APPICATION OF COMMERCIAL ENZYMES FOR JICAMA PULP TREATMENT IN JUICE PRODUCTION

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ABSTRACT: *Jicama or Yam bean (Pachyrhizus erosus) tuber is a popular tropical legume in Viet Nam. It contains glucids, different amino acids, vitamins and minerals. Until present, jicama has not been used as a principal raw material in food industry in our country. Enzymatic treatment of raw material is a well-known technique for increasing the extraction yield in fruit and vegetable processing. The aim of this work is to investigate effects of enzymatic treatment of jicama pulp on the extraction yield, and sugar content in the jicama juice production. The highest extraction yield was 92,7% when jicama pulp was treated with the mixture of Pectinex Ultra SP (pectinase), Celluclast 1.5L (cellulase) and Termamyl 120L (alpha amylase). In this case, the total and reducing sugar contents in the jicama juice were 95.79g/L and 57.38g/L, respectively. Jicama juice could be used as a soft-drink or a medium for fermented beverage and microbial metabolite production.*
Key words: *amylase, cellulase, hemicellulase, pectinase, Pachyrhizus erosus, enzymatic treatment*

1. INTRODUCTION

The jicama or yam bean (*Pachyrhyzus erosus L.*) tuber is a leguminous root which is produced in many tropical and subtropical areas. Jicama has a good composition balance between carbohydrates and protein (Mercado-Silva et al.,1998). Yam bean tuber contains sugar and most essential amino acids. It also contains different vitamins such as niacin, riboflavin, thiamine, and minerals such as magnesium and sodium (Abud-Archila, 2007).

Jicama juice can be produced from jicama. This juice can be used as a soft-drink or a medium for fermented beverage or microbial metabolite production. In our country, jicama has been used in preparation of different foods at home scale. Until present, jicama has not been used as a principal raw material in food industry.

In juice production, application of commercial enzymes is considered as a well-known technique for facilitating pulp pressing, increasing juice extraction and improving juice clarification (Kashyap et al., 2001).

In this paper, jicama was used as a main raw material for juice processing. The objective of this research focused on the application of different commercial enzymes in jicama pulp treatment for increasing the extraction yield.

2. MATERIAL AND METHOD

2.1 Material

2.1.1 Jicama

Jicama (*Pachyrhyzus erosus*) was purchased from Tan Xuan market in Ho Chi Minh City. This material originated from Mekong delta was harvested during the spring 2008. The principal chemical composition of jicama used in this study was as follows: water 90.5%, starch 2.5%, total reducing sugar 4.7%, pectin 0.58%, cellulose 0.41%, ash 0.38% w/w.

2.1.2 Commercial enzymes

Pectinex Ultra SP (pectinase), Celluclast 1.5L (cellulase), Viscozyme (endo-1,3/1,4 beta glucanase), Termamyl 120L (alpha amylase), and AMG 300L (glucoamylase) used in this research were originated from Novo Nordisk Ltd. (Denmark). The optimal temperature and pH of these commercial enzymes are presented in Table 1.

2.1.3. Chemicals

All chemicals used in this study were originated from Merck (EU).

2.2 Enzyme treatment

The procedure for jicama juice processing in this study was as follows: Jicama → Washing with water → Peeling → Cutting into slices (2*2cm) → Pulping → Adjusting pH and temperature → Supplementing commercial enzymes → Enzymatic treatment → Pressing → Filtration → Jicama juice.

Table 1. Temperature and pH optima of the commercial enzymes

Enzyme	Temperature (°C)	pH
Pectinex Ultra SP	50 °C	4,5
Celluclast 1.5L	50 °C	4,5
Viscozyme	50 °C	4,5
AMG 300L	60 °C	4,5
Termamyl 120L	90 °C	6,2

In each experiment, jicama pulp was treated by one commercial enzyme or a mixture of two or three different enzymes. The enzymatic treatment was carried out at suitable pH and temperature according to each experiment. At the end of enzymatic treatment, the enzymes were inactivated by heating the sample in a water bath. After pressing and filtration, the obtained jicama juice was used for further analysis.

2.3 Analytical methods

- Moisture and dry matter in jicama was determined by drying method (Bradley, 2003)
- Soluble solid was measured by a refractometer and expressed in °Brix (Bradley, 2003).
- Total sugar was quantified by spectrophotometric method using phenol-acid sulfuric reagent (BeMiller, 2003).
- Reducing sugar was determined by spectrophotometric method using 3.5 dinitrosalisylic acid reagent (BeMiller, 2003).
- The extraction yield E (% w/w) was calculated by the following formula: $E = S_1/S_2$; where: S_1: content of soluble solid in the obtained juice (g), S_2: content of dry matter in the jicama sample used in the experiment (g)

2.4. Statistical treatment

The presented results were the average of three independent experiments. The obtained results were subjected to analysis of variance (ANOVA), $p<0.05$ using Statgraphics plus, version 3.2.

