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EFFECT OF SOME FACTORS ON THE HYDROLYSIS PROCESS OF SWEET POTATO STARCH BY SPEZYME ALPHA TO PRODUCE ISOMALTOOLIGOSACCHARIDE (IMO)

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Abstract. Isomalto-oligosaccharide (IMO), a mixture of glucooligosaccharides linked by α-(1 → 6) and/or a low proportion of α-(1 → 4), α-(1 → 3) (nigerooligosaccharide) or α-(1 → 2) glycosidic bonds (kojioligosaccharide), included glucose oligomers linked together by α-D-(1,6) glycoside bonds such as: isomaltose, panose, isomaltotetose, and isomaltotetose. IMO is considered as prebiotics found in several traditional foods such as rice miso, soybean sauce and sake, etc. This functional ingredient is attracting much attention from researchers and manufacturers because of its great health benefits such as beneficial effects on bowel functions and metabolism, bifidogenic effects, anti-cariogenic properties, immunity improvement, and techno-functional properties that helps IMO to apply widely in food technology. IMO is produced from hydrolysed starch that have been branched to form isomaltooligosaccharide using enzymes. In this article, the effect of some factors on hydrolysis of sweet potato starch by Spezyme Alpha to form oligosaccharide with degree of polymerization (DP) from 2 to 6 before being branched was studied. The optimum hydrolysis conditions were at 85 ^{o}C for 60 min., starch solution of 10 %, enzyme concentration of 0.03 % and pH = 5.8, after branching, the product contained 61.83 % IMO. It indicated that sweet potato starch could be used as a potential material for IMO synthesis.

Keywords: DP, Hydrolysis, IMO, Spezyme Alpha, sweet potato starch.

Classification numbers: 1.3.1; 1.3.2.

1. INTRODUCTION

Isomalto-oligosaccharide (IMO), a mixture of glucooligosaccharides linked by α-(1 → 6) and/or a low proportion of α-(1 → 3) (nigerooligosaccharide) or α-(1 → 2) glycosidic bonds (kojioligosaccharide), is found in commercial IMO syrup and is produced through various enzyme pathways [1 - 2]. IMO is currently of interest in the food and pharmaceutical industries with many

functional properties beneficial to health, notably prebiotic function [3], low-calorie sweetener [4], anti-constipation [5], and cholesterol level regulation [6]. IMO has a low glycemic index [7] and has appeared in long-standing traditional products such as honey [8], sake [9], and soy sauce [10]. In many countries, research and production of IMO have been carried out for many years [2, 11] and mainly on cassava, banana and mango starch. Several significant studies have been focused on IMOs produced from some different materials such as maize starch [12], chestnut [13], banana flour [2], rice crumbs [14], and cassava starch [15]. However, no research has been conducted on sweet potatoes.

Sweet potatoes are suitable for the Vietnamese climate, they are easy to grow, have a short harvest time and a wide range of varieties available. In recent years, there are many varieties of sweet potato with high productivity as well as high starch yield. Furthermore, Vietnamese sweet potato is in the top 10 in the world, however the price is completely precarious due to dependence on Chinese traders. Sweet potato products have not yet taken advantage of its inherent nutritional benefits, and valuable products from sweet potatoes are still limited. Therefore, this study was carried out to produce IMO from sweet potato starch to increase the economic efficiency of sweet potato roots, with the desire to increase income as well as create career opportunities for Vietnamese labours.

2. MATERIALS AND METHODS

2.1. Materials

Sweet potato starch (Hoang Long species or Tuong ban No 59 founded by Eng. Quach Ngoc An) was supplied by Food Technology Laboratory of the School of Biotechnology and Food Technology, Hanoi University of Science and Technology, Ha Noi, Viet Nam. Spezyme Alpha from DuPont, USA. Enzyme Transglucosidase, *Aspergillus niger*, from Megazyme.

2.2. Composition analysis

The determination of protein content was carried out according to TCVN 8125 : 2009, fiber content according to TCVN 4329 : 2007, ash content according to TCVN 9939 : 2013, lipid content according to TCVN 10730 : 2015, and amylose content according to TCVN 5716-1 : 2008. The average degree of polymerization (DP) and dextrose equivalent (DE) were determined by the method of Klucinec, 1998 [16].

