GIDA (1994) 19 (2) 89-92

ENZYMATIC HYDROLYSIS AND PRODUCTION OF ETHANOL FROM POTATO STARCH

PATATES NİŞASTASININ ENZİMATİK HİDROLİZİ VE ETANOL ELDESİ

Yekta GÖKSUNGUR, Ulgar GÜVENÇ
Ege University, Department of Food Engineering, İzmir-TÜRKİYE

SUMMARY: Ethanol was produced from potato starch suspension containing 23 % DM. The main process stages were; liquefaction with bacterial α-amylase, saccharification with glucosidase and fermentation of the obtained syrup with baker's yeast. Similar alcohol concentrations were obtained in the processes with 2 and 24 hours of saccharification (10.4 % and 10.6 % respectively) before fermentation.

ÖZET: Patates nişastasından, α-amilaz ile sıvılaştırma, amiloglikozidaz ile şekerlendirme ve elde edilen şurubun ekmek mayası ile fermentasyonu basamakları ile etanol elde edilmiştir. 2 saat ve 24 saat süren şekerlendirmeden sonra yapılan fermentasyonlarda, yaklaşık aynı oranlarda (sırasıyla % 10.4 ve % 10.6) etanol elde edilmiştir.

INTRODUCTION

Starch is a polymeric carbohydrate composed of glucose units and is extracted in granular form from different plant roots, tubers or grains. The commercial sources of starch are the seeds of cereal grains (corn, wheat, sorghum, rice), tubers (potato), and roots (tapioca, sweat potato, arrowroot). The properties of the starch vary with the plant source from which it is derived (HARKEMA, 1991).

Starch consists of two components, that are polymers of glucose: Amylose and amylopectin. Amylose, which normally makes up 20 to 25 % of starch weight, is a linear polymer of glucose units joined by alpha-1,4 bonds. The linear polymer contains up to 6000 glucose units. Blue color reaction with iodine is given by amylose. Amylopectin makes up 75-80 % of most starch types. It is a branched polymer in which the glucose units are joined by alpha, 1-4 bonds in the linear molecule sections and by alpha, 1-6 bonds at the branching points which typically occur at every 20 to 30 glucose units. Amylopectin molecule which is one of the largest molecules in nature has about 2 million glucose units (ANON., 1981; BATUM, 1993).

When starch containing biomass such as grain, cassava or potatoes is chosen as raw material for ethanol production, enzymes are used to convert the starch into sugars which are then fermented to ethanol by yeast. In this process; gelatinization, liquefaction, saccharification and fermentation steps are followed.

Gelanization is very important since enzymes are only active either on gelatinized or mechanically damaged starch granules. Starch granules are insoluble in water below approximately 55°C. When a suspension of starch is heated beyond a critical temperature, the granules start absorbing water and swell to many times their original volume. The temperature at which this occurs is known as the pasting or gelatinization temperature which ranges roughly from 60°C to 85°C depending on the type of starch. True solubilization of all starch molecules occurs when the paste is cooked at 100-160°C. When cooked starch paste is allowed to stand, retrogradation can take place which is manifested by the formation of a gel or a precipitate (HARKEMA, 1991).

In the liquefaction step amylose and amylopectin in gelatinized starch are hydrolyzed into soluble dextrins. For this purpose *Bacillus subtilis* or thermostable *Bacillus licheniformis* amylase is used. As a result of this process, the viscous starch paste is transformed into a thin, fluid solution that can be cooled to ambient temperature without gelling or retrogradation.

During saccharification dextrins are broken down into glucose by amyloglucosidase enzyme. This enzyme is another amylase that catalyzes the hydrolysis of 1-4 linkages from the ends of dextrin molecules. Glucoamylases can also hydrolyze 1-6 bonds, but at a much slower rate than the 1-4 bonds. The efficiency of the saccharification reaction can be improved by incorporating a specific amylopectin debranching enzyme pullulanase which hydrolyzes the 1-6 bonds in amylopectin molecules (BOYCE, 1986; NORMAN, 1982).

After 95-96% conversion of dextrins into glucose, alcoholic fermentation is accomplished by yeasts. In some processes both saccharification and fermentation proceeds together. After liquefaction, the mash is cooled to fermentation temperature (25-30°C) and the entire amount of saccharifying enzyme and yeast inoculum is added to the fermenter (ANON., 1981).

In this study ethyl alcohol was produced from starch with two different processes (2 and 24 hours of saccharification were used before fermentation) and ethyl alcohol yields of these processes were compared.