3. RESULTS AND DISCUSSION

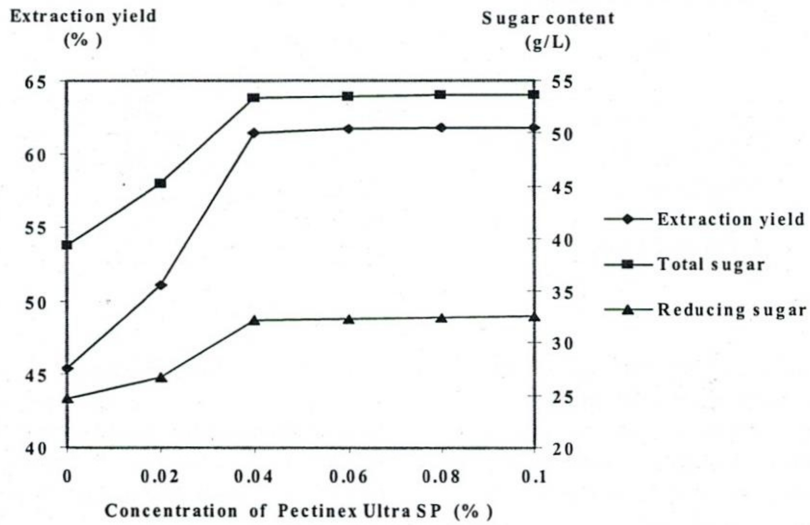
Cell wall of plant contains different polysaccharides such as pectin, cellulose and hemicellulose (Horvaùth – Kerbai, 2006). In addition, jicama contains starch. In this research, pectinase, cellulase, hemicellulase and amylase were used in jicama juice processing for increasing the extraction yield.

3.1. Effect of each commercial enzyme on jicama pulp treatment

Firstly, the influence of enzyme concentration on the extraction yield and sugar content in the jicama juice was examined. Various concentrations of different commercial enzyme were used for jicama pulp treatment. The pH and temperature of jicama pulp were adjusted to the optimal values of each commercial enzyme. The treatment time was fixed for 60min. From the obtained results, suitable concentration of each commercial enzyme was determined and used for the next step. Secondly, treatment time of jicama pulp was varied and optimized.

3.1.1. Application of Pectinex Ultra SP (pectinase)

Pectin is an essential structural component of fruit and vegetable, where in combination with hemi-cellulose it binds single cells to form the plant tissues. Enzymatic treatment is required to break down the pectin and to enable precipitation or sedimentation of the resulting pectic substances (Taylor, 2005). The effect of Pectinex Ultra SP on jicama pulp treatment was presented in Figure 1.



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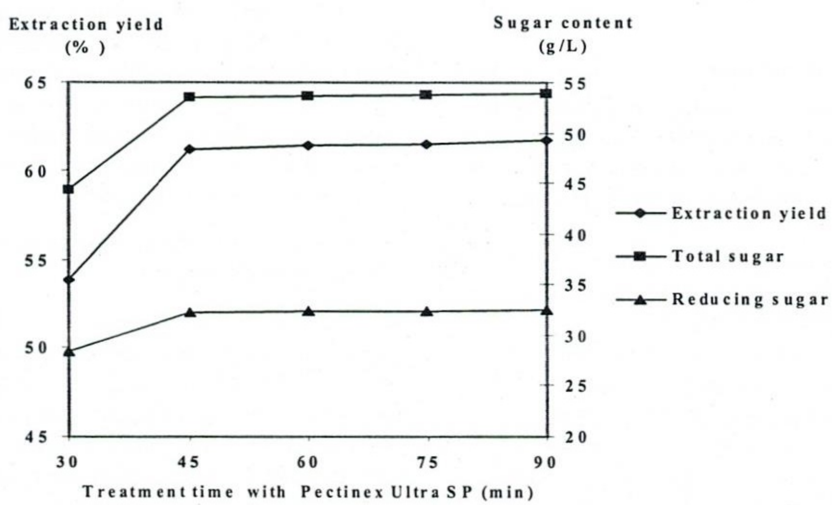

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Figure 1. Effect of Pectinex Ultra SP on extraction yield and sugar content in jicama juice

From Figure 1a, it can be noted that in the control sample, the extraction yield, total and reducing sugar content were 45.4% w/w, 39.3g/L and 24.7g/L, respectively. On the contrary, a remarkable increase in extraction yield and sugar content in the jicama juice when Pectinex Ultra SP was used in the treatment. According to Demir et al., (2001), partial or complete disintegration of pectin in the plant tissues increased significantly the extraction yield and soluble solid content in the fruit juice.

