Annex 10-5

IMPROVING CORN MALTING AND PREPARATION OF MALT EXTRACT USING EXOGENOUS AND ENDOGENOUS AMYLASES

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ABSTRACT

Addition of gibberellic acid (0.1×10^{-3} g kg^{-1}) to the steeping water containing 150 ppm sodium metebisulphite increased malting of corn and its endogenous amylase activity. Malting and production of endogenous amylase of corn were further improved by the incorporation of Tween 80 (1.0 g l^{-1}) to steeping water. Moisture, reducing sugar and protein contents, and endogenous amylase activity of corn malt powder were 100.0 g kg^{-1}, 660.0 g kg^{-1}, 90.0 g kg^{-1} and 480.0 x 102 U kg^{-1} malt powder, respectively. When 5.0 g kg^{-1} α – amylase (exogenous amylase) was supplemented, 97.6% of the endogenous starch was hydrolyzed at 2 hours. From 1.0 kg corn malt powder, 4.0 l clear malt extract was obtained. Total sugars, dextrose equivalent (DE), calcium, and protein contents of malt extract were 180.0 g l^{-1}, 75.6%, 0.2 g l^{-1} and 4.0 g l^{-1} respectively.

KEY WORDS: Malting, endogenous amylase, exogenous amylase, corn malt powder.

INTRODUCTION

Cereal malts are important sources of amylases for fermentation process in brewing industry. Several studies were made by others on malting rice[1], wheat[2], barley, oat[2] and millet[2]. Malting process provides correct environment within the grain for the synthesis of hydrolytic enzymes and controlled action of these enzymes to act on the reserve proteins and carbohydrates of the endosperm. During malting, presence of additives such as hypochlorite, lime water, formaldehyde, sodium metabisulphite[3],

SO_2[4], ethanol[5], etc. reduced microbial growth and contamination. Gibberellic acid is added to overcome dormancy of seeds, accelerate malting process[6], increase respiration rate of the embryo[7,8], and stimulate the rate of hydrolytic enzymes production[2]. Penetration of gibberellic acid is accelerated by lightly brushing or abrasing the grain i.e. by creating small faults in the pericarptesta[2,6]. The dose of gibberellic acid is critical because at certain concentrations malt may be over modified, its colour may be affected and the sugar / dextrin and amino acid / protein ratios may be increased to higher levels[6]. Hence, it is necessary to select the optimum gibberellic acid concentration while improving its entry into the cells of corn grains.

In this study a surfactant was used as an alternative to abrasing of the grains. Here, we report the work carried on malting of corn in the presence of different concentrations of gibberellic acid with and without Tween 80, and preparation of malt extract from the malted corn with a commercially available exogenous α – amylase.

MATERIALS AND METHODS

Materials

Corn (Zea mays, L.) was purchased from local market and α – amylase (Termamyl 60LR, 67.5 KNU g^{-1}) was from NOVO Industries, Denmark. Gibberellic acid, sodium metebisulphite and Tween 80 were of analytical grade.

Malting of corn

Effect of gibberellic acid on malting

Corn (1.0 kg) was steeped in tap water (2.0 l) containing 150 ppm sodium metebisulphite and different concentrations of gibberellic acid (0.0×10^{-3}, 0.001×10^{-3}, 0.1×10^{-3} and 1.0×10^{-3} g kg^{-1}) at room temperature for 18 hours. After draining the water, malting was continued by keeping the grains in moistened cloth bag kept in dark room. The grains were mixed regularly during malting and sprayed with aqueous solution of sodium metabisulphite (150 ppm) containing the respective concentrations of gibberellic acid. Samples were taken daily and malting was arrested by drying the germinated grains at 40°C for 2 days. Moisture content of the malted corn was determined[9]. Percentage of malting was determined as follows: Malting (%) = No. of seeds malted / Total no. of seeds taken x 100. Malt powder was prepared as follows for analysis.