2.3. Determination of oligosaccharide composition

RID high-pressure liquid chromatography system (HPLC 1290 – Agilent) was used with HyperRez XP analysis column, Carbohydrate Na^+, (7.7 × 300 mm) with guard column corresponding to HyperRez XP, Carbohydrate Na^+, (7.7 × 50 mm) from Thermo Scientific (United States). Standard linear oligosaccharide standards include glucose, maltose, maltotrinose (DP3), maltotetraose (DP4), maltopentaose (DP5), maltohexaose (DP6), maltoheptaose (DP7), maltooctaose (DP8), maltononaose (DP9), maltodecaose (DP10), and linear maltooligosaccharide (DP = 10 - 40). The mobile phase was H_2O (100 %) with a flow rate of 0.15 mL/min, the sample injection volume was 20 µL.

2.4. Statistical analysis

All tests were performed at least in duplicate. Analysis of variance was performed using Ducan's multiple-range test on Microsoft Excel 2010 and SPSS software. Significance was defined at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Effect of hydrolysis time on starch hydrolysis product by Spezyme Alpha

The hydrolysis process was carried out at a temperature of 80 ^{o}C, pH = 5.6, Spezyme Alpha concentration of 0.03 % db, substrate concentration of 10 % (db), and hydrolysis time of 30 - 90 minutes. These parameters were chosen based on recommended information from the manufacturer and our preliminary investigations [19]. DP and DE of the product after hydrolysis are shown in Table 1.

Table 1. Effect of hydrolysis time on the degree of polymerization and dextrose equivalents of sweet potato starch hydrolysates by Spezyme Alpha.

Hydrolysis time (min)	Average DP	DE
30	7.97 ± 0.55^{a}	12.35 ± 0.88^{e}
60	6.73 ± 0.07^{b}	14.59 ± 0.15^{f}
90	4.74 ± 0.21^{c}	20.76 ± 0.94^{g}

Note: Values followed by the same letter in the same column were not significantly different at p = 0.05.

It is clearly seen that when the hydrolysis time increased gradually from 30 minutes to 90 minutes, DP of hydrolysates after hydrolysis decreased from 7.97 to 4.74. When the hydrolysis time increased from 30 minutes to 60 minutes, DP of hydrolysates decreased by 1.24, DP after hydrolyzing for 90 minutes reduced by 1.99 compared to that for 60 minutes. Thus, the longer the hydrolysis time, the shorter the oligosaccharides chains. If the hydrolysis time continued to increase, the chain length would decrease very slowly [17]. The statistical analysis indicated the significant difference in the results and DP decreased as hydrolysis time increased. However, an increase in time also caused an increase in energy costs, thus, in order to save time and energy along with the hydrolysis goal of obtaining a product with low polymerization, the 60-minute hydrolysis time was suitable for the following branching reaction by transglucosidase enzyme. When hydrolysing cassava starch [18] at 90 ^{o}C for 30 minutes with alpha amylase concentration of 0.02 %, the DE obtained was 12.6. In this study, with the same hydrolysis time of 30 minutes, the Spezyme Alpha concentration was 0.03 %, the reaction temperature of 80 ^{o}C, the DE resulting from sweet potato starch hydrolysates reached 13.3.

The effects of hydrolysing time on the oligosaccharides composition in sweet potato hydrolysate by Spezyme Alpha are shown in Figure 1.

Figure 1 showed that hydrolysis time affected the composition of oligosaccharides, at hydrolysis time of 30 minutes, the minimum total content of DP = 2 to DP = 6 (abbreviated as DP2-6) was 40.45 % and the maximum total content of DP = 7 to DP =10 (denoted as DP7-10) was 15.63 %. After 60 minutes of hydrolysis, DP2-6 reached 59.92 %, DP7-10 reached 12.4 %. The best hydrolysis results were obtained at 90 minutes when DP2-6 reached 69.85 % and DP7-10 reached 9.38%. As the hydrolysis time increased, short-chain oligosaccharides (DP2-6) gradually increased, while longer-chain oligosaccharides (DP7-10) gradually decreased.

Accordingly, the chromatographic results demonstrated that the longer the hydrolysis time, the lower the oligosaccharide content [17].

