за умов ступінчатого двостадійного нагріву сусла було кращим у порівнянні з одностадійним режимом. Вміст нерозчиненого крохмалю в дозрілій бражці був 0.03 проти 0.13 г/100 см^3 в контролі, показники незброджених цукрів в бражці відповідали регламентованим, а вміст спирту збільшився на 0.2 %.

Ключові слова: кукурудза; крохмаль; гідроліз; ферментний препарат; альфа-амілаза; ферментативна активність; цукри

**Corresponding author: e-mail: dankoek77@gmail.com*

doi: 10.15421/jchemtech.v31i3.280974

Introduction

The determining factor in the efficiency of the production of ethyl alcohol from starch-containing raw materials is the hydrolysis of starch into monosugars by enzymatic preparations of microbial origin. The positive aspects of this process are the possibility of carrying out the reaction at low temperatures with obtaining a hydrolyzate with a high content of fermentable substances.

The theory and practice of enzymatic hydrolysis of starch is characterized by the study of the chemical composition of polysaccharides of cereal crops and the kinetics of their hydrolysis. Features of enzymatic hydrolysis require determination of interrelationships of kinetic, diffusion, and hydrodynamic processes that affect on the course of the process and yield of fermentable sugars.

The current state of the theoretical foundations of hydrolysis largely solves these problems and makes it possible to effectively influence to the production of ethyl alcohol from starch-containing raw materials using current technologies. However, the need to further increase the profitability of ethyl alcohol production requires research into the activity of commercial enzyme preparations of alpha-amylase and glucoamylase and the study of individual factors affecting on the course of the enzymatic kinetics of starch components that are hydrolyzed at different rates. Recently, amylolytic enzyme preparations have been widely used in the technology of ethyl alcohol from starch-containing raw materials [1–3]. Hydrolysis of starch is carried out in two stages by the enzymes alpha-amylase and glucoamylase, which are characterized by high specificity in relation to the substrate and the type of chemical reaction [4–6]. At the first stage of enzymatic dilution, 1–4 glucosidic bonds of amylose are destroyed by alpha-amylase. Further hydrolysis is characterized by the destruction of the 1–6 glucosidic bond of amylopectin to glucose by the enzyme glucoamylase [7–9]. The products of starch hydrolysis include dextrin or maltodextrin, maltose and glucose. Dextrins are mixtures of polymers of d-glucose units linked by α-(1 → 4) or α-(1 → 6) glycosidic bonds. The percentage of obtained products depends on the conditions used for the reaction such as duration and strength/amount of reagents used.

The formation of enzyme-substrate complexes plays a decisive role in the mechanism of enzymatic catalysis in general and starch in particular. The speed of this enzymatic reaction largely depends on the chemical composition and availability of the substrate for enzyme action. Under the conditions when a multicomponent substrate is used in the hydrolysis reaction, which includes cereal grain and preparations containing a complex of enzymes, the enzyme-substrate interaction becomes more complex, which affects the reaction rate [10; 11]. Amylose lipid complexes (AMLs) are likely to form during liquefaction of ground corn in the dry grind process. AML will form under high temperature (≥ 85 °C) and excess water conditions, due to interaction of gelatinized starch with corn lipids. AMLs are resistant to α-amylase action, resulting in a decrease in starch available for enzymatic hydrolysis. This affects sugar available for fermentation and the final ethanol yield. It was investigated that increasing the dilution temperature to 105 °C reduced the number of formed amylose-lipid complexes by 3.5 times [12]. In addition, the efficiency of obtaining fermentable sugars depends on the stability of enzymes and the inhibitory effect of hydrolysis products on them. Studies of the formation of sugars under the conditions of variable parameters of the course of starch hydrolysis during three hours of reaction established the change in the reaction rate, which decreases as glucose is formed, which used [13; 14] in their experiments. It is established that no α-amylase activity was detected when glucose was used as the carbon source in the influent medium, indicating that α-amylase is catabolically repressed by glucose. Contrastingly, maltose and starch induce synthesis of α-amylase, with starch being the best α-amylase induce [15]. Most of the enzymatic processes slow down with the duration of their implementation, which is due to a decrease in the concentration of the substrate, inactivation of the enzyme at a suboptimal temperature and concentration of hydrogen ions in the medium. The study of the activity of enzymes in a biological system and the study of the influence of individual factors on the course of enzymatic catalysis is the subject of numerous scientific studies [16–20]. So it was investigated, decreases in resistant starch after high temperature liquefaction were 55 % to 74 %, whereas low temperature liquefaction decreases were 11 % to 43 % [20]. However, data on the efficiency of starch hydrolysis under conditions of variable parameters such as temperature, pH, activity and dose of enzyme in the practical technology of ethyl alcohol is almost not taken into account. According to the starch