Effect of Tween 80 on malting

In this experiment, to tap water containing 150 ppm sodium metabisulphite, different concentrations of gibberellic acid and Tween 80 (1.0 g l^{-1}) were added. Corn grains were malted as described above.

Preparation of malt powder

Malted corn dried at 40°C for 2 days was grounded to fine powder in a domestic mill where the temperature did not exceed 40°C. Malt powder was analyzed for moisture content[9] (by drying at 80°C to constant weight), total sugar[9] (by acid hydrolysis and determining the reducing sugar content using dinitrosalicylic acid method), reducing sugars[10](by dinitrosalicylic acid method), protein[9] (by Kjeldhal method) and endogenous amylase activity (as described below).

Corn was powdered using domestic mill and the corn flour was analyzed for total sugar[9], protein[9] and moisture[9] contents.

Determination of malt amylase activity

Soluble enszyme was extracted by mixing 1.0 g of malt powder with distilled water (4.0 ml) for 30 minutes and the strained extract was centrifuged. The supernatant was used as enzyme source. Supernatant (1.0 ml) was incubated with 2.5 ml of 40.0g l^{-2} soluble starch at pH 4.0[11] and 60°C for 30 minutes and the reducing sugars were determined [10]. One unit of amylase is the amount of enzyme which releases 1.0 μ mole of glucose in 1.0 min at 60°C and pH 4.0. The activity of malt amylase in presented as U kg^{-1} malt powder.

Effect of exogenous amylase (α – amylase) on the hydrolysis of residual starch present in malt powder

Malt powder suspended in water (250.0 g l^{-1}; at pH 4.0) was incubated at optimum temperature for 30 minutes After increasing the pH to 7.0[12], different concentrations of α – amylase (0.05, 0.5 and 5.0 g kg^{-1}) were added and incubated at 95°C for 4 hours. The samples were withdrawn at 30 minutes intervals, the pH was reduced to 3.5, heated to 95°C (to stop the exogenous amylase activity)[13], centrifuged and the supernatants were analyzed for reducing[10] and total sugars[9] and the dextrose equivalent[13] was calculated. From the samples taken at 30 minutes intervals, reducing[9] and total reducing sugars[9] were determined and the percentage of starch hydrolyzed[13] was calculated. A control experiment was carried out without the addition of α amylase.

Preparation of malt extract

Corn malt powder in suspension (1.0 kg in 4.0 l water, pH 4.0) was first incubated at 60°C for 30 minutes and cooled. Then pH was adjusted to 7.0, incubated with optimized amount of exogenous amylase (α – amylase) at 95°C for required period. The hydrolysate was extracted using a screw press. Residue was washed with water (1.0 l) and the 1st and 2nd extracts were analyzed for total[9] and reducing sugars[10], proteins[9], and calcium[14]. The proteins[9], residual total sugars[9] and moisture[9] contents of the residue were determined.

RESULTS AND DISCUSSIONS

Effect of gibberellic acid and of Tween 80 on malting of corn

Sodium metabisulphite is an antibacterial agent and gibberellic acid is a growth promoting hormone. Therefore to tap water containing sodium metabisulphite, gibberellic acid of different concentration was added. These solutions were used to steep corn as well as to spray during malting to keep the moisture content between 390.0 and 400.0 g kg^{-1}. When the concentration of gibberellic acid added to steeping water was increased from 0.0 to 0.1 x 10^{-3}g kg^{-1}, there was 38.8% improvement in malting. A further increase in the gibberellic acid concentration to 1.0 x 10^{-3} g kg^{-1} did not improve malting (Table 1). In the absence of gibberellic acid endogenous amylase activity increased from 2nd day and reached the maximum on the 6th day (Figure 1). Endogenous amylase activity in the grains was high in the presence of gibberellic acid. At 0.1 x 10^{-3}g kg^{-1} gibberellic acid and above, the highest amylase activity was obtained on the 3rd day (Figure 1). Lineback et al[2] observed that amylase activity in malted wheat, oat, and pearl millet increased during malting and tended to become constant during the later stages of malting (after 8 to 14 days). Gibberellic acid improved the malting of wheat and oat grains[8]. However, prolonged exposure affected malting[6]. In our experiment the activity was constant after the 3rd day. Malting, therefore, can be arrested on the 3rd day.