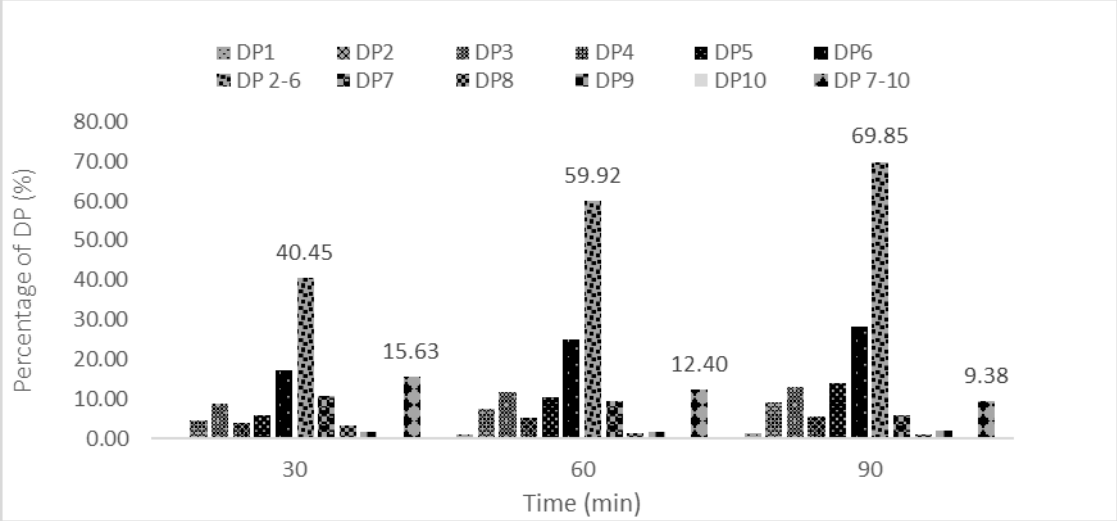

Figure 1. Effect of hydrolysis time on the composition of oligosaccharides in sweet potato starch hydrolysate by using Spezyme Alpha.

3.2. Effect of starch concentration on starch hydrolysis products by Spezyme Alpha

The sweet potato starch hydrolysis process was carried out at 80 ^{0}C, pH = 5.6, Spezyme Alpha concentration of 0.03 % of the dry matter, substrate concentration of 5-15 % (calculated on dry matter), and hydrolysis time of 60 minutes. Average DP and DE of products after hydrolysis are shown in Table 2. It was observed that when the sweet potato starch concentration increased, the DP increased while the DE of the hydrolysates decreased. When concentration of sweet potato starch was 5 %, the best hydrolysis results were obtained with DP = 3.88 and DE = 25.33, and the lowest hydrolysis was observed at sweet potato starch concentration of 15 % with DP = 5.75 and DE = 17.77. Statistical analysis indicated that DP and DE values at different substrate concentrations were significantly different. Increasing concentration of starch led to a decrease in DP, meaning that the ability of hydrolysis decreased, the chain length of oligosaccharide increased with lowering concentration. This can be explained by the increase in viscosity of the solution, resisting the enzyme activity. However, if the hydrolysis was carried out at low concentrations, it did not give high economic efficiency. In the study on cassava starch [18], with hydrolysis time of 30 minutes, temperature of 90 ^{o}C, alpha amylase (Clearflow AA) concentration of 0.02 %, starch concentration of 10 %, the DE value of hydrolysate reached 9.4. In this study, the hydrolyzing conditions of sweet potato were also with the same starch concentration of 10 %, hydrolysis time of 30 minutes, the Spezyme Alpha concentration was 0.03 %, the reaction temperature was 80 ^{0}C, the resulting DE was obtained at 12.35. Thus, at the same concentration of 10 %, sweet potato starch produced hydrolysate with higher DE, which was more favorable for IMO branching process. Starch hydrolysis products were detected using HPLC to determine the individual components. The results (Figure 2) showed that the DP2-6 content increased gradually when the sweet potato starch concentration decreased, the minimum DP2-6 content was 53.45 % when the sweet potato starch concentration was 15 % and the maximum DP2-6 content was 62.95 % when the sweet potato starch concentration was 10 %.

However, the DP7-10 content increased gradually with the substrate concentration, reaching 9.31 % and 18.23 % at the starch concentration of 5 % and 15 %, respectively. Thus, for the purpose of hydrolysis to create a solution with low DP to facilitate branching and achieve economic efficiency, it was recommended to choose a starch concentration of 10 %.

Table 2. Effect of starch concentration on the degree of polymerization and dextrose equivalents of sweet potato starch hydrolysates by Spezyme Alpha.

