APPLICATION OF COMMERCIAL MICROBIAL ENZYME PREPARATION (FUNGAMYL 800L AND CEREMIX 2XL) IN MASHING PROCESS FROM MALT AND SWEET POTATO

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ABSTRACT: *Sweet potato is an interesting adjunct in brewing because of its high sugar and starch contents. However, in 60% malt and 40% sweet potato mashing, the extraction yield, reducing sugar and amino nitrogen contents in the obtained wort were much lower than those in 100% malt mashing. In this paper, the commercial Fungamyl 800L (with beta-amilase activity) and Ceremix 2XL (with alpha-amilase, protease and beta-glucanase activities) were alternatively added to the mash. In these cases, the extraction yield increased, the saccharification time decreased, the reducing sugar and amino nitrogen contents of wort were augmented. Ceremix 2XL gave a better mashing effect than Fungamyl 800L because of its catalytic ability on different substrates of plant tissue. The physio – chemical characteristics of the 60% malt – 40% sweet potato wort (Ceremix addition dose: 0,4% of raw material mass) were similar to those of 100% malt wort.*

1. INTRODUCTION

Although not essential in brewing, adjuncts are used in most countries and brewing regions and provides benefits in extract cost, beer quality and new product development (Lloyd W.J.W., 1986). Our recent study showed that sweet potato can be used in brewing because of its high sugar and starch contents (Ton Nu Minh Nguyet et al., 2003). However, enzyme activities in sweet potato and other adjuncts are much lower than those in barley malt. Different commercial enzymes are therefore used for ameliorating the quality of the wort (Moll M., 1991; Kunz W., 1999).

In many coutries, 20-40% of malt replacement by adjuncts is currently employed (Moll M., 1991). This research focussed on the application of Fungamyl 800L (with alpha-amilase activity) and Ceremix 2XL (enzyme mixture with alpha-amilase, protease and beta-glucanase activities) in the 60% malt – 40% sweet potato mashing process. The concentration and the catalytic times of Fungamyl 800L and Ceremix 2XL were varied for decreasing the saccharification time, increasing extraction yield, reducing sugar and amino nitrogen contents of the obtained wort.

2. MATERIALS AND METHODS

Materials

Malt: Malt supplied by an Australian company was used in this study (Moisture: 4.5%, extraction yield: 75.4%).

Sweet potato (Impomoea batatas L.): fresh sweet potato (Variety: *Coastal Red*) supplied by a Dalat farm was used in this study (Moisture: 66.7%, glucid: 29.9%, reducing sugar: 6.3%, total protein: 1.1% m/m).

Microbial enzymes: Commercial Fungamyl 800L (alpha-amylase activity: 800 units/g) and Ceremix 2XL with different enzyme activities (alpha-amylase: 80 Kilo-Novo units/g; beta-glucanase: 300 units/g and protease: 0.33 Anson units/g) were supplied by Novo Nordisk Fermented Ltd. (Denmark)

Mashing process: was carried out by decoction method (Moll M., 1991; Kunze W., 1999)

- *100% malt mash:* Malt and water were heated to 50°C and maintained at this temperature for 15 min. Then 1/3 mass of the mash was separated, heated to 72°C and maintained at this temperature for 15 min. This portion was rapidly heated to 100°C and boiled for 15 min. After all, it was mixed with the main mash and the temperature was adjusted to 63°C for 15 min. The mixture was then heated to 72°C for total saccharification. Saccharification time was then noted. The mash was filtered, boiled with hops for an hour, filtered again and adjusted to 11°Pt specific gravity. The obtained wort was used for determination of extraction yield, reducing sugar and amino nitrogen contents.

- *60% malt and 40% sweet potato mash:*

Adjunct mashing: Sweet potato, malt (15% of adjunct mass) and water were heated to 72°C and maintained at this temperature for 15 minutes. After that, the mash was heated to 100°C and boiled for 15 min.