To improve malting, it was decided to supplement the steeping water containing sodium metabisulphite and different concentrations of gibberellic acid with the detergent, Tween 80. Here too, the moisture content was maintained in the range between 390.0 and 400.0 g kg^{-1} as before. When Tween 80 (1.0 g l^{-1}) was incorporated in addition to different concentrations of gibberellic acid, malting was improved (Table 1) as compared with the respective controls. Gibberellic acid concentrations at and above 0.1 x 10^{-3} g kg^{-1} decreased malting time to 3 days and increased amylase

Table 1. Effect of different concentrations of gibberellic acid and Tween 80 on malting of corn grains at 4th day

Medium		
Giberellic acid ($x\ 10^{-3}$ g kg^{-1})	Tween 80 (g kg^{-1})	Malting* (%)
0.00	0.0	50
0.00	1.0	78
0.01	0.0	85
0.01	1.0	94
0.10	0.0	88
0.10	1.0	98
1.00	0.0	88
1.00	1.0	98

*Malting (%) = No. of seeds malted / Total no. of seeds taken x 100.

Table 2. Composition of malt extract 1 and 2 and the residue obtained from 1.0 kg malt powder in suspension (1.0 kg in 4.0 l).

Parameters monitored	Malt extract 1	2	Rresidue
Volume / Weight (1.0 / kg)	3.0	1.0	0.54
Redusing sugar (g l^{-1})	136.0	43.9	ND
Total sugar (g l^{-1} / g kg^{-1})	180.0	58.0	170.0
Dextrose equavalent (DE)	75.6	75.6	ND
Protein (g l^{-1} / g kg^{-1})	15.0	1.3	136.5
Calcium (g l^{-1} / g kg^{-1})	0.2	0.2	ND
Moisture (g kg^{-1})	79.2	93.0	58.0

ND - Not determined

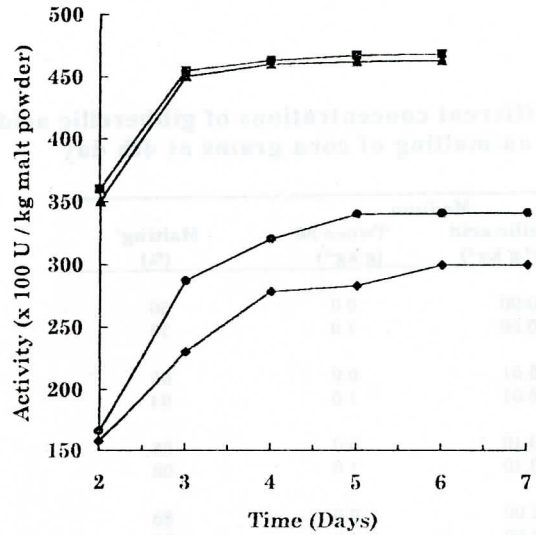

Figure 1. Endogenous amylase activity as a function of germination time in the presence of different concentrations of gibberellic acid (g kg^{-1}). Assay conditions are in the text. Gibberelic acid (◆) 0.0 x 10^{3}: (●) 0.01 x 10^{-3}; (▲)0.1 x 10^{-3}; and (■) 1.0 x 10^{-3} g kg^{-1}.

production to 480 x 10^{2} U kg^{-1} malt powder at 3rd day (Table 1 and Figure 2). This result indicated that Tween 80 has probably improved the permeability of the cell membrance to gibberellic acid. The dose of gibberellic acid applied in malting of barley[3] was 0.025 – 0.25 mg kg^{-1}. The results indicated that , for malting of corn grains it is sufficient to steep them for 18 hours in tap water containing 150 ppm sodium metabisulphite, 0.1 x 10^{-3} g kg^{-1} gibberellic acid and 1.0 g l^{-1} Tween 80. To continue malting, grains can be kept in moistured cloth bag placed in dark room, while spraying with the same solution used for soaking and malting can be arrested on the 3rd day.