biotransformation technologies operating in the industry in the production of ethyl alcohol, the necessary completeness of saccharification of dextrins is not ensured, which is a limiting factor in the course and duration of fermentation.

We conducted a study of the kinetics of hydrolysis of corn starch by enzymes of the amylase complex depending on their dosage in the conditions of a one and two-stage process in the production of ethyl alcohol.

Material and methods

The objects of research were amylolytic enzyme preparation "Amilex 4T" of the company "Danisko", wort prepared from corn, processes of thermo-enzymatic hydrolysis of starch and fermentation of wort, mature wort.

The research methods generally accepted in the alcohol industry were used in the work [21; 23]. Conditional starch content was determined polarimetrically according to the Evers method [22; 23].

Amylolytic activity (AA) characterizes the action of alpha-amylase in enzyme preparations, is determined from the rate of the enzymatic reaction of starch hydrolysis, which is determined from the amount of starch hydrolyzed in the process of this reaction. The unit of amylolytic activity (AA) is taken as the number of enzyme that, under specified conditions (temperature 30 °C, pH 4.7–4.9, action time 10 min), catalyzes the hydrolysis of 1 g of soluble starch to non-iodine-stained dextrins [24]. The activity of alpha-amylase was determined by the colorimetric method [25]. Alcohol content in fermented distillates was determined hydrometrically [21], the mass concentration of unfermented carbohydrates – by the photoelectric colourimetric anthrone method [26].

The optimal schedule of temperature and pH of enzyme preparations "Amilex 4T" and "Diazim SSF" are shown in table 1 [27]

Table 1

The optimal schedule of temperature and pH of enzyme preparations

Name of the enzyme preparation	Specific gravity, g/cm^3	Temperature range, °C	pH range
Amilex 4T	1.15–1.25	60–100	4.8–7.0
Diazim SSF	1.12–1.15	30–65	3.0–5.5

To determine the degree of hydrolysis of starch in the dilution process, depending on the dose of the diluting enzyme, the 73 g of ground corn with starchiness of 65% was mixed with water, the volume of the mixture was brought to 250 cm^3. The enzyme preparation "Amilex 4T" of the company "Danisco" was added at the rate of 0.5; 1.0; 1.5; 2.0 units AA/g of starch, kept for three hours. After every hour of dilution, the content of sugars formed and alcohol-soluble carbohydrates. The dynamics of starch hydrolysis by the number of sugars formed was determined by the method of high-performance liquid chromatography (HPLC) on a Shimadzu Lab Solutions chromatograph.

The technological mode of fermentation and the parameters of the mature brew were carried out by the fermentation test method. Corn meal was mixed with water in a conical flask with a volume of 500 mL, the volume of the mixture was brought to 250 mL, and dilution was carried out according to the technological regimes chosen in the experiments. The diluted mass was cooled to a temperature of 30°C, the saccharifying enzyme preparation "Diazim SSF" of the "Danisco" company was added at the rate of 6 units/g of starch, 0.01 mL of the proteolytic enzyme preparation "Alfalase AFP" of the "Danisco" company, an antiseptic and 10 mL of yeast suspension. After that, the flasks were closed with an acid seal and fermentation was carried out at a temperature of 30 °C for 80 hours. To prepare the yeast suspension for each flask, a measurement of 1 g of dry yeast "Thermosacc" was diluted in 10 mL of water, kept for 20–25 minutes and introduced into the flask with wort.

The experimental data were obtained in triplicate and processed by the methods of mathematical statistics.