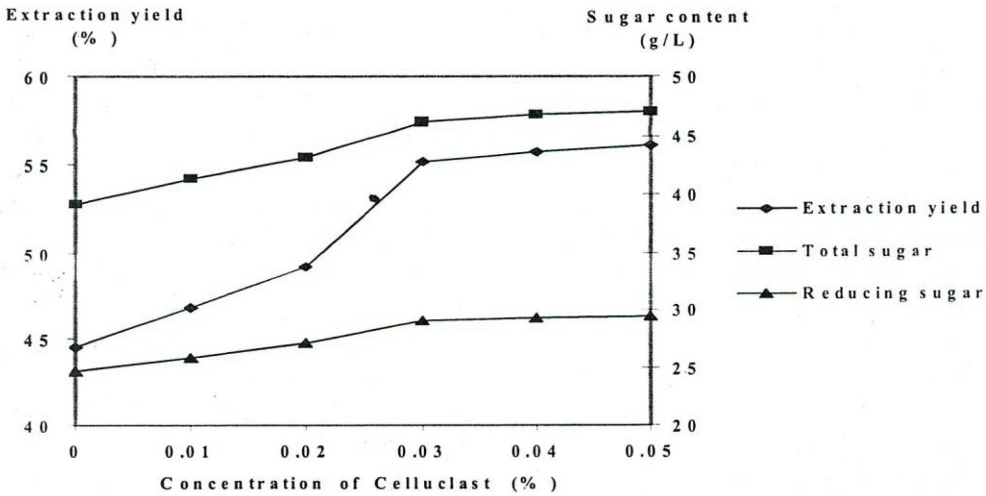
When the content of Pectinex Ultra SP used in jicama pulp treatment increased from 0 to 0.04% w/w, the extraction yield augmented sharply from 45.4% w/w to 61.5%. In addition, the total and reducing sugar contents increased from 39.3g/L to 53.8g/L and 24.7g/L to 32.2g/L, respectively. However, increase in concentration of commercial pectinase from 0.04 to 0.10% did not augment the extraction yield and sugar content in the obtained juice. So, the suitable content of Pectinex Ultra SP used in jicama processing was 0.04% w/w.

Similar phenomenon was observed by Kashyap et al (2001) in apple juice processing. Pectic enzymes were used in apple pulp treatment for facilitating pressing and juice extraction. Without pectinase treatment, the slimy pectin particles became saturated with juice which was then difficult to extract from the pulp. They blocked drainage channels in the pulp through which the juice passed. The application of pectinase increased notably the extraction yield in apple juice processing.

The results in figure 1b show that the appropriate time of jicama pulp treatment with Pectinex Ultra SP was 45min. The extraction yield was 61.2% w/w. If the treatment time augmented from 45 to 90 min., the extraction yield and sugar content in jicama juice increased insignificantly. Similarly, the degradation rate of carrot mash by a commercial pectinase was found to depend on the time of incubation. The most conspicuous effect was the very rapid degradation in the first 20 min.; after 20 min. incubation, a maximum was reached and remained unchanged on further incubation. This effect can be explained by accumulation of an enzymatically resistant core and limitation of the substrate (Demir et al., 2001).

3.1.2 Application of Celluclast 1.5L (cellulase) and Viscozyme (endo-1,3/1,4 beta glucanase)

Cellulose and hemicellulose are the major components of cell wall. Different nutrients such as sugar, amino acids, vitamins... are found inside the cell wall of fruit and vegetable. Cellulase and hemicellulase can be used to disintegrate plant cell wall in juice processing (Taylor, 2005).



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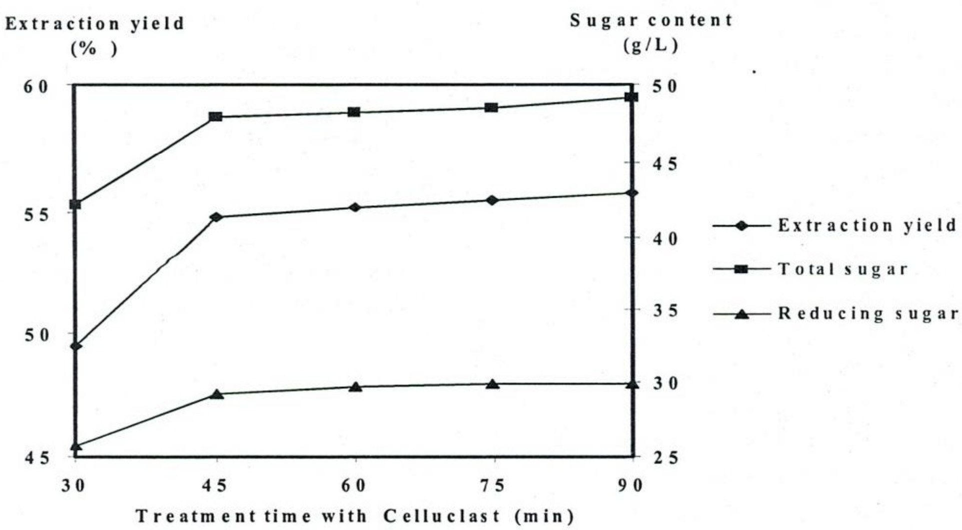