Malt mashing (without exoenzyme use): Malt and water were heated to 50°C and maintained at this temperature for 15 min. Then, adjunct and malt mashes were mixed together and the temperature was adjusted to 63°C for 15 min. The mixture was heated to 72°C for total saccharification. The following procedure of the malt-adjunct mashing was similar to that of the 100% malt mashing.

Malt mashing (with exoenzyme use): Malt, water and Fungamyl 800L (or Ceremix 2XL) – with different concentrations were heated to 50°C. The time for proteolytic stand was 15 min. (or 10, 20, 30 min. if using Ceremix 2XL). Then, adjunct and malt mashes were mixed together and the temperature was adjusted to 60°C for 10, 20 or 30 min. (or to 63 °C for 15 min. if using Ceremix 2XL). The mixture was heated to 72°C for total saccharification. The following procedure of the malt-adjunct mashing was similar to that of the 100% malt mashing.

Analytical methods (Analytica EBC, 1987; Helrich K. 1992)

Saccharification time: Saccharification time at 72°C was determined by iodine solution (0.02N). Sample was taken out and tested each minute until unchanged color of iodine solution.

Extraction yield: Extraction yield was calculated by the ratio of total extract of the obtained wort to total dry weight of brewing materials. Total extract was measured at 20°C by densimeter method.

Reducing sugar: Reducing sugar content was quantified by spectrophotometric method using DNS (3,5-DiNitroSalisilic acid) reagent.

Amino nitrogen: Amino nitrogen content was determined by spectrophotometric method using Ninhydrin reagent.

3. RESULTS AND DISCUSSION

3. 1. Control samples

In this research, 2 control samples were carried out. The first control sample was mashed from 100% malt, the second control sample - from 60% malt and 40% sweet potato without microbial enzyme utilization (table 1).

Table 1: Mashing process in the control samples

Mash, N^0	Malt-sweet potato ratio, % (m/m)	Saccharification time, min.	Extraction yield, % (m/m)	Reducing sugar, g/L	Amino nitrogen, mg/L
1	100 – 0	4	75.32	93.9	285.4
2	60 – 40	15	71.52	73.6	189.3

Table 1 shows that adjunct utilization augmented the saccharafication time, reduced the extraction yield, sugar and amino nitrogen contents in the obtained wort. Therefore, utilization of commercial enzymes in malt – adjunct mashing was essential for decreasing the saccharification time and improving the extraction yield and the wort quality.

3. 2. Malt and sweet potato mashing with commercial Fungamyl 800L

15 mashes with different concentrations of Fungamyl 800L and its catalytic times were carried out. The optimum of catalytic temperature was 60°C. The experimental plan is presented in table 2. The results are visualized in figures 1, 2, 3 and 4.

It can be noted that the higher the Fungamyl concentration and the longer the catalytic time; the shorter the saccharification time and the higher the extraction yield and the reducing sugar content. It was explained by the amylase catalysis of Fungamyl 800L on starch. If the Fungamyl 800L concentration was 0.4% and the catalytic time was 20 min, the saccharification time, extraction yield and reducing sugar content in the obtained wort were 4 min, 76% and 96g/L respectively. These data were similar to those of the 100% malt mash (Table 1, mash N°1). In addition, Fungamyl improved slightly the proteolysis. However, the amino nitrogen content in wort was quite low (240mg/L). In order to increase the amino nitrogen content, commercial protease should be used in mashing process.