Moisture, total sugar and protein contents and endogenous amylase activity of the malt powder prepared by the above were 100 g kg^{-1}, 669 g kg^{-1}, 90.0 g kg^{-1} and 480 x 10^{2} U kg^{-1}, respectively. From this it can be concluded that 86.3% of the total reducing sugar and 81.8% of the protein (measured as nitrogen) were retained by the grain after germination.

Effect of exogenous amylase (α – amylase) on the hydrolysis of residual starch present in malt powder

Malt powder contained 13.9% of the total starch hydrolyzed by endogenous amylase (Figure 3) with a DE of 66.5% (Figure 4). Hence to improve starch hydrolysis, malt powder suspension was incubated for 0.5 hour at optimum pH (4.0)[11] and temperature

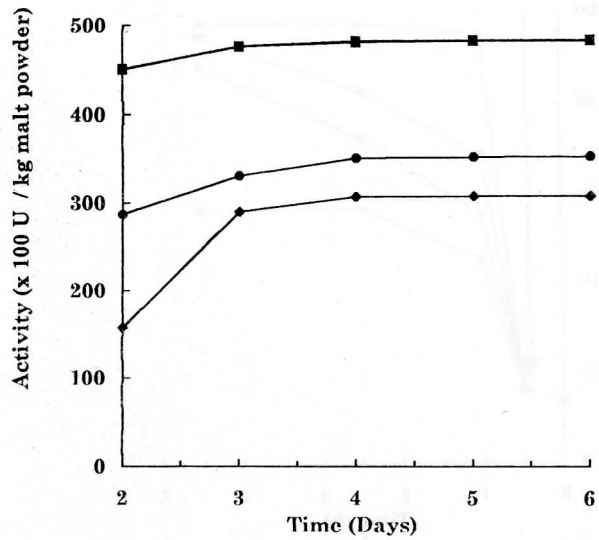

Figure 2. Endogenous amylase activity as a function of germination time in the presence of different concentrations of gibberellic acid (g kg^{-1}) supplemented with Tween 80 (1.0 g kg^{-1}). Assay conditions are in the text. Gibberellic acid (◆) 0.0 x 10^{-3}; (●)0.01 x 10^{-3}; (▲)0.1 x 10^{3}; and (■) 1.0 x 10^{-3} g kg^{-1}.

(60°C) of malt amylase, which increased the DE to 95.7% (Figure 4) and total starch hydrolysis to 16.2% (Table 2). Since the increase in starch hydrolysis by endogenous amylase was small, exogenous amylase (α – amylase) of different concentrations was added and incubated at pH 7.0 and 95°C. The results indicated that pH 4.0 and 60°C were not suitable to the endogenous amylase when compared to pH 7.0 and 95°C. Changes in starch hydrolysis (%) and DE are shown in Figures 3 and 4. In control the DE was 35.5% (at 1hour) and it further decreased to 31.5% (at 3.5 hours). At high exogenous α – amylase concentration (5.0 g kg^{-1}), the extent of hydrolysis was 94.9% in the first half hour and the rate limiting endogenous saccharifying enzymes (endogenous amylase)during this time had increased the DE to 68.2. Increase in DE, thereafter, was small due to the denaturation of the endogenous saccharifying enzymes at 95°C. When α – amylase (exogenous amylase) in low concentration was added, a decrease in DE was clearly observed and it then slowly increased. This could be due to slow release of dextrins and thus, decreased concentration of substrate was made available for the endogenous amylase activity before its denaturation at 95°C.

Even though 0.5 and 5.0 g kg^{-1} exogenous amylase gave almost same percentage of starch hydrolysis at 5.5 hours (Figure 3), 5.0 g kg^{-1} exogenous amylase was used in malt extract preparation considering the time factor and DE.