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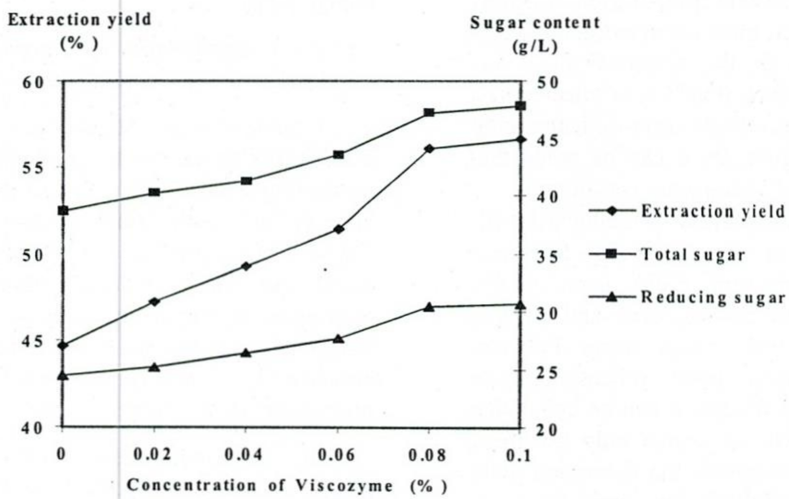
Figure 2. Effect of Celluclast 1.5L on extraction yield and sugar content in jicama juice



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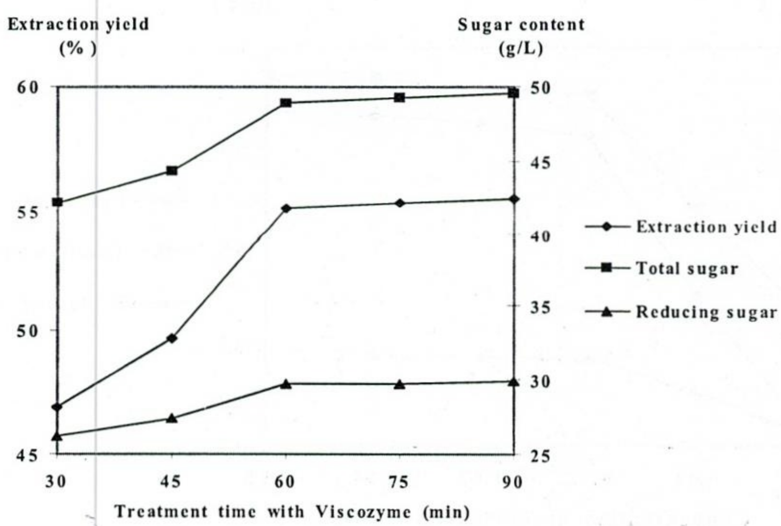

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Figure 3. Effect of Viscozyme on extraction yield and sugar content in jicama juice

The effect of Celluclast 1.5L and Viscozyme on jicama pulp treatment is presented in Figures 2 and 3. Figure 2a shows that increase in Celluclast 1.5L concentration from 0 to 0.03% w/w augmented significantly the extraction yield and sugar content in jicama juice. However, when the enzyme content increased from 0.03 to 0.05% w/w, the extraction yield augmented only from 55.2% to 56.3%. So the appropriate content of Celluclast 1.5L in jicama pulp treatment was 0.03% w/w. From Figure 2b, the treatment time of Celluclast 1.5L was 45min. The extraction yield was 54.8% w/w.

