Synergistic Action of α-Amylase and Glucoamylase on Raw Corn

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Optimum ratio of glucoamylase to α-amylase for synergistic hydrolysis of starch in corn flour was 1.8 AGU/1.0 KNU. The rate of hydrolysis of starch in dry milled corn was faster than that of waxy maize starch and wet processed corn. Hydrolysis of the starch in dry milled corn was the most efficient compared with that of wet milled, corn steeped in water or NaOH.
(AGU = Amyloglucosidase Unit; KNU = Kilo Novo Unit)

Synergistische Wirkung von α-Amylase und Glucoamylase auf Rohmais. Das optimale Verhältnis von Glucoamylase zu α-Amylase für die synergistische Hydrolyse von Stärke in Maismehl betrug 1,8 AGU/1,0 KNU. Die Hydrolysegeschwindigkeit der Stärke in trocken vermahlenem Mais war größer als die von wachsiger Maisstärke und naßvermahlenem Mais. Die Hydrolyse der Stärke in trockenvermahlenem Mais war die wirksamste verglichen mit derjenigen von naßvermahlenem, in Wasser oder NaOH gequollenem Mais.
(AGU = Amyloglucosidase-Einheit; KNU = Kilo-Novo-Einheit)

1. Introduction

Hydrolysis of starch is the first step in many of its industrial conversions into food, beverages and chemicals. The conversion of starch in starch containing materials such as corn grits and corn flour without previous isolation of starch had attracted the interest of scientists as well als industrialists for decades within glucose industries [1]. The interest was due to considerable savings in the investment costs. For direct hydrolysis of starch in corn either wet [2] or dry milled [3] corn can be used. By dry milling of corn, products such as grits, meal and flour are obtained [3]. In wet milling of corn the raw material is pretreated by steeping to soften the kernel [2].
Different studies have been made to compare the hydrolysis of starch from different sources [4, 5] and by different enzymes [5]. However, no comparisons were made of the hydrolysis of wet and dry milled starch preparations. The purpose of this study is to explore the synergistic action of α-amylase and glucoamylase on the hydrolysis of starch in corn flour (dry milled) and wet milled corn.

2. Experimental

2.1 Materials

Waxy maize starch was a gift form Stadex (Sweden). Corn was purchased from local market. Corn was pulverized in a domestic grinder. α-Amylase (Termamyl 60 L®, activity 67.5 KNU · g^{-1}) and glucoamylase (Spiritamylase 150 L®, activity 159.9 AGU · g^{-1}) were from NOVO Industri (Denmark). The activities of α-amylase and glucoamylase were determined as described in references [6] and [7].

2.2 Analytical methods

The reducing sugar produced by the hydrolysis of starch in corn flour was determined by 3.5 dinitrosalicylic acid method and represented in terms of glucose [8].

2.3 Optimum ratio of glucoamylase and α-amylase activities

Corn flour (16% w/w) was hydrolysed at 70°C in 0.025 M acetate buffer pH 4.6, by different ratios of α-amylase and glucoamylase for 3 h and the reducing sugars formed were measured [9].

2.4 Comparison of the hydrolysis of waxy maize starch and corn flour by enzyme mixture

Corn flour (16% w/w) in suspension (0.025 M acetate buffer, pH 4.5) was hydrolysed by optimized amounts of α-amylase and glucoamylase mixture at 70°C for 5 h and samples were analyzed for the product intermittently [8]. Similarly waxy maize starch in suspension (16% w/w) and corn flour in suspension (18.5% w/w) were treated. At 5 h dry weights of the residues obtained with 16% (w/w) suspensions of waxy maize starch and corn flour were determined.

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2.5 Hydrolysis of starch in wet and dry milled corn

Corn (80 g) steeped in 420 g of 0.1N NaOH or water containing 150 ppm sodium metabisulphite for 60 h was washed well with distilled water and with 0.025M acetate buffer, pH 4.6. Homogenized sample in buffer (16% w/w) (wet milled) was incubated with mixture of α-amylase and glucoamylase at 70°C. Under similar conditions, corn flour (dry milled) suspension (16% w/w) was hydrolysed.