Results and discussion

The current level of biotechnology of starch hydrolysis and fermentation of the formed sugars into ethyl alcohol is quite high and is based on separate technological stages of hydrolysis of raw materials and fermentation of the hydrolyzate by yeast. Under the conditions of alcohol production, the hydrolysis of grain raw materials is carried out at a temperature of 90 °C in order to release starch from plant cells and convert it into a soluble state [24]. Depending on the composition of hydrolyzed enzyme preparations and the parameters of the course of starch hydrolysis, the technological process can be carried out with different rates of formation of final sugars.

At the first stage of the work, the influence of the temperature, which corresponds to the technological regimes of the dissolution stage, on the activity and stability of alpha-amylase of the enzyme preparation "Amilex 4T" was investigated.

As it can be seen from Fig. 1, the increase in temperature in the first hour accelerates the catalytic reaction, i.e. the rate of substrate conversion. However, at the same time, the rate of irreversible denaturation of the protein increases, which leads to a decrease in the concentration of the enzyme and causes the reaction to slow down. If the process was carried out at a temperature of 90 °C, the loss of activity was 73 % already after the first hour of exposure and after three hours of exposure, the activity of the enzyme preparation was only 15.4 % of the initial level. The dilution regime at a temperature of 50–70 °C for one hour and raising it to 90 °C and holding for two hours ensured the preservation of the enzyme activity, which was 62.9 % of the initial one. This indicates a more intensive saccharification of dextrins by glucoamylase. It is obvious that this temperature regime contributes to the preservation of enzymes for the entire period of fermentation. Thus, with alpha-amylase activity of 1750 units/ml, after dissolution at a temperature of 65 °C for an hour, it does not change, and after two hours of dilution at a temperature of 90 °C, it decreases by 1100 units. Thus, during the fermentation period, the activity of alpha-amylase is quite high and is 650 units/ml against 250–280 units/ml when dissolving starch at a temperature of 85–90 °C for 3 hours. This factor is particularly important when processing high-amylose corn, which requires a longer time to hydrolyze starch to low molecular weight sugars.

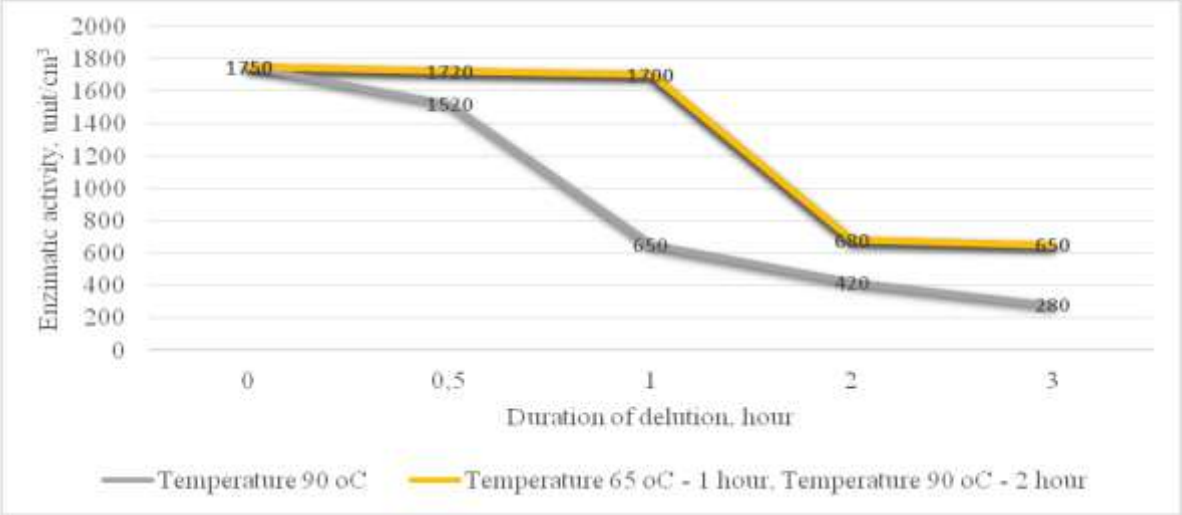

Fig. 1 Thermal stability of alpha-amylase depending on the duration of dilution

At the same time, enzymatic processes are characterized not only by the intensity of starch degradation, but also by the rate of formation of reaction products. The dynamics of the influence of enzyme : substrate weight ratio on the degree of starch hydrolysis are shown in Table 2. 47.45 g of starch was introduced into the hydrolysis reaction.