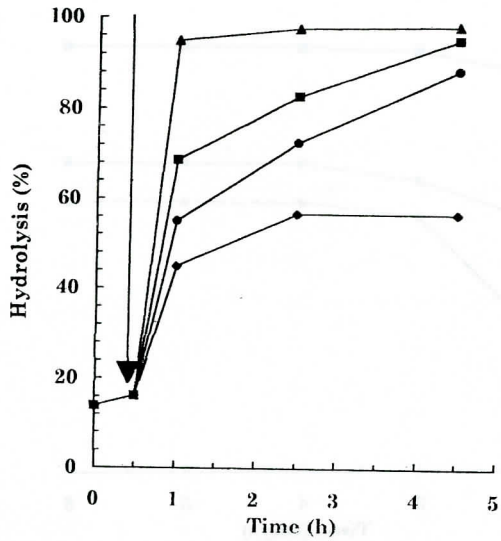

Figure 3: Effect of endogenous and exogenous (α-) amylase on starch hydrolysis in malt powder. Endogenous amylase acted for 0.5 h at pH 4.0 and 60°C. then pH was raised to 7.0, different amount of exogenous amylase was added and incubated at 95°C. Exogenous amylase (◆) 0.0; (●) 0.05; (■) 0.5;and (▲) 5.0g kg^{-1}. A-Addition of exogenous amylase.

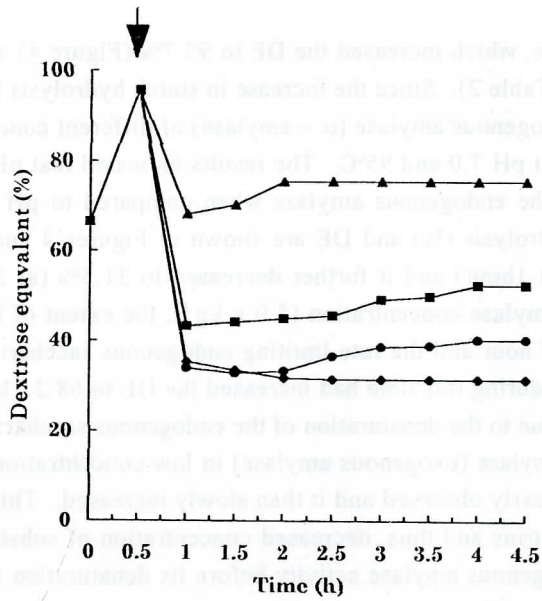

Figure 4: Effect of endogenous and exogenous (α-) amylase on change in dextrose equivalence in malt powder. Exogenous amylase acted for 0.5 h at pH 4.0 and 60°C. Then pH was raised to 7.0, different amount of exogenous amylase was added and incubated at 95°C. Exogenous amylase (◆) 0.0; (●) 0.05; (■) 0.5;and (▲) 5.0g kg^{-1}. A – Addition of exogenous amylase.

Malt extract

From 1.0 kg of malt powder in 4.0 l tap water, 3.0 l of malt extract and with 1.0 l of tap water, 1.0 l of 2nd malt extracts were obtained. Composition of the extracts and the residue is given in Table 2. Of the total sugar in malt powder (660.0 g kg^{-1}) 90.6% was recovered in malt extract. The DE of the extracts 1 and 2 were same (76.6%). The residue which is rich in protein (25.3%) is a good feed for animals and birds.

CONCLUSION

Malting of corn was improved by steeping the grains in water containing sodium metabisulphite, gibberellic acid (0.1 x 10^{-3} g kg^{-1}) and Tween 80 (1.0 g l^{-1}). A malt extract with DE 75.6% and 21% dry substance was prepared. Work is in progress to prepare malt drinks from the malt extract.

ACKNOWLEDGEMENTS

The authors thank the University of Jaffna and the International Science Program (Sweden) for the financial assistance.

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