EFFECT OF UTILIZATION OF MICROBIAL ENZYME COMPLEX IN MASHING PROCESS FROM MALT AND SWEET POTATO

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***ABSTRACT**: Utilization of 40% sweet potato in brewing increased mashing time and decreased the wort quality. The use of microbial enzymes for acceleration of mashing process and improvement in wort quality was therefore essential. Commercial Neutrase 0.5L with endoprotease activity (from Novo Nordisk Fermented Ltd.) was added to the 60% malt-40% sweet potato mash. The higher the enzyme content and the longer the catalytic time, the higher the extraction yield and the higher the amino nitrogen content in the obtained wort. However, the concentrations of sugar and amino nitrogen in the malt-sweet potato wort were lower than those in the 100% malt wort. The use of enzyme complex: Termamyl 120L with alpha-amylase activity (from Novo Nordisk Fermented Ltd.) and Neutrase 0.5L gave a better effect. The extraction yield of the 60% malt-40% sweet potato mash with Termamyl 120L and Neutrase 0.5L was higher than that of 100% malt mash. The physio-chemical characteristics of the two obtained worts were similar.*

1. INTRODUCTION

In brewing, adjunct is defined as any carbohydrate source other than barley malt which provides sugars to wort. The reasons for adjunct use in brewing are many and varied: decreasing the product cost, improving beer stability by diluting non-starch constituents which can contribute to hazes, modifying the carbohydarte spectrum of the wort and controlling fermentability, modifying flavour profiles... Nowadays, adjunct utilization has become a popular phenomenon in world brewing industry (Lloyd W. J. W., 1986, Le Van Viet Man et.al., 2001).

Brewing adjuncts are classified according to their major ingredient as delivered to the brewery: starch-rich and sugar-rich adjuncts. In Vietnam, starch-rich adjunct such as rice has been considered as a traditional adjunct in brewing. However, our recent studies showed that sweet potato has been a perspective raw matrerial with high sugar and starch contents, low cost (Ton Nu Minh Nguyet et al., 2003). From the technological point of view, the structure of starch granules in sweet-potato endosperm is more porous than that in rice endosperm, so the starch saccharification can be carried out more rapidly and easily (Hudson J.R., 1984; Kalunhans K.A., 1994).

The ratio of malt and adjunct used in brewing varies from country to country, from brewery to brewery and among individual beers in a single brewery. However, many researches showed that high adjunct ratio could decrease the amino nitrogen content in the wort. This may affect negatively to the fermentation performances and the beer quality (Lloyd W. J. W., 1986; Le Van Viet Man et.al., 2001; Ton Nu M. N. et.al., 2003). In Vietnam, adjunct ratio used in brewing varies from 10 to 40%.

In this research, the adjunct ratio was fixed (40% of brewing material mass). This paper focussed on the effect of the application of microbial enzyme complex in mashing process from 60% malt and 40% sweet potato. Different microbial enzymes were added to

the mash for wort quality improvement. The exoenzymes were used in both single and mixture types.

2. MATERIALS AND METHODS

Materials

- Malt: malt was supplied by an Australian company (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: Termamyl 120L with alpha-amylase activity and Neutrase 0.5L with protease activity (from Novo Nordisk Fermented Ltd., Denmark) were used in mashing process from 60% malt and 40% sweet potato. The Termamyl 120L and Neutrase 0.5L doses used in this reasearch were calculated by percent of adjunct mass and percent of total raw material mass respectively.
- Hop (granule type) was supplied by an Australian company (Moisture: 12%, alpha-acid content: 12% m/m).

Mashing process was carried out from 60% malt and 40% sweet-potato by decoction method.

Adjunct mash: 2 cases

- All sweet potato, 15% malt and water were heated to 72°C and maintained at this temperature for 15 minutes. Then, the mash was heated to 100°C and boiled for 15 minutes or
- All sweet potato, commercial Termamyl 120L (if use) and water were heated to 90°C and maintained at this temperature for 15 min. Then, the mash was heated to 100°C and boiled for 15 minutes.

Malt mash:

Malt, water and commercial Neutrase 0.5L (if use) were heated to 50°C. The proteolysis time was varied from 15 to 30 minutes according each experiment (cited in the results).