Table 2

Dynamics of the influence of the enzyme:substrate weight ratio on the degree of starch hydrolysis

Consumption of alpha-amylase, un./g of starch	Duration of dilution, hours	Sugar content, g/100 cm³				Sugars were formed, g per volume of 250 cm³	Amount of sugars, in % to the introduced starch
		Maltose and malto-dextrins	Fructose	Glucose	Sum of mono- and disugars		
0.5	1	3.66±0,15	0.09±0.005	0.62±0.03	4.37±0.2	10.92±0.6	23.0±0.9
	2	3.71±0,15	0.10±0.005	0.67±0.03	4.48±0.2	11.20±0.6	23.6±0.9
	3	3.92±0,15	0.12±0.005	0.71±0.03	4.75±0.25	11.87±0.65	25.0±1.0
1	1	3.41±0,15	0.12±0.005	1.05±0.05	4.58±0.2	11.45±0.65	24.1±1.0
	2	3.43±0,15	0.12±0.005	1.11±0.05	4.66±0.25	11.65±0.65	24.5±1.0
	3	3.45±0,15	0.17±0.005	1.12±0.05	4.72±0.25	11.8±0.65	24.9±1.0
1.5	1	3.72±0,15	0.19±0.005	1.16±0.05	5.07±0.3	12.67±0.7	26.7±1.0
	2	3.84±0,15	0,21±0.005	1.18±0.05	5.23±0.3	13.07±0.7	27.5±1.1
	3	3.96±0,15	0.23±0.005	1.21±0.05	5.40±0.3	13.5±0.72	28.4±1.1

Continuation of the table 2

2	1	3.50±0,15	0.16±0.005	1.12±0.05	4.78±0.25	11.95±0.65	25.2±1.0
	2	3.52±0,15	0.18±0.005	1.14±0.05	4.84±0.25	12,1±0.65	25.5±1.0
	3	3.56±0,15	0.19±0.005	1.17±0.05	4.92±0.3	12.3±0.65	25.9±1.0

The obtained results show that the carbohydrate composition of the wort is represented by maltose and maltodextrins (3.4–3.96 g/100 cm^3), and the glucose content is much lower – up to 1.21 g/100 cm^3. Which is explained by the shortest relaxation time, i.e. the length of time during which mono- and di-sugars are formed will always be shorter than for hydrolyzates with a higher degree of polymerization.

The main number of sugars is formed during the first hour of the process – 5 g/100 cm^3 or 26.7 % to the introduced starch, which corresponds to the theory of starch hydrolysis by alpha-amylase, which destroys 1,4-bonds [3;6]. As it can be seen from the data in Table 2, with a decrease in the dose of the introduced enzyme alpha-amylase, the process of hydrolysis of amylose is more intense. Yes, at a dose of 2 units of activity, 4.78 g/100 cm^3 of sugars are formed for 1 hour, which is 25.2 % of the introduced starch, and at a dose of 1.5 units of activity for 1 hour, 5.07 g of sugars or 26.7 % of the introduced starch are formed. In our opinion, this can be explained by the fact that the excess of the enzyme preparation interacts with the substrate with the formation of enzyme-substrate complexes that do not participate in starch hydrolysis. With a further decrease in the dose of the enzyme preparation, the number of sugars formed drops and amounts to 4.58 g and 4.37 g, this is 24.1 % and 23.0 % of the introduced starch. Thus, the optimal dose of the diluting enzyme alpha-amylase is 1.5 units/g of starch.

Figure 2 shows the dynamics of the influence of the enzyme : substrate weight ratio on the specific rate of substrate hydrolysis, which is determined by the number of sugars formed in 1 minute.