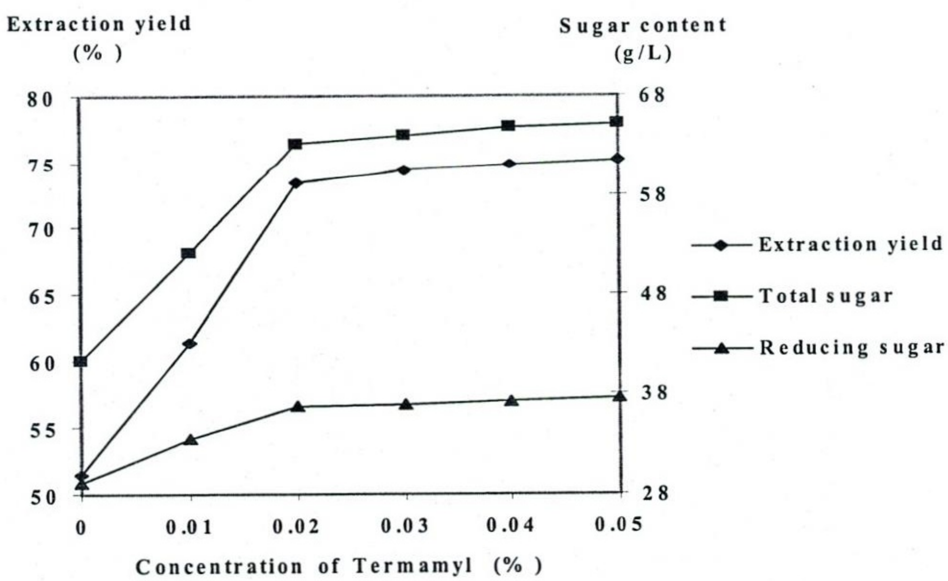
Figure 3a indicates that when Viscozyme concentration was 0.08%, the extraction yield was 56.1%. If the enzyme content used in jicama treatment was higher, increase in extraction yield and sugar content in the obtained juice was insignificant. Therefore, 0.08% was selected as a suitable Viscozyme concentration in jicama pulp treatment. From figure 3b, it can be noted that the treatment time of Viscozyme was 60min.

In summary, application of Celluclast 1.5L and Viscozyme in jicama pulp treatment increased the extraction yield from 45.4% (control sample) to 54.8% w/w and 56.1%, respectively. However, when using Pectinex Ultra SP in jicama juice processing, the extraction yield was 61.2%. It can be concluded that pectin hydrolysis in jicama pulp treatment augmented more effectively the extraction yield than hydrolysis of cellulose and hemicellulose in the jicama tissues. This result is in good agreement with Sreenath et al. (1995) who carried out the enzymatic treatment of some varieties of mango pulp.

3.1.3 Application of Termamyl 120L (α-amylase) and AMG 300L (glucoamylase)

Jicama contains starch (Amya-Llano et al., 2008). This starch can be gelatinized during juice processing and can give rise to precipitation and haze effects in the final product. According to Taylor (2005), amylase can be used to break down starch and overcome such problem. In addition, hydrolysis of starch increases sugar content and improves sweet flavour of the juice. Until present, amylases have widely been used in apple juice processing. (Kashyap et al., 2001).

In this experiment, alpha amylase and glucoamylase were used in jicama pulp treatment. The results are given in Figures 4 and 5.



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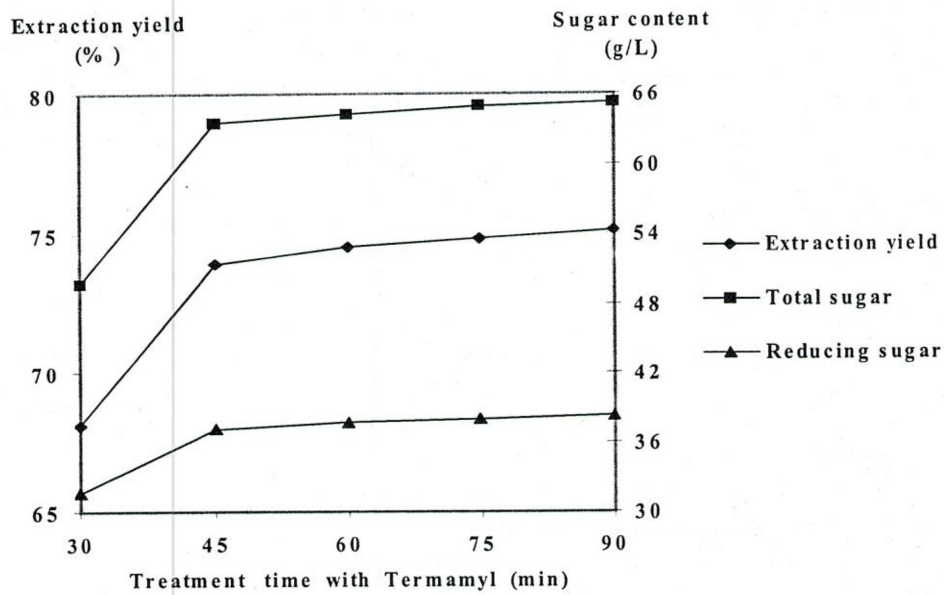