Table 2: Malt-sweet potato mashing with commercial Fungamyl 800L

Catalytic time: 10 min		Catalytic time: 20 min		Catalytic time: 30 min	
Mash, N^0	Fungamyl content, % of total material mass	Mash, N^0	Fungamyl content, % of total material mass	Mash, N^0	Fungamyl content, % of total material mass
3	0.0	8	0	13	0.0
4	0.1	9	0.1	14	0.1
5	0.2	10	0.2	15	0.2
6	0.3	11	0.3	16	0.3
7	0.4	12	0.4	17	0.4

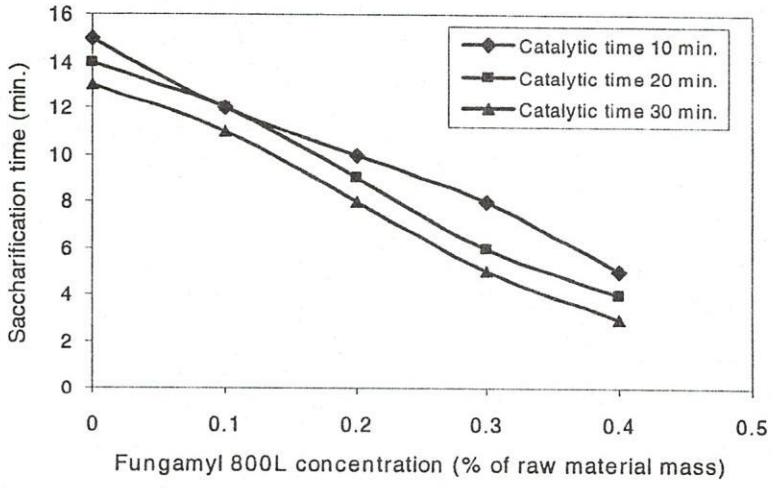

Figure 1: Influence of Fungamyl 800L on saccharification time in 60% malt – 40% sweet potato mashing

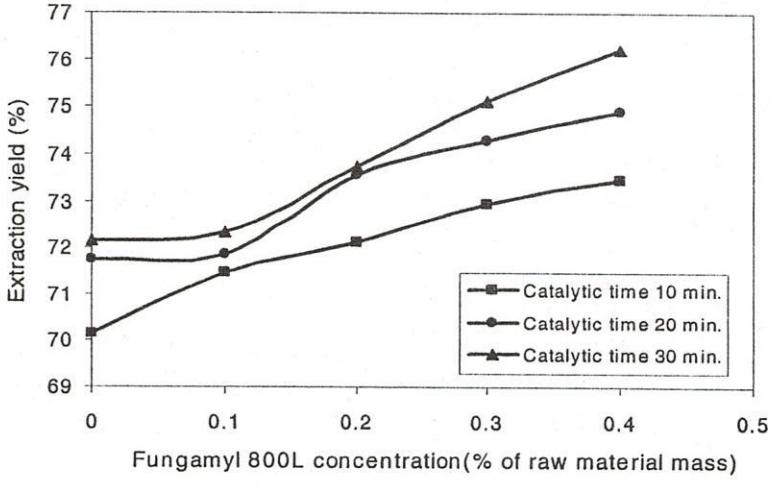

Figure 2: Influence of Fungamyl 800L on extraction yield in 60% malt – 40% sweet potato mashing

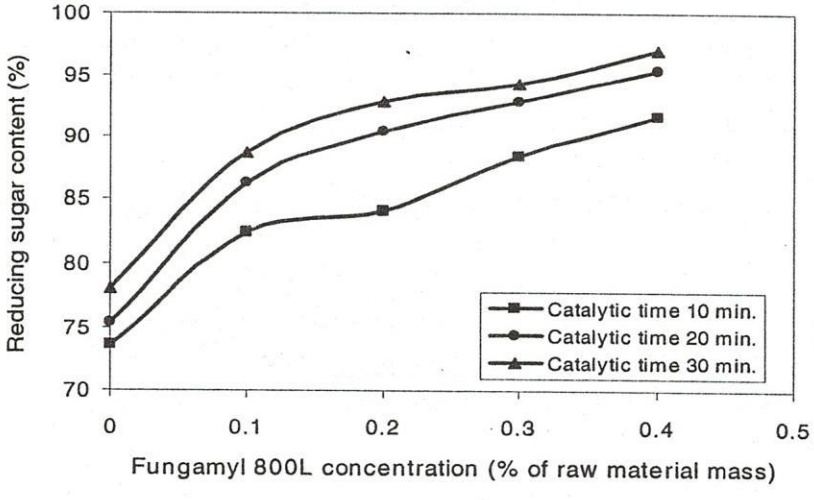

Figure 3: Influence of Fungamyl 800L on reducing sugar content in the wort from 60% malt - 40% sweet potato