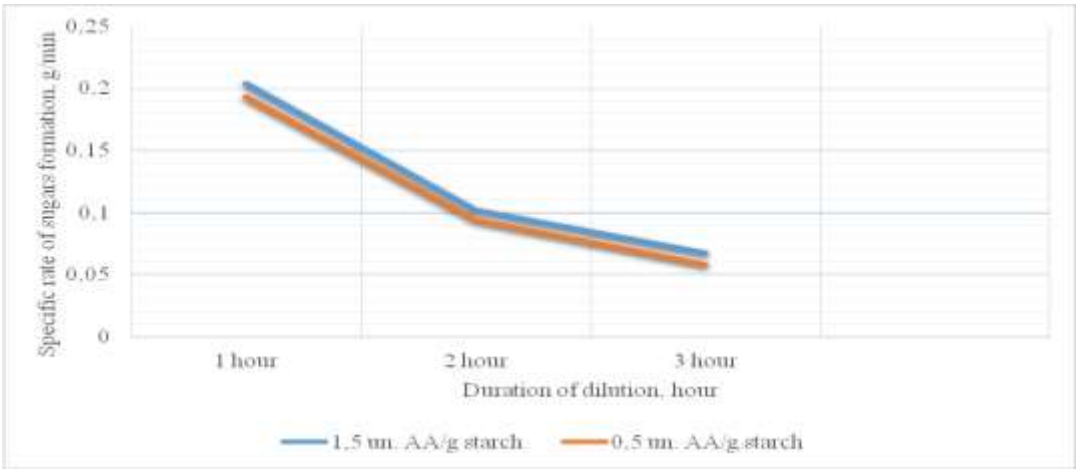

Fig. 2. The dynamics of the influence of the enzyme:substrate weight ratio (E/S) on the specific rate of substrate hydrolysis, in g/min.

In the first hour of dilution, the largest number of sugars was formed, the formation rate of their was maximum and it consist 0.2 g per minute. With an increase in the duration of dilution, the specific rate of formation of sugars decreased and was 0.102 and 0.067 g/minute at the 2nd and 3rd hours, respectively. This dependence persists for any amount of added diluting enzyme preparation. Thus, with an increase in the time, the enzyme is in the medium, its enzymatic activity decreases and, accordingly, the rate of sugar formation decreases, which was only 33 % of the initial rate by the 3rd hour. This is explained by the inhibition of the enzyme preparation by hydrolysis products.

At the same time, enzymatic processes are characterized not only by the intensity of starch degradation, but also by the rate of formation of reaction products [28]. Therefore, the influence of the dosage of the diluting enzyme preparation "Amilex 4T" on the accumulation of alcohol-soluble carbohydrates in the wort was investigated. The choice of this indicator was due to the nature of the action of alpha-amylase, namely its ability to hydrolytically split 1-4 glycosidic bonds at any point of the polyglycosidic chain of amylose and amylopectin with the formation of dextrins [29]. Therefore, the products of this reaction are fractions of dextrins of different molecular weight and a small

amount of glucose [30]. Alcohol-soluble carbohydrates include the fraction of maltodextrins (number of monomers in the chain < 5) [29]. Thus, the number of alcohol-soluble carbohydrates, as the theoretically dominant product of enzymatic hydrolysis of starch, gives a more complete picture of the kinetics of processes occurring in the process of liquefaction of starch. The dynamics of the accumulation of alcohol-soluble carbohydrates depending on the duration of dilution and the enzyme : substrate weight ratio (E/S) (Figure 3).

As it can be seen from Fig. 3, the highest concentration of alcohol-soluble carbohydrates was obtained when 1.5 units of AA/g starch of the diluting enzyme preparation alpha-amylase were added to the medium. When the concentration of the enzyme preparation in the solution increased to 2 units of AA/g of starch, the content of alcohol-soluble carbohydrates in the wort was 30–35 % lower compared to the dosage of 1.5 units of AA/g of starch throughout the dilution process. This may indicate the formation of unproductive enzyme-substrate complexes and inhibition of the diluting enzyme preparation.