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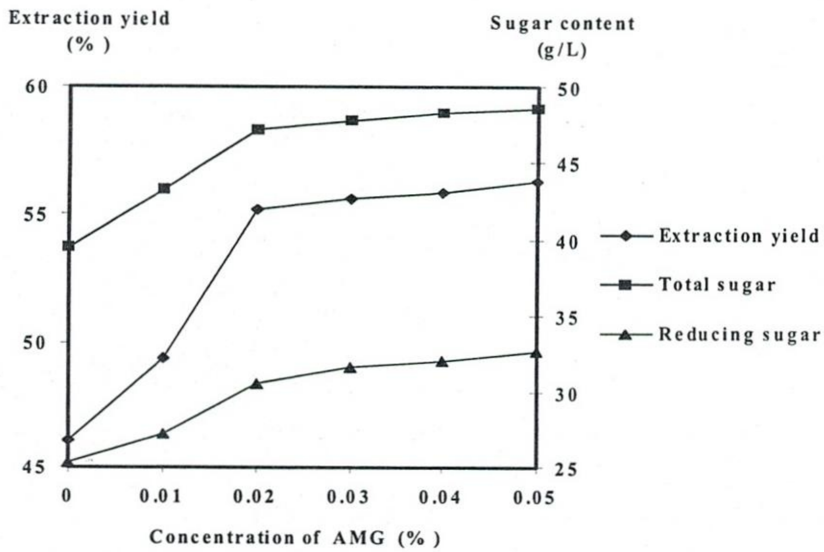
Figure 4. Effect of Termamyl 120L on extraction yield and sugar content in jicama juice

When concentration of Termamyl 120L augmented from 0 to 0.02%, the extraction yield and sugar content in the obtained juice increased significantly. In this sample, jicama pulp was treated at 90°C (optimum temperature of Termamyl 120L). During this process, the pulp was heated to dissolve the starch granules and release the amylose and amylopectin molecules. The starch granules were extensively fragmented so that alpha amylase easily liquefied it into soluble and short chain dextrins (Synowiecki, 2007; Van der Maarel et al., 2002). However, increase in concentration of Termamyl 120L from 0.02-0.10%, the extraction yield rose moderately. From Figure 4b, the suitable time for pulp treatment was 45min. The extraction yield, total and reducing sugar content were 73.5% w/w, 63.5g/L and 37.2g/L, respectively.



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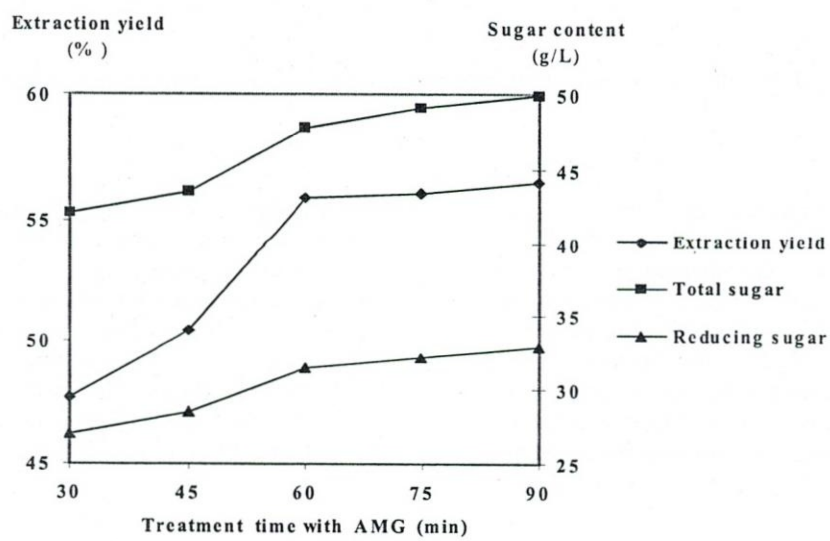

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Figure 5. Effect of AMG on extraction yield and sugar content in jicama juice

Similar phenomenon was also observed for AMG 300L in Figure 5a. However, the extraction yield and sugar content in jicama juice when using AMG 300L were much lower than those when using Termamyl 120L. It was due to an incomplete liquefaction of starch at 60°C – optimum temperature of AMG 300L. This led to a partial saccharification of starch and the sugar content in the obtained juice was lower. The appropriate content of AMG 300 used in the treatment was 0.02% w/w. From Figure 5b, 60min. was selected as suitable treatment time of AMG 300L. In this case, the extraction yield, total and reducing sugar content were 55.9% w/w, 47.8g/L and 31.6g/L, respectively.

In conclusion, using alpha amylase gave the highest extraction yield and sugar content in the jicama juice. Liquefaction of starch was an important step in jicama pulp treatment. In the next experiment, alpha amylase was used with pectinase or cellulase or hemicellulase for improving the extraction yield in jicama juice processing: Termamyl 120L liquefied starch; Pectinex Ultra SP or Celluclast 1.5L or Viscozyme broke down the plant tissues.