The adjunct mash and malt mash were mixed together and the temperature was adjusted to 63°C and maintained at this temperature for 15 minutes. The mixture was then heated to 72°C for total saccharification. The filtered wort and hop were boiled at 100°C for 1 hour. After boiling, the wort was centrifuged, adjusted to 11°Pt specific gravity and used for determination of extraction yield, reducing sugar and amino nitrogen contents.

A control sample was carried out from 100% malt by decoction method: the boiling mash with 4 stands (50, 63, 72 and 100°C) and the non-boiling mash with 3 stands (50, 63 and 72°C).

All mashes were realized in duplicate.

Analytical methods

- Saccharification time was determined by iodine test (Analytica EBC, 1987).
- Reducing sugar content was quantified by spectrophotometric method, using DNS reagent (3,5-DiNitroSalysilic acid), (Helrich K., 1992).
- Free amino nitrogen content was determined by spectrophotometric method, using ninhydrin reagent (Analytica EBC, 1987).

3. RESULTS

3.1. Application of commercial Neutrase 0.5L with protease activity in 60% malt and 40% sweet-potato mashing

In this experiment, six mashes were realized. The first mash from 100% malt was the control sample (No exogenous enzyme was used). The other five mashes were prepared from 60% malt and 40% sweet-potato. Commercial Neutrase 0.5L with different contents - 0, 0.05, 0.10, 0.15 and 0.20% of brewing raw material mass - was alternatively added to the five mashes. The proteolytic stand of all six mashes was 15 minutes. The saccharification time and extraction yield of each mash are given in table 1.

Table 1. Saccharification times and extraction yield of the six mashes

Mash N°	Raw materials	Neutrase 0.5L content, (%)	Saccharification time, (min)	Extraction yield, (%)
1	100% malt (control)	0	3	75.49
2	60% malt – 40% sweet potato	0	14	69.12
3	60% malt – 40% sweet potato	0.05	8	71.50
4	60% malt – 40% sweet potato	0.10	7	75.52
5	60% malt – 40% sweet potato	0.15	6	76.53
6	60% malt – 40% sweet potato	0.20	4	77.89

Our obtained results showed that if the sweet potato ratio used in brewing was 40%, the saccharification time and the extraction yield decreased (Table 1, mash N° 1 and 2) . It is due to low enzyme activity in the adjunct. Addition of microbial protease Neutrase 0.5L to the malt mash ameliorated significantly the mashing process. The higher the exogenous protease content, the lower the saccharification time and the higher the extraction yield.

If the Neutrase 0.5L content was 0.10% of brewing material mass, the obtained extraction yield (75.52%) was similar to that of the control mash from 100% malt (75.49%). Higher content of microbial protease gave better result on extraction yield.

In brewing, saccharification time is determined as the catalytic time at 72°C for malt alpha-amylase in the malt mash. For determining saccharification time, iodine test has been used (Analytica EBC, 1987). It should be affirmed that the saccharification time depends principally to the amylase activity in the mash. However, our results showed that utilization of microbial protease decreased notably the saccharification time. But the saccharification time of the malt-adjunct mash was always higher than that of 100% malt mash.

The reducing sugar and free amino nitrogen contents in the 100% malt wort were 94.5g/L and 285.7mg/L respectively. These physio-chemical characteristics of the malt-adjunct worts are visualized in figures 1 and 2. The higher the microbial protease added to the malt mash, the higher the reducing sugar and free amino nitrogen contents in the obtained wort.

It is reported that cereal endosperm includes many starch granules. Each starch granule includes many amylose and amylopectin molecules. Every starch granule has an exocell that contains protein and cellulose. The linkage between the starch granules in the endosperm is maintained by protein, pectin and hemicellulose (Kalunhans K.A. et al.,

1992; Kalunhans K.A. 1994). High degree of proteolysis damages completely the linkage between the strach granules and the exocell of each granule in the endosperm. This leads to total liberation of starch molecules. Therefore, malt amylases contact more easily with their substrates. That is the reason for augmentation of reducing sugar content in the obtained wort. The similar results were also obtained in the research focussed on the application of barley adjunct and microbial enzymes in brewing (Kalunhans K.A. 1994).