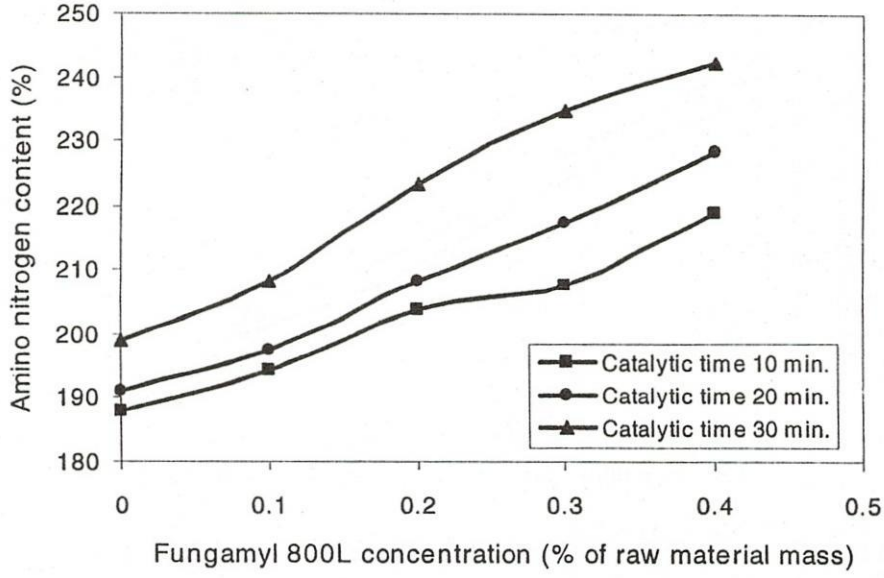

Figure 4: Influence of Fungamyl 800L on amino nitrogen content in the wort from 60% malt and 40% sweet potato

3. 3. Malt and sweet potato mashing with commercial Ceremix 2XL

15 mashes with different concentrations of Ceremix 2XL and its catalytic times were realized. The optimal temperature of Ceremix 2XL was 50°C. The experimental plan is showed on table 3.

The results are given in figures 5, 6, 7 and 8. With alpha-amylase (80 Kilo-Novo units/g), beta-glucanase (300 units/g) and protease activities (0.33 Anson units/g), Ceremix 2XL could hydrolyze starch and other macromolecule substrates such as protein and beta-glucane. The use of Ceremix 2XL decreased the notably the saccharification time (figure 5). In addition, the extraction yield and the concentrations of sugar and free amino nitrogen augmented (figure 6, 7 and 8). With the catalytic time of 30 min., increase in Ceremix 2XL concentration from 0% to 0.4% of brewing material mass (Table 3) augmented the extraction yield from 72.39% to 76.13% (Figure 6), the saccharification time reduced from 15 min. to 2 min. (Figure 5). Moreover, this microbial enzyme ameliorated the physio–chemical characteristics of the wort. The reducing sugar and amino nitrogen contents increased to 95g/L and 272mg/L respectively (figures 7 and 8). These values were similar to those of the 100% malt wort (Table 1, mash N°1).