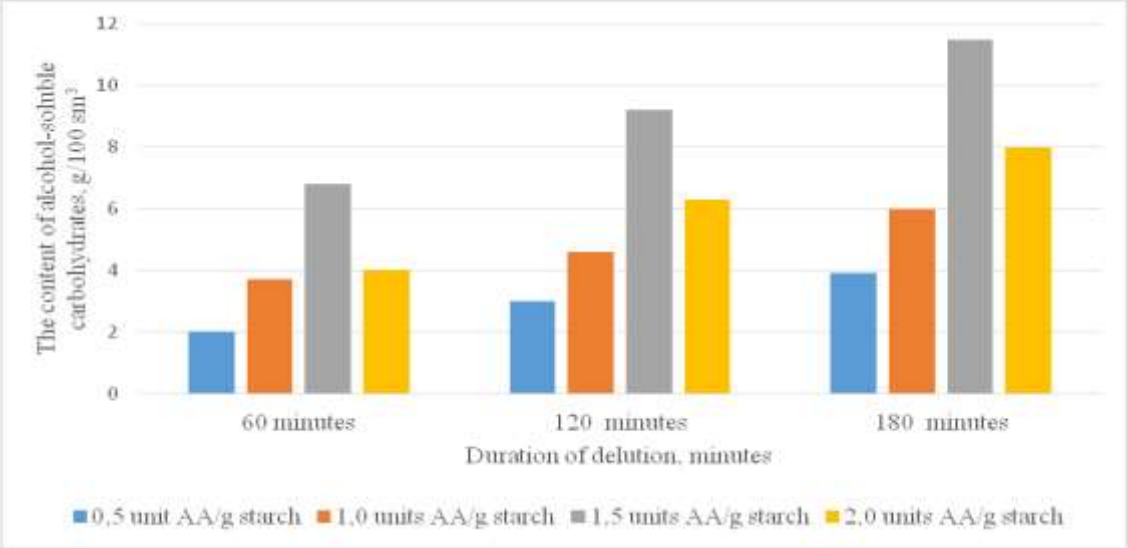

Fig. 3. Dynamics of the influence of the enzyme:substrate weight ratio (E/S) on the accumulation of alcohol-soluble carbohydrates.

To determine the influence of the starch dilution mode on the ethanol yields and conversion of starch into sugars, the fermentation of diluted corn wort was carried out with the addition of alpha-amylase in the amount of 1.5 units AA/g of starch under one-stage and two-stage temperature regimes (table 3).

Table 3

The influence of the starch dilution regime on the conversion into sugars and ethanol content

Indicator	Temperature regimes	
	One stage t – 87–90 °C, 3 hours	Two stages t – 65 °C, 1 hour t – 90 °C, 2 hours
Unfermented carbohydrates, g/100 ml:		
- General	0.49±0.02	0.32±0.02
- Water soluble	0.35±0.02	0.28±0.02
- Undissolved starch	0.13±0.02	0.03±0.001
- Alcohol soluble	0.16±0.02	0.17±0.02
- Dextrins	0.19±0.02	0.11±0.02
Alcohol content, % (by volume)	8.0±0.05	8.2±0.05

From the given data, it can be seen that the dissolution of starch under the conditions of step heating of the dough was better; the content of undissolved starch in the matured brew was significantly lower, 0.03 against 0.13 g/100 ml in the control. The content of alcohol-soluble carbohydrates differed within the error of determination, and the content of dextrins was 0.11 against 0.19 g/100 ml in the control, and the alcohol content increased by 0.2 %. It is obvious that the two-stage temperature regime of starch

dilution contributes to the preservation of enzymes for the entire period of fermentation.

Conclusion

1. The complete use of starch introduced into the technological process depends on the preservation of the activity of the alpha-amylase enzyme due to the variable parameters of the temperature regime of starch hydrolysis.

2. If the process was carried out at a temperature of 90 °C, the loss of activity was 73 % already after the first hour of exposure and after three hours of exposure, the activity of the enzyme preparation was only 15.4 % of the initial level. The dilution regime at temperatures of 50–70 °C for one hour and raising it to 90 °C and holding for two hours ensured the preservation of the enzyme activity, which was 62.9 % of the initial one.

3. The most effective dose of alpha-amylase, which ensures the necessary and sufficient level of formation of alcohol-soluble sugars, is achieved with an enzyme consumption of 1.5 units of activity per 1 g of starch.

4. An increase in the consumption of alpha-amylase to 2.0 units per 1 g of starch causes its overspending and negatively affects the degree of dilution and formation of ethyl alcohol.