It can be concluded that utilization of exogenous protease activated the malt amylase activity and increased the reducing sugar content in malt – adjunct wort.

However, in all cases, the reducing sugar and free amino nitrogen contents in the 60% malt and 40% sweet potato wort were much lower than those in the 100% malt wort. To improve the physio-chemical characteristics of malt-adjunct wort, using a complex of microbial enzymes is therefore essential.

3.2. Application of commercial Neutrase 0.5L with protease activity and Termamyl 120L with alpha amylase activity in 60% malt and 40% sweet-potato mashing

In the next experiment, five mashes were conducted. Commercial Termamyl 120L was added to all adjunct mashes (0.2% of adjunct mass). Neutrase 0.5L with different contents - 0, 0.05, 0.10, 0.15 and 0.20% of total raw material mass - was alternatively added to the five malt mashes. The proteolytic stand was fixed for 15 minutes. Table 2 presents the saccharification time and the extraction yield of the five mashes.

Table 2. Saccharification times and extraction yield of the five mashes from 60% malt and 40% sweet-potato

Mash N°	Termamyl 120L content, (%)	Neutrase 0.5L content, (%)	Saccharification time, (min)	Extraction yield, (%)
7	0.2	0	14	69.12
8	0.2	0.05	2	71.50
9	0.2	0.10	2	74.90
10	0.2	0.15	2	80.87
11	0.2	0.20	2	80.90

It is shown that utilization of complex: Termamyl 120L with alpha-amylase activity and Neutrase 0.5L with protease activity increased the extraction yield and decreased significantly the shaccarification time. The saccharification time in 60% malt and 40% adjunct mashes with enzyme complex was only 2 minutes – shorter than that in 100% malt mash (3 minutes, table 1).

Figures 3 and 4 show the reducing sugar and free amino nitrogen contents in the five mashes.

Increase in Neutrase 0.5L content augmented notably both reducing sugar and free amino nitrogen contents in the wort. It is reported that maltose is a principal reducing sugar in wort (Moll M., 1991). Both alpha-amylase and protease do not catalyze the hydrolytic reactions for maltose formation. Perhapes, the catalysis of alpha-amylase and protease on amylose, amylopectin and protein liberated the substrates for malt beta-amylse and

facilitates the contact between the beta-amylase and its substrate. Therefore, the reducing sugar content increased.

It can be noted that addition of enzyme complex (amylase and protease) to the mash gave a better effect than addition of single enzyme.

With the contents 0.2% for both Termamyl 120L and Neutrase 0.5L, the reducing sugar and free amino nitrogen concentrations in the wort were 92.4g/L and 257mg/L respectively. In comparaison with the 100% malt wort, the sugar contents were similar (92.4g/L and 94.5g/L). However, the free amino nitrogen content in the malt-adjunct wort was quite lower (257mg/L and 285.7mg/L). To improve this characteristics, increase in proteolytic yield is essential.

In the last experiment, 4 mashes were realized with addition of Termamyl 120L (0.2%) and Neutrase 0.5L (0.2%). The proteolytic stand varied from 15 to 30 minutes. The saccharification time and extraction yield are presented on table 3.

Table 3 shows that prolongation of the proteolytic stand did not affect notably to the saccharification time and extraction yield.

Table 3. Saccharification times and extraction yield of the four mashes from 60% malt and 40% sweet-potato (Using Termamyl 120L – 0.2% and Neutrase 0.5L – 0.2%)

Mash N°	Time of proteolytic stand (minutes)	Saccharification time, (min)	Extraction yield, (%)
12	15	2	80.9
13	20	2	80.9
14	25	2	81.3
15	30	2	81.3

Figures 5 and 6 show that increase in proteolysis time augmented slightly the reducing sugar concentration but improved significantly the free amino nitrogen content in the wort. If the proteolysis time was 30 minutes, the amino nitrogen content in the malt-adjunct wort was 280.1mg/L, nearly equivalent to that in the 100% malt wort (285.7mg/L).