Table 3: Malt-sweet potato mashing with commercial Ceremix 2XL

Catalytic time: 10 min		Catalytic time: 20 min		Catalytic time: 30 min	
Mash N^0	Ceremix content, % of total materials	Mash N^0	Ceremix content, % of total materials	Mash N^0	Ceremix content, % of total materials
18	0.0	23	0	28	0.0
19	0.1	24	0.1	29	0.1
20	0.2	25	0.2	30	0.2
21	0.3	26	0.3	31	0.3
22	0.4	27	0.4	32	0.4

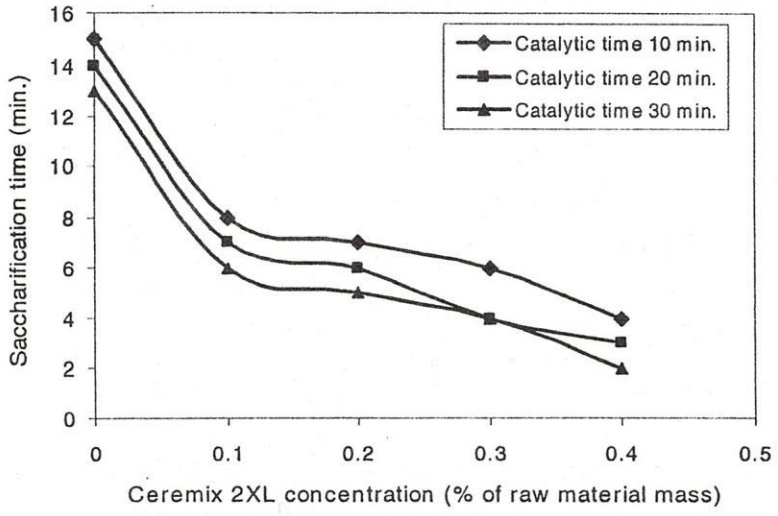

Figure 5. Influence of Ceremix 2XL on saccharification time in 60% malt – 40% sweet potato mashing

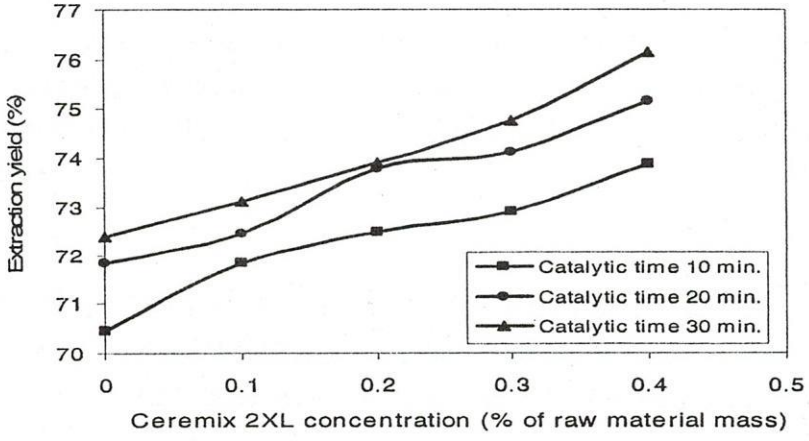

Figure 6. Influence of Ceremix 2XL on extraction yield in 60% malt – 40% sweet potato mashing

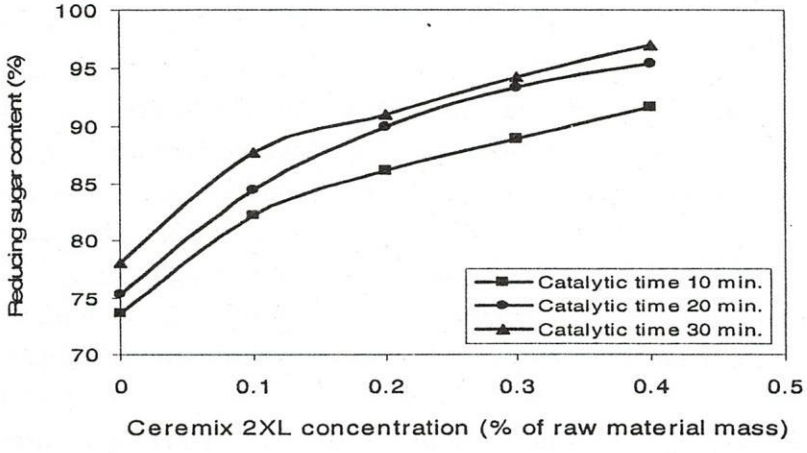

Figure 7. Influence of Ceremix 2XL on reducing sugar content in the wort from 60% malt and 40% sweet potato