References

[1] Hua, X., Yang, R. (2015). Enzymes in starch processing. In book Enzymes in Food and Beverage Processing. *Boca Raton*. *CRC Press*., 139–170. https://dx.doi.org/10.1201/b19408

[2] Bednarska, K. A. (2015). Kinetic modelling of enzymatic starch hydrolysis. Wageningen: Wageningen University. https://edepot.wur.nl/343988

[3] Dziedzic, S. Z., Kearsley, M. W. (1995). *Handbook of starch hydrolysis products and their derivatives*. Boston, USA: Springer

[4] Paul, J. S., Gupta, N., Beliya, E.b., Tiwari, S., Jadhav, S. K. (2021). Aspects and Recent Trends in Microbial α-Amylase: a Review. *Applied Biochemistry and Biotechnology, 193*(8), 2649–2698. https://dx.doi.org/10.1007/s12010-021-03546-4

[5] Ganti, S. M., Johnston, D. B., Rausch, K. D., Tumbleson, M. E., Singh, V. (2011) Starch hydrolysis modeling: application to fuel ethanol production. *Bioprocess Biosyst Eng., 34*(7), 879–890. https://dx.doi.org/10.1007/s00449-011-0539-6

[6] Vidal, B., Rausch, K. Tumbleson, M. E., Singh, V. (2009). Kinetics of Granular Starch hydrolysis in Corn Dry-Grind Process. *Starch-Starke, 61*(8), 448–456. http://dx.doi.org/10.1094/CCHEM-86-2-01

[7] Alonazi, M. K, Aida, B.-H.-a., Yacine, A., Abir Ben, B. (2021). Alpha amylase from bacillus pacificus associated with brown algae turbinaria ornata: Cultural conditions, purification, and biochemical characterization. *Processes, 9*(1), 1–13. https://dx.doi.org/10.3390/pr9010016.

[8] Srichuwong, S., Sunarti, T. C., Mishima, T., Isono, N., Hisamatsu, M. (2005). Starches from different botanical sources I: Contribution of amylopectin fine structure to thermal properties and enzyme digestibility. *Carbohydrate Polymers, 60*(4), 529–538. https://dx.doi.org/10.1016/j.carbpol.2005.03.004

[9] Chin, P. M., Ingledew, W. M. (1993). Effect of recycled laboratory backset on fermentation of wheat mashes. *Journal of Agricultural and Food Chemistry, 41*(7), 1158–1163. https://dx.doi.org/10.1021/jf00031a028

[10] Bailey, D. F., Ollis, J. E. (1988). *Biochemical Engineering Fundamentals*. New York, USA: McGraw-Hill.

[11] Bialas, W., Czerniak, A., Szymanowska-Powalowska, D. (2014). Kinetic modeling of simultaneous saccharification and fermentation of corn starch for ethanol production. *Acta Biochimica Polonica, 61*(1), 153–162. http://dx.doi.org/10.18388/abp.2014_1938.

[12] Bhatia, G., Juneja, A., Johnston, D., Rausch, K., Tumbleson M. E., Singh, V. (2021). Characterization of Amylose Lipid Complexes and Their Effect on the Dry Grind Ethanol Process. *Starch/Staerke, 73*(7-8). doi: 10.1002/star.202100069

[13] Vernon-Carter, E. J., Alvarez-Ramirez, J., Meraz, M., Garcia-Diaz, S. (2019). Gaining insights into alpha-amylase inhibition by glucose through mathematical modelling and analysis of the hydrolysis kinetics of gelatinized corn starch dispersions. *International Journal of Biological Macromolecules, 132*, 766–771. http://dx.doi.org/10.1016/j.ijbiomac.2019.03.226

[14] Vernon-Carter, E. J., Alvarez-Ramirez, J., Bello-Perez, L. A., Reyes, I., Hernandez-Jaimes, C. (2018). Inhibition of the amylolytic hydrolysis of starch by ethanol. *Food Hydrocolloids, 90*, 285–290. http://dx.doi.org/10.1016/j.foodhyd.2018.12.046