Substrate concentration	Average DP	DE
5%	3.88 ± 0.08^{a}	25.33 ± 0.49^{e}
10%	4.80 ± 0.13^{b}	20.46 ± 0.57^{f}
15%	5.75 ± 0.20^{c}	17.77 ± 0.65^{g}

Note: Values followed by the same letter in the same column were not significantly different at p = 0.05..

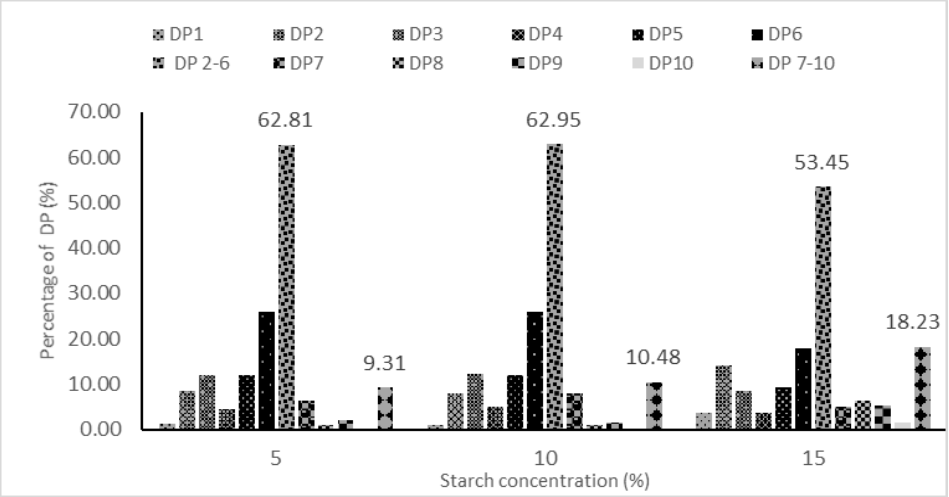

Figure 2. Effect of starch concentration on the composition of oligosaccharides in sweet potato starch hydrolysate by Spezyme Alpha.

3.3. Effect of Spezyme Alpha concentration on starch hydrolysis products

Table 3. Effect of α-amylase concentration on the degree of polymerization and dextrose equivalent of sweet potato starch hydrolysates.

Enzyme concentration (% db)	Average DP	DE
0.01 %	10.35 ± 0.18^{a}	9.48 ± 0.16^{f}
0.02 %	6.62 ± 0.13^{b}	14.82 ± 0.28^{g}
0.03 %	4.68 ± 0.13^{c}	20.96 ± 0.58^{h}
0.04 %	5.74 ± 0.07^{d}	17.10 ± 0.21^{i}
0.05 %	5.27 ± 0.03^{e}	18.61 ± 0.09^{k}

Note: Values followed by the same letter in the same column were not significantly different at p = 0.05.

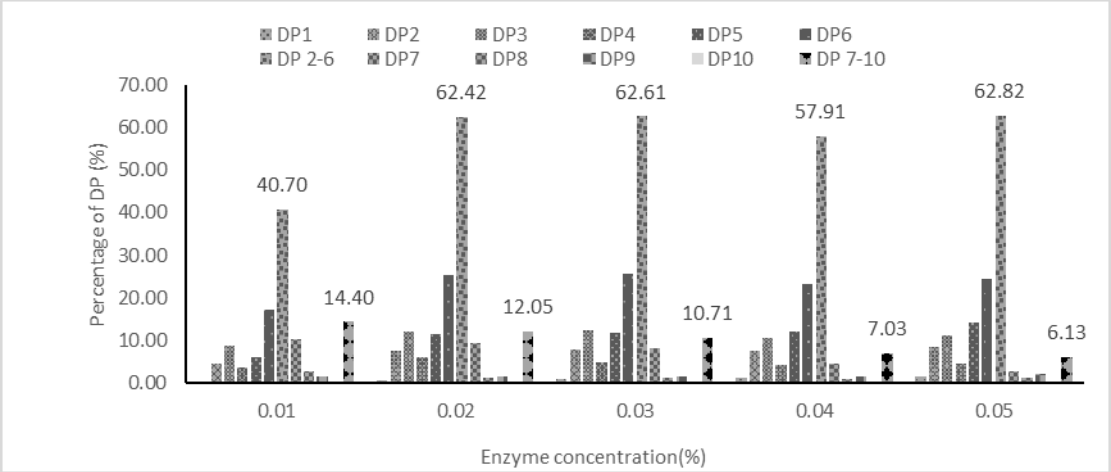

Figure 3. Effect of enzyme concentration on oligosaccharides composition in sweet potato starch hydrolysate.