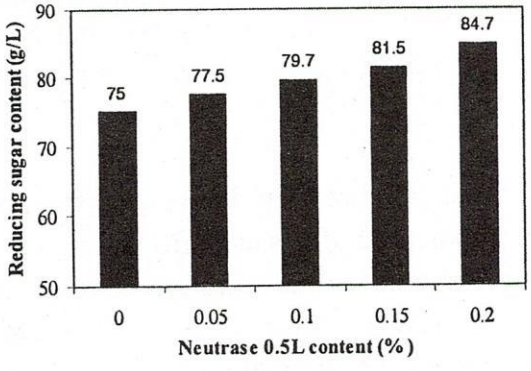

Fig 1. Influence of commercial Neutrase 0.5L content with protease activity on the reducing sugar concentration in 60% malt – 40% sweet potato wort

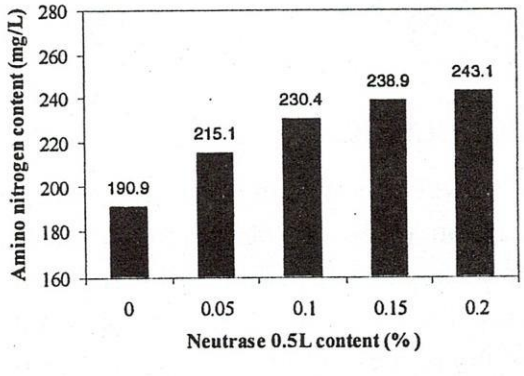

Fig 2. Influence of commercial Neutrase 0.5L content with protease activity on the free amino nitrogen concentration in 60% malt – 40% sweet potato wort

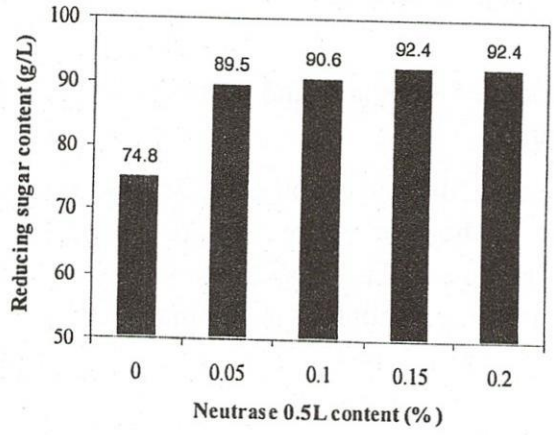

Fig 3. Influence of commercial Neutrase 0.5L content on the reducing sugar content in 60% malt – 40% sweet potato wort (Using Termamyl 120L: 0.2% of adjunct mass

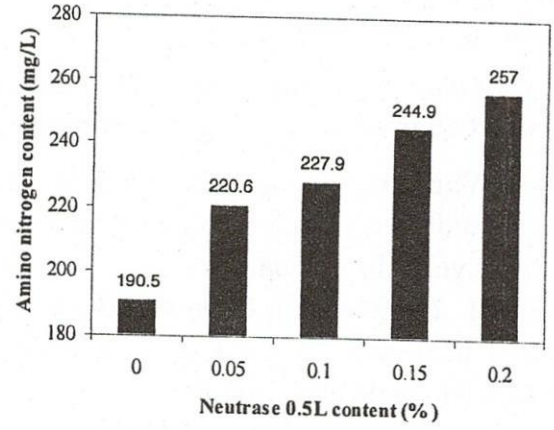

Fig 4. Influence of commercial Neutrase 0.5L content on the free amino nitrogen content in 60% malt – 40% sweet potato wort (Using Termamyl 120L: 0.2% of adjunct mass)

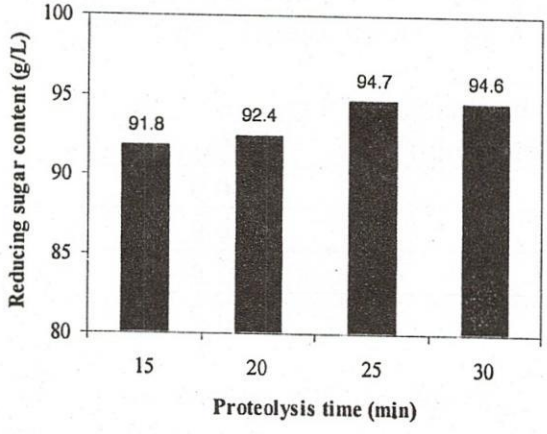

Fig 5. Influence of proteolysis time on the reducing sugar content in 60% malt – 40% sweet potato wort (Using Termamyl 120L: 0.2% of adjunct mass and Neutrase 0.5L: 0.2% of brewing raw material mass)