MATERIAL AND METHODS

Materials

In this study potato starch (AVEBE), α-amylase (Termamyl 120 L, NOVO), amyloglucosidase (AMG 300 L, NOVO), and baker's yeast (*Saccharomyces cerevisiae,* PAKMAYA) were used.

Methods

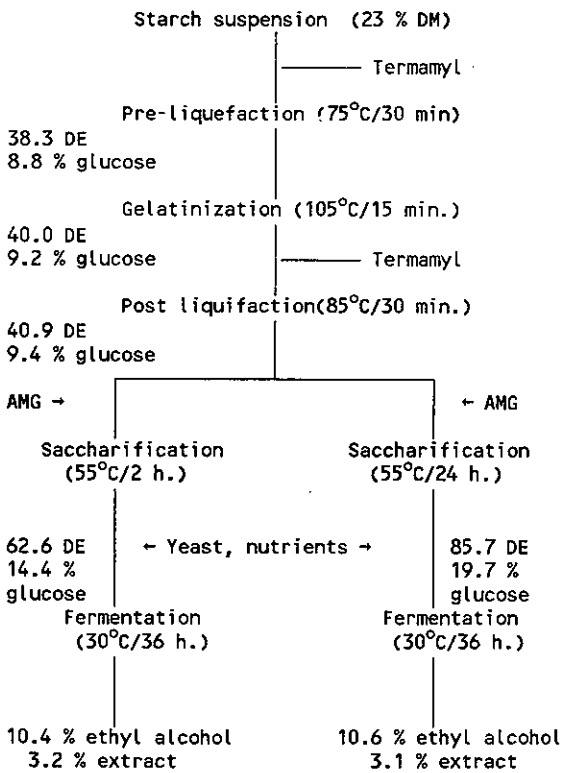

Figure 1. Production of ethyl alcohol from starch

Production of ethyl alcohol from potato starch is given in Figure 1. Starch suspension containing 23 % dry matter was prepared and the pH of suspension was adjusted to 6.5 with 1 N HCl. After addition of Termamly (0.5 % of starch weight), the suspension was held at 75°C/30 min. for pre-liquefaction. After gelatinization at 105°C for 15 minutes, post-liquefaction (85°C/30 min) was accomplished with a fresh dosage of Termamly (0.5 % of starch weight). The liquefied starch was cooled to 55°C and after pH was adjusted to 5.0 with 1 N HCl, saccharifying enzyme AMG (0.25 % of starch weight) was added. The saccharification time was 2 and 24 hours for two different samples. After saccharification, the solution was cooled to 30°C and yeast nutrients (0.1 % $(NH_4)_2HPO_4$, 0.3 % $MgSO_4$, 0.5 % peptone, 0.3 % yeast extract) were added. Baker's yeast was added to fermentation medium and fermentation time was 36 hours.

Reducing sugar during saccharification and fermentation was determined according to DNS metnod (DUBOIS et al., 1956).

Ethyl alcohol and extract during fermentation was determined according to picnometric method (TÜRKER, 1969).

Dextrose equivalent was defined as reducing sugars expressed as dextrose and calculated as a percentage of dry substance (OSTERGAARD, 1982).

RESULTS AND DISCUSSION

The main process steps and conditions in the production of alcohol from potato starch are summarized in Figure 1. Termamyl which is a thermostable α-amylase produced by a selected strain of

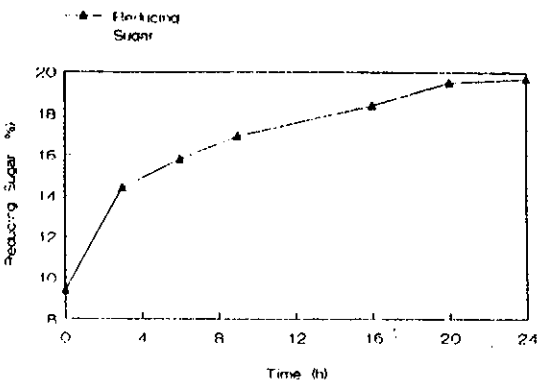

Figure 2. The increase in reducing sugar content during 24 hours of saccharification