3.2 Effect of mixture of two commercial enzymes on jicama pulp treatment

In this experiment, three samples were carried out. The experimental plan is presented in Table 2. Jicama pulp treatment included 2 steps. For each step, pH and temperature of the pulp were adjusted to optimal values of the commercial enzyme used in the treatment (see Table 1).

Table 2. Application of mixture of two commercial enzymes in jicama pulp treatment

Sample \ Step	Step 1	Step 2
Pectinex Ultra SP – Termamyl 120L	Pulp treatment with 0.04% Pectinex Ultra SP for 45min.	Pulp treatment with 0.02%Termamyl 120L for 45min
Celluclast - Termamyl 120L	Pulp treatment with 0.03% Celluclast 1.5L for 45min.	
Viscozyme - Termamyl 120L	Pulp treatment with 0.08% Viscozyme for 60min.	

Table 3. Effect of mixture of two commercial enzymes on jicama pulp treatment

Mixture	Pectinex Ultra SP – Termamyl 120L	Celluclast – Termamyl 120L	Viscozyme – Termamyl 120L
Extraction yield (%)	85,2[a]	80,3[b]	77,7[c]
Soluble solid (°Brix)	9,8[a]	9,2[b]	9,0[c]
Total sugar (g/L)	86,27[a]	73,31[b]	69,56[c]
Reducing sugar (g/L)	50,87[a]	45,12[b]	42,67[c]

Different letters in each row mean significant differences ($P < 0.05$)

The results in Table 3 show that the maximal extraction yield reached 85.2% when using both Pectinex Ultra SP and Termamyl 120L. Similarly, the contents of total and reducing sugars in the juice processed with Pectinex Ultra SP - Termamyl 120L treatment were the highest.

It can be noted that jicama pulp treatment by both commercial enzymes Pectinex Ultra SP and Termamyl 120L gave an extraction yield up to 85.2% w/w, whereas when using one commercial enzyme Pectinex Ultra SP or Termamyl 120L, the extraction yield reached 61.2% w/w or 73.5% w/w only. In conclusion, using mixture of Pectinex Ultra SP and Termamyl 120L in jicama juice production gave better results than application of each enzyme.

This result is in accordance with a study of Kashyap et al (2001). Kashyap stated that apple pulp treatment with mixture of pectinase and amylase gave higher juice yield and improved the colloidal stability of the final product.

In the next experiment, both Pectinex Ultra SP and Termamyl 120L were used with another commercial enzyme in jicama pulp treatment.

3.3 Effect of mixture of three commercial enzymes on jicama pulp treatment

In this experiment, jicama pulp was treated with mixture of three commercial enzymes: Pectinex Ultra SP – Celluclast - Termamyl 120L and Pectinex Ultra SP – Viscozyme – Termamyl 120L. In these cases, two commercial enzymes were used for breaking down polysaccharide complex of plant tissues into small molecules, and another commercial enzyme was used for starch hydrolysis.

In the first step, Pectinex Ultra SP and Celluclast or Pectinex Ultra SP and Viscozyme were added to the jicama pulp for enzymatic treatment because the optimal pH and temperature of the 3 commercial enzymes were similar. In the second step, the pulp was treated by Termamyl 120L.

Table 4. Effect of mixture of 3 commercial enzymes jicama pulp treatment

Mixture	Pectinex Ultra SP – Celluclast – Termamyl 120L	Pectinex Ultra SP – Viscozyme - Termamyl 120L
Extraction yield (%)	92,7[a]	87,4[b]
Soluble solid (°Brix)	11,1[a]	10,6[b]
Total sugar (g/L)	95,79 [a]	89,42 [b]
Reducing sugar (g/L)	57,38 [a]	52,87 [b]

Different letters in each row mean significant differences (P < 0.05)

Table 4 shows that mixture of pectinase, cellulase and amylase gave the best results: the extraction yield was extremely high 92.7%. In addition, the obtained juice was rich in total and reducing sugars (95.79g/L and 57.38g/L). This juice can be therefore used as a soft drink or a medium in fermentation technology.

It can be deducted that when using one or two or three commercial enzymes in jicama pulp treatment, the extraction yield increased 28.1% w/w or 39.8% w/w or 47.3% w/w, respectively in comparison with the control sample (without enzymatic treatment). Moreover, the contents of total and reducing sugars in the jicama juice processed with mixture of three enzymes were remarkably higher than that in the juice processed with single enzyme or mixture of two enzymes.