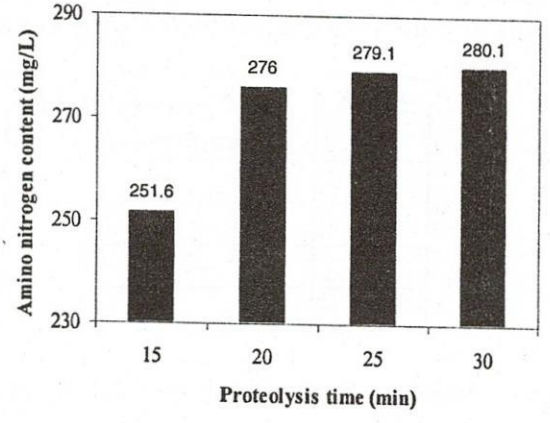

Fig 6. Influence of proteolysis time on the free amino nictogen content in 60% malt –40% sweet potato wort (Using Termamyl 120L: 0.2% of adjunct mass and Neutrase 0.5L: 0.2% of brewing raw material mass)

4. CONCLUSION

Sweet potato can be considered as a new adjunct in brewing. However, sweet potato utilization (40% of total raw material mass) augmented the saccharification time and decreased the extraction yield, reducing sugar and free amino nitrogen contents in the obtained wort. Addition of microbial enzymes to the malt-adjunct mash accelerated the mashing process and improved the wort quality. Use of commercial enzyme complex gave a better effect than use of single enzyme. The extraction yield of the 60% malt-40% sweet potato mash with Termamyl 120L (0.2% of adjunct mass) and Neutrase 0.5L (0.2% of total raw material mass) was higher than that of 100% malt mash. The physio-chemical characteristics of the two obtained worts were similar.

HIỆU QUẢ VIỆC SỬ DỤNG TỔ HỢP CÁC CHẾ PHẨM ENZYME VI SINH VẬT TRONG QUÁ TRÌNH NẤU DỊCH NHA TỪ MALT VÀ KHOAI LANG

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TÓM TẮT: *Sử dụng thế liệu khoai lang với tỉ lệ 40% trong sản xuất bia sẽ kéo dài thời gian quá trình nấu và làm giảm chất lượng dịch nha thu được. Do đó, việc bổ sung các chế phẩm enzyme vào mẻ nấu có sử dụng thế liệu với tỉ lệ cao là rất cần thiết. Trong nghiên cứu này, chúng tôi đã sử dụng chế phẩm Neutrase 0.5L với hoạt tính endoprotease (hãng Novo Nordisk) trong mẻ nấu dịch nha từ 60% malt và 40% khoai lang. Khi tăng hàm lượng chế phẩm sử dụng và thời gian xúc tác, hiệu suất trích ly và hàm lượng nitơ amin trong dịch nha thu được tăng theo. Tuy nhiên, nồng độ đường khử và nitơ amin của dịch nha nấu từ hỗn hợp malt và khoai lang luôn thấp hơn mẫu đối chứng được nấu từ 100% malt đại mạch. Việc sử dụng tổ hợp chế phẩm enzyme: Termamyl 120L với hoạt tính alpha-amylase (hãng Novo Nordisk) và Neutrase 0.5L cho kết quả tốt hơn. Hiệu suất trích ly của quá trình nấu dịch nha từ 60% malt và 40% khoai lang được bổ sung đồng thời hai chế phẩm Termamyl 120L và Neutrase 0.5L sẽ cao hơn mẫu đối chứng được nấu từ 100% malt đại mạch. Khi đó, các chỉ tiêu hóa lý của hai mẫu dịch nha thu được là tương đương nhau.*

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