[15] Cha´vez-Camarillo, G. M., Lopez-Nuñez, P. V., Jime´nez-Nava, R. A., Aranda-Garcı´a, E., Cristiani, U. E. (2022)/ Production of extracellular α-amylase by single-stage steady-state continuous cultures of Candida wangnamkhiaoensis in an airlift bioreactor. *PLoS ONE, 17*(3), e0264734. https://dx.doi.org/10.1371/journal.pone.0264734

[16] Azmi, A. S., Malek, M. I. A., Puad, N. I. M (2017). A review on acid and enzymatic hydrolyses of sago starch. *International Food Research Journal, 24*, 265–273. http:/www.ifrj.upm.edu.my

[17] Al-Amri, A. A., Al-Ghamdi, M. A.a., Khan, J. A.a., Altayeb, H. N.a., Alsulami, H.A., Sajjad, M. B., Baothman, O. A. A., Nadeem? M. S. (2022)/ Escherichia coli expression and characterization of α-amylase from geobacillus thermodenitrificans dsm-465. *Brazilian Journal of Biology, 82*. https://dx.doi.org/10.1590/1519-6984.239449

[18] Margetić, A., Dojnov, B., Vujčić, M., Mišić, M., Božić, N., Vujčić, Z. (2023). Modified simultaneous saccharification and fermentation for the production of bioethanol from highly concentrated raw corn starch. *Fuel, 338*. https://dx.doi.org/10.1016/j.fuel.2022.127363

[19] Cripwell, R. A. a., Favaro, L., Viljoen-Bloom, M., Van Zyl, W. H. (2020). Consolidated bioprocessing of raw starch to ethanol by Saccharomyces cerevisiae: Achievements and challenges. *Biotechnology Advances*, 42. https://dx.doi. org/10.1016/j.biotechadv.2020.107579

[20] Sharma, V., Rausch, K., Graeber, J., Schmidt, S., Buriak, P., Tumbleson M.E., Singh, V. (2010). Effect of resistant starch on hydrolysis and fermentation of corn

starch for ethanol. Applied *Biochemistry and Biotechnology, 160*(3), 800–811. doi: 10.1007/s12010-009-8651-7

[21] Polygalina, G. V. (1999). [*Technochemical control of alcohol and liquor production*]. Moskva, Russian Federation: Kolos (in Russian).

[22] Mitchell, G. A. (1990). Methods of Starch Analysis. *Starch-Stärke, 42*(4), 131–134. https://dx.doi.org/10.1002/star.19900420403.

[23] Marynchenko, V.O., Domaretskyi, V.A, Shyyan, P.L., Shvets, V.M., Tsygankov, P.S., Gholner, I.D. (2003) [*Alcohol technology*]. Vinnytsya, Ukraine: Podillya-2000.

[24] SOU 15.9-37-241:2005 [*Enzyme preparation for the alcohol production. Methods for determination of amylolytic activity*]. Ministerstvo agrarnoi polityky Ukrainy, Kyiv. (In Ukrainian).

[25] Albalasmeh, A. A., Berhe, A. A., Ghezzehei, T.A. (2013). A new method for rapid determination of carbohydrate and total carbon concentrations using UV spectrophotometry. *Carbohydrate Polymers, 97*(2), 253–261 https://dx.doi.org/10.1016/j.carbpol.2013.04.072https://tristan.org.ua/ru/produktsiya/fermentnye-preparaty

[26] Langenaeken, N., De Schepper, C., De Schutter, D., Courtin, C.M. (2019). Different gelatinization characteristics of small and large barley starch granules impact their enzymatic hydrolysis and sugar production during mashing. *Food Chemistry, 295*, 138 – 146. doi 10.1016/j.foodchem.2019.05.045

[27] Šokarda Slavić, M., Margetić, A., Dojnov, B., Vujčić, M., Mišić, M., Božić, N., Vujčić, Z (2023). Modified simultaneous saccharification and fermentation for the production of bioethanol from highly concentrated raw corn starch. *Fuel, 338*. doi 10.1016/j.fuel.2022.127363

[28] Lugowoj, S., Balcerek, M. (2022) Polysaccharides of Starchy and Lignocellulose Materials and their Use in Ethanol Production: Enzymes and other Factors Affecting the Production Process. *Applied Food Biotechnology, 9*(2), 157–172. doi: 10.22037/afb.v9i2.37355