3 Results and Discussion

3.1 Optimum ratio of glucoamylase and α-amylase activities

Increasing the ratio of glucoamylase to α-amylase Increased the glucose production from corn flour. When the ratio was increased from 0 to 1.8 AGU/KNU, glucose formed was increased almost linearly from 6 to 13.1% (w/w) (Figure 1). Further increase in the ratio of enzymes (*i. e.* above 1.8 AGU/KNU) had not improved the hydrolysis. The hydrolysis efficiency was

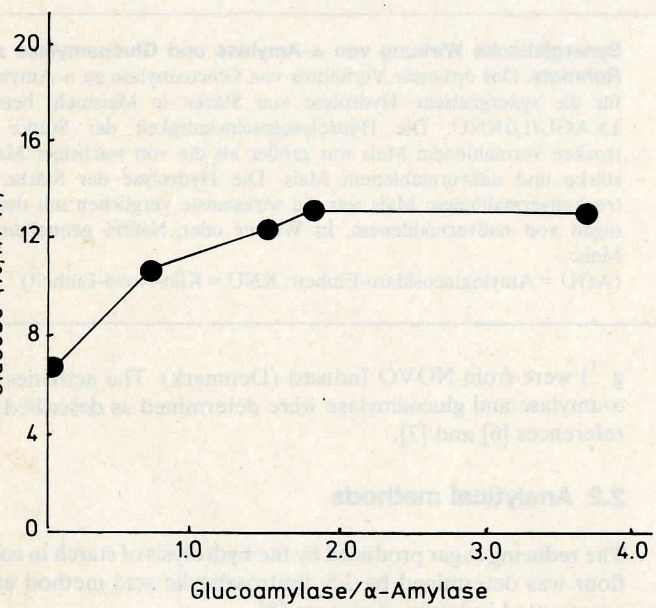

Figure 1. Ratios of the activites of glucoamylase and α-amylase as a function of glucose production from 16% (w/w) corn flour suspension (0.025M acetate buffer, pH 4.6) at 70°C and 3 h.

99.2% (Table 1). The optimum ratio between α-amylase and glucoamylase for the hydrolysis of corn flour was 1.8 AGU/KNU. The ratio was same as the ratio of the enzymes obtained for waxy maize starch [9]. This ratio was chosen for rest of the experiments. In commercial practice starch hydrolysis requires more than 24 h to obtain 93.5 to 97.3% of DE [10]. The work reported in this paper required 3 h to obtain a DE of 99.2% at 70°C and pH 4.6 by simultaneous liquefaction and saccharification, and avoiding gelatinization. Thus, in this process not only the time but also the energy was conserved. Limiting the reaction time also gives additional advantages such as avoidance of the reversion problem usually encountered with commercial glucoamylase preparations, which contain transglucosylase as a contaminant, and the inhibition of glucoamylase by glucose [11].

Table 1.
Comparison of the Hydrolysis of Waxy Maize Starch and Corn Flour Suspensions (16%, (w/w) in 0.025M Acetate Buffer (pH 4.6) by the Mixture of α-Amylase and Glucoamylase at 70°C.

Substrate	Hydrolysis efficiency (%)	Glucose yield (%)	Residue (%)
Waxy maize starch	100	100	1.9
Corn flour	99.2	81.9	28.3

$$\text{Hydrolysis efficiency (\%)} = \frac{\text{Glucose obtained by enzyme hydrolysis}}{\text{Glucose obtained by acid hydrolysis}} \times 100$$

$$\text{Glucose yield (\%)} = \frac{\text{Glucose obtained}}{\text{waxy maize starch/corn flour used}} \times 100$$

$$\text{Residue (\%)} = \frac{\text{Residue left}}{\text{waxy maize starch/corn flour used}} \times 100$$

3.2 Comparison of hydrolysis of corn flour and waxy maize starch

When 16% (w/w) suspensions of corn flour and waxy maize starch were hydrolyzed, glucose obtained were 13.1% and 15.3%, respectively (Figure 2). Thus, the glucose yield is 81.9% and 100% for corn flour and waxy maize starch, respectively