4. CONCLUSION

It can be affirmed that the combination of Pectinex Ultra SP, Celluclast 1.5L and Termamyl 120L gave the highest juice yield in jicama pulp treatment. In addition, this enzymatic treatment increased notably the total and reducing sugar contents in the jicama juice. Each of three commercial enzymes above has a specificity in hydrolysis of cell wall and starch in jicama pulp. Enzymatic treatment of jicama pulp by mixture of Pectinex Ultra SP, Celluclast and Termamyl 120L doubled the juice yield in comparison with the control sample without enzymatic treatment.

SỬ DỤNG CÁC CHẾ PHẨM ENZYME ĐỂ XỬ LÝ PHẦN THỊT CỦ ĐẬU TRONG SẢN XUẤT THỨC UỐNG

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TÓM TẮT: *Củ đậu (Pachyrhizus erosus) là một loại nông sản phổ biến tại Việt nam. Củ đậu được xem là nguồn thực phẩm cung cấp glucid, nhiều loại acid amin, vitamin và khoáng chất. Cho đến nay, củ đậu vẫn chưa được sử dụng như là một nguyên liệu chính trong công nghiệp thực phẩm ở nước ta. Xử lý nguyên liệu bằng enzyme được xem là một giải pháp kỹ thuật phổ biến để làm tăng hiệu suất thu hồi chất chiết trong chế biến rau quả. Mục đích của nghiên cứu này là khảo sát sự ảnh hưởng của quá trình xử lý enzyme đến hiệu suất thu hồi chất chiết và hàm lượng đường thu được trong dịch chiết từ củ đậu. Hiệu suất thu hồi chất chiết đạt giá trị cao nhất là 92.7% khi sử dụng tổ hợp 3 chế phẩm enzyme Pectinex Ultra SP (pectinase), Celluclast 1.5L (cellulase) và Termamyl 120L (alpha amylase) để xử lý phần thịt củ đậu. Khi đó, hàm lượng đường tổng và đường khử trong dịch chiết lần lượt là 95.79g/L và 57.38g/L Dịch chiết từ củ đậu có thể được sử dụng như một loại thức uống hoặc làm môi trường để sản xuất thức uống lên men và các sản phẩm trao đổi chất từ vi sinh vật.*

REFERENCES

[1]. Abud-Archila M., Vaùzquez-Mandujano D.G., Ruiz-Cabrera M.A., Grajales-Lagunes A., Moscosa-Santillaùn M., Ventura-Canseco L.M.C., Gutieùrrez-Miceli F.A., Dendooven L., Optimization of osmotic dehydration of yam bean (*Pachyrhizus erosus*) using an orthogonal experimental design, *Journal of Food Engineering,* 84, pp. 413 – 419 (2007)

[2]. Amaya-Llano S.L., Martínez-Alegría A.L., Zazueta-Morales J.J., Martínez-Bustos F., Acid thinned jicama and maize starches as fat substitute in stirred yogurt, *Food Science and Technology,* 41, pp. 1274-1281 (2008)

[3]. BeMiller J.N., Carbohydrate Analysis, *Food Analysis*, 3rd edition, Kluwer academic /Plenum Publisher, New York (2003)

[4]. Bradley R.L.Jr., Moisture and total solids analysis, *Food analysis*, 3rd edition, Kluwer Academic/Plenum Publisher, New York (2003)

[5]. Demir N., Acar J., Sariogêlu K., Mutlu M., The use of commercial pectinase in fruit juice industry. Part III: Immobilized pectinase for mash treatment, *Journal of Food Engineering,* 47, pp. 275 – 280 (2001)

[6]. Horvaùth - Kerbai E., Manufacturing fruit beverages, Handbook of Fruits and Fruit Processing, Blackwell Publishing, New york (2006)

[7]. Kashyap R.D., Vohra K.P., Chopra S., Tewari R., Applications of pectinase in the commercial saector: a review, *Bioresource Technology,* 77, pp. 215 – 227 (2001)

[8]. Mercado-Silva E., Garcia R., Heredia-Zepeda A., Cantwell M., Development of chilling injury in five jicama cultivars, *Postharvest Biology and Technology*, 13, pp. 37 – 43 (1998)

[9]. Sreenath H.K., Sdarshana Krishna K.R., Santhanam K., Enzymatic liquefaction of some varieties of mango pulp, *Lebensm. Wiss. u. Technol.,* 28, pp. 196-200 (1995)

[10]. Synowiecki J., The use of starch processing enzymes in the food industry, *Industrial enzymes*, Springer, Amsterdam (2007)

[11]. Taylor B., Fruit and juice processing, *Chemistry and technology of soft drink and fruit juices*, 2nd edition, Blackwell Publishing, Oxford (2005)

[12]. Van der Maarel M.J.E.C., Van der Veen B., Uitdehaag J.C.M., Leemhuis H., Dijkhuizen L., Properties and applications of starch-converting enzymes of the alpha amylase family,*Journal of Biotechnology,* 94, pp. 137 – 155 (2002).