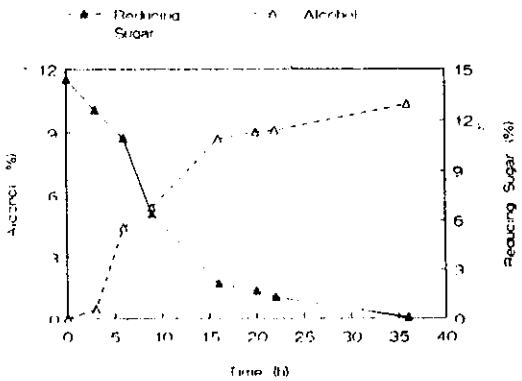

Figure 3. Changes in reducing sugar and alcohol contents during fermentation

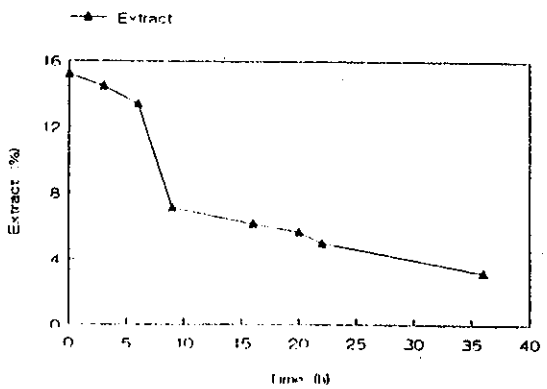

Figure 4. Decrease in extract content during fermentation

Bacillus licheniformis was used for liquefaction. Termamyl hydrolyzes alpha 1,4 linkages in starch almost at random and the breakdown products formed are mainly soluble dextrins and oligosaccharides (ANON., 1981). In order to prevent excessive mash viscosities to occur at any time during cooking, part of the liquefaction treatment was carried out before complete gelatinization had been attained and this part was referred to as pre-liquefaction. After gelatinization, post-liquefaction was accomplished in which starch was further hydrolized to low moleculer weight dextrins. At the end of post-liquefaction, liquefied starch with DE of 40.9 was obtained.

The dextrins formed in the liquefaction process were further hydrolyzed to glucose by means of AMG during saccharification. AMG is a glucoamylase produced by a selected strain of *Aspergillus niger*. It catalyzes the hydrolysis of 1-4 linkages in starch and single molecules of glucose are cleaved in a stepwise manner from the one end of the starch molecule. Glucoamylases can also hydrolyze 1-6 bonds, but at a much slower rate than the 1-4 bonds (BOYCE, 1986).

The increase in reducing sugar content during 24 hours of saccharification is given in Figure 2. After 24 hours, a syrup with 19.7 % glucose (DE=85.7) was obtained. After fermentation of this syrup, 10.6 % alcohol was produced and 3.1 % extract left unfermented. The majority of this extract are the dextrins containing alpha-1,6 glucosidic linkages which are resistant to enzymatic attack.

After 2 hours of saccharification, changes in reducing sugar and alcohol contents during fermentation are given in Figure 3. In this case part of the saccharification was carried out similtaneously with the fermentation process and 10.4 % alcohol was produced. This short time pre-saccharification was found to increase fermentation rate (ANON., 1981). Decrease in extract content during fermentation is given in Figure 4. At the end of fermentation 3.2 % extract was left unfermented. It is recommended to use a debranching enzyme such as pullulanase with glucoamylase to decrease extract and increase alcohol contents of the final product.

It is advisable to use the less time and energy consuming process in which part of the saccharification was carried out together with fermentation since same percentage of alcohol was obtained as in the second process where fermentation was accomplished after 24 hours of saccharification.

REFERENCES

ANONYMOUS, 1981. Novo enzymes in the production of ethanol from starch containing crops. Novo Industry AS; Bagsvaerd-Denmark, 47 pp.

BATUM, M., 1993. Industrial applications of enzymes. Enzymes as Industrial Tools. Ege University Biotechnology Centre, İzmir, Turkey, 229-271.

BOYCE, 1986. Novo's Handbook of Practical Biotechnology. Novo Industry A/S Enzymes Division; Bagsvaerd-Denmark, 125 pp.

DUBOIS, M., GILLES, K.A., HAMILTON, J.K., REBERS, P.A., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem., 28, 350.

HARKEMA, J., 1991. General properties and applications of starch and starch products in the food industry. The Third International Congress on Food Industry, Food Additives; Kuşadası, Turkey, 216-229.

NORMAN, B.E., 1982. A novel *Bacillus* pullulanase-its properties and application in the glucose syrups industry. Amylase Symposium; Osaka, Japan, pp. 36.

OSTERGAARD, J., 1982. Enzymes in the carbohydrate industry. Intermational Symposium on the Use of Enzymes in Food Technology; Versailles, France, pp.23.

TÜRKER, İ., 1969. Laboratuar Tekniği. Ankara Üniversitesi Basımevi, Ankara, 380 pp.