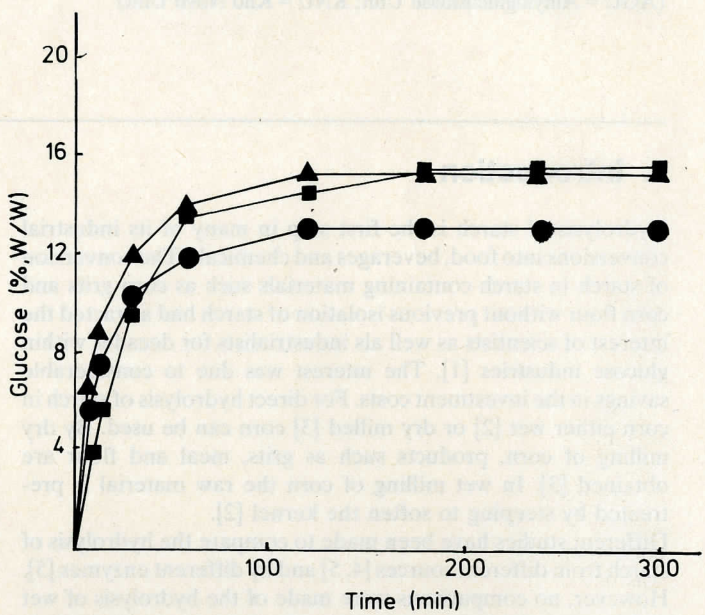

Figure 2. Hydrolysis of starch in corn flour and waxy maize starch by enzyme mixture at 70°C (α-amylase 0.225 KNU ml^{-1} and glucoamylase 0.4 AGU ml^{-1}). 16% (w/w) (■) waxy maize starch and (●) corn flour, and 18.5% (w/w) (▲) corn flour. (The samples were suspended in 0.025M acetate buffer, pH 4.6).

(Table 1). The difference in glucose production was due to the difference in starch content between 16% (w/w) suspensions of waxy maize starch and corn flour. (Starch content in waxy maize starch was 86.3% and in the corn flour was 74.5%). When 18.5% (w/w) corn flour suspension containing the same amount of starch as 16% (w/w) waxy maize starch suspension was hydrolysed, glucose obtained was 15.2% (w/w) and hydrolysis efficiency was 99.2%. Enzyme mixture hydrolyzed the corn flour faster than waxy maize starch during the initial stages (Figure 2). This could be due to the absence of calcium and chloride in purified starch preparation [12]. In our experiment neither calcium nor chloride were added to the reaction mixture, which may explain the low rate of hydrolysis of waxy maize starch. Residue from the hydrolysis of corn flour was more than that of the waxy maize starch as the former contains cellulose, hemicellulose and proteins as impurities.

3.3 Comparison of the hydrolysis of wet and dry milled corn

Maximum glucose produced by the hydrolysis of wet milled corn steeped in NaOH and water at 3 h was 3.6% (w/w) and 6.4% (w/w), respectively, and the hydrolysis efficiency was 27.3% and 46.2%, respectively (Figure 3). When dry milled corn (corn flour) was hydrolyzed, not only the hydrolysis efficiency

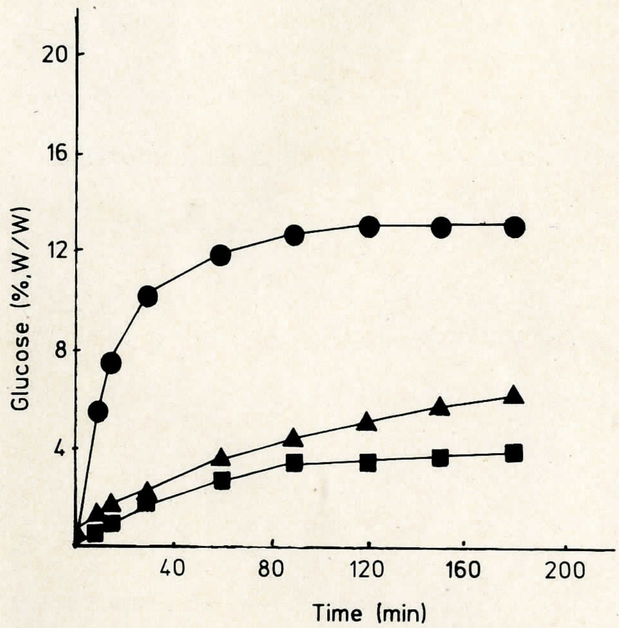

Figure 3. Hydrolysis of starch in 16% (w/w) (●) corn flour (dry milled) and (■) wet milled corn steeped in NaOH and (▲) in water by enzyme mixture (α-amylase 0.225 KNU ml^{-1} and glucoamylase 0.4 AGU ml^{-1}) at 70°C. The suspensions are in 0.025 M acetate buffer, pH 4.6.

was high (99.2%) but also the time taken for the hydrolysis was short (3 h). On the other hand when corn was wet milled and hydrolyzed, the process required 63 h and glucose yield was 27.5–46.8% of that of dry milled corn. The wet milled corn, corn steeped in water had 0.5% (w/w) glucose at zero time and was better hydrolyzed than that steeped in NaOH (Figure 3). These effects could be due to the formation of endogenous amylase during germination of corn while steeped in water and the synergistic activity of endogenous amylase and added mixture of enzymes on starch hydrolysis. The corn steeped in NaOH was a control in which contamination would be minimal and proteins would be solubilized.

4 Conclusion

This work indicates the synergistic action of α-amylase and glucoamylase on raw dry milled corn is economical and efficient, even though the concentration of the enzymes used for the process are more than those previously reported for purified starch preparations. To make this process economically feasible, cheap down-streem processing methods should be studied.

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