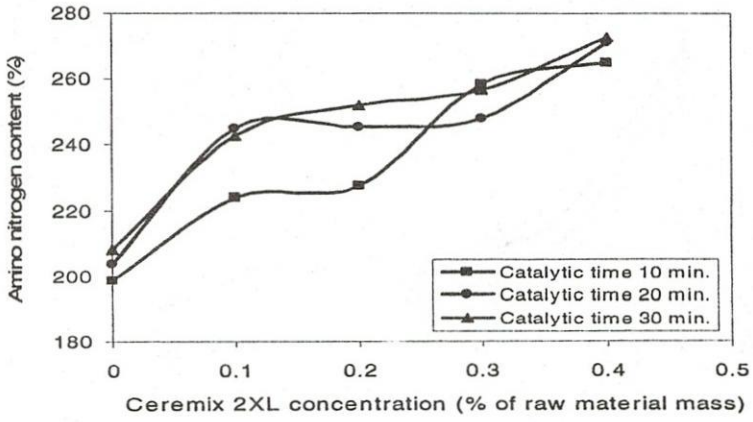

Figure 8. Influence of Ceremix 2XL on amino nitrogen content in the wort from 60% malt and 40% sweet potato

4. CONCLUSION

In 60% malt - 40% sweet potato mashing process, the use of microbial amylase decreased the saccharification time and augmented the extraction yield, the sugar and free amino nitrogen conentrations in the obtained wort. However, the use of enzyme complex with amylase, protease and beta-glucanase activities gave a better effect on the technological parameters of the mashing process and the physio-chemical characteristics of the obtained wort.

NGHIÊN CỨU ỨNG DỤNG CHẾ PHẨM ENZYME VI SINH VẬT (FUNGAMYL 800L VÀ CEREMIX 2XL) TRONG QUÁ TRÌNH NẤU DỊCH NHA TỪ HỖN HỢP MALT VÀ KHOAI LANG

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TÓM TẮT: *Khoai lang là một thế liệu triển vọng trong sản xuất bia do chứa nhiều đường và tinh bột. Tuy nhiên, trong quá trình nấu dịch nha từ 60% malt và 40% khoai lang, độ trích ly chất chiết từ nguyên liệu, hàm lượng đường khử và nitơ amin trong dịch nha thu được thấp hơn nhiều so với các giá trị tương ứng trong quá trình nấu dịch nha từ 100% malt đại mạch. Trong nghiên cứu này, các chế phẩm Fungamyl 800L (với hoạt tính beta-amilase) và Ceremix 2XL (với hoạt tính alpha-amilase, protease và beta-glucanase) lần lượt được bổ sung vào nồi nấu. Khi đó, độ trích ly tăng và thời gian đường hóa giảm xuống. Hàm lượng đường khử và nitơ amin trong dịch nha thu được cũng tăng lên. Chế phẩm Ceremix 2XL cho kết quả tốt hơn chế phẩm Fungamyl 800L vì nó có thể xúc tác thủy phân nhiều loại cơ chất khác nhau trong cấu trúc mô thực vật. Các chỉ tiêu hóa lý của dịch nha được nấu từ 60% malt và 40% khoai lang (có sử dụng chế phẩm Ceremix 2XL với hàm lượng 0,4% so với khối lượng nguyên liệu) và của dịch nha được nấu từ 100% malt đại mạch là tương đương nhau.*

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