The hydrolysis of sweet potato starch was conducted at 80 ^{o}C, pH = 5.6, enzyme concentrations were 0.01 - 0.05 % (db), sweet potato starch concentration was 10 %, and hydrolysis time was 60 minutes. The average DP and DE of the products after hydrolysis are given in Table 3.

Table 3 and Figure 3 show that the average DP and DE changed significantly when there were sharp changes in α – amylase concentration. Especially, when the concentration of α-amylase increased from 0.01 % to 0.03 % (db), the average DP of hydrolysate decreased sharply from 10.35 to 4.68 and DE increased rapidly from 9.48 to 20.96. When enzyme concentration increased to 0.04 % and 0.05 % (db), DP tended to increase slightly from 4.68 to 5.27 and DE decreased slightly from 20.96 to 18.61. This can be explained that increasing enzyme concentration too high leads to the inhibition of the reaction due to the interaction between the enzyme molecules. Statistical analysis showed that the DP and DE values (Table 3) were significantly different. When hydrolysing cassava starch [18], at 90 ^{0}C for 30 minutes, the concentration of alpha amylase (Clearflow AA) was 0.03 % (g/g starch), the DE of hydrolysate was obtained at 13.3. In this study, hydrolyzing sweet potato starch at 80 ^{o}C for 30 minutes by Spezyme Alpha (with concentration of 0.03 % based on starch), the DE of sweet potato starch hydrolysate reached 12.35. The hydrolysate components were detected by HPLC. The results shown in Figure 3 described that at an enzyme concentration of 0.01 % (db) the minimum DP2-6 content reached 40.70 % and the maximum DP7-10 content reached 14.4 %, indicating an incomplete hydrolysis, where long-chain oligosaccharides were still in large concentrations. When the enzyme concentrations were 0.02 % - 0.05 % (db), contents of hydrolysate with DP from 2 to 6 ranged from 57.91 % to 62.82 %, however, contents of those with DP7 to DP10 at enzyme concentrations of 0.04 % and 0.05 % were 7.03 % and 6.13 %, lower than those at enzyme concentrations of 0.02 % - 0.03 %, which were 12.05 % and 10.71 %, respectively. Thus, the appropriate hydrolytic enzyme concentration in the range from 0.02 % to 0.03 % is more favorable. This concentration is also in the range that the manufacturer had recommended [19]. From Table 3 and Figure 3, it was seen that the highest content was of hydrolysate DP 2-6, so the enzyme concentration was chosen to be 0.03 %.

3.4. Effect of pH on the hydrolysis process of sweet potato starch by Spezyme Alpha

The hydrolysis was carried out at a substrate (sweet potato starch) concentration of 10 % (db), 80 ^{o}C for 60 minutes, pH = 5.2 - 6.0, enzyme concentration of 0.03 % (db). The pH range was chosen based on the operating conditions recommended by the manufacturer and our preliminary investigations [19]. The average DP and DE of the products after hydrolysis are shown in Table 4.

Table 4. Effects of pH on the degree of polymerization and dextrose equivalents of sweet potato starch hydrolysates by Spezyme Alpha.

pH	Average DP	DE
5.2	7.37 ± 0.15^{a}	13.33 ± 0.27^{e}
5.4	8.20 ± 0.05^{b}	11.97 ± 0.08^{f}
5.6	6.78 ± 0.13^{c}	14.48 ± 0.28^{g}
5.8	6.23 ± 0.07^{d}	15.76 ± 0.18^{h}
6.0	6.88 ± 0.09^{c}	14.27 ± 0.19^{g}

Note: Values followed by the same letter in the same column were not significantly different at p = 0.05.

Table 4 shows that as pH changed, the average DP and dextrose equivalent also changed. Thus, pH had a great effect on the hydrolysis reaction of α - amylase in Spezyme Alpha [19-20]. At pH of 5.2 - 6.0, the average DP ranged from 6.23 to 8.2, DE ranged from 11.97 to 15.76. It was also indicated that at pH 5.8, the DP of 6.23 was the lowest, that meant the highest level of hydrolysis or the shortest oligosaccharide chain length. The highest DE value at pH 5.8 was 15.76. Enzyme activity was lowest at pH 5.4 and pH 5.2 with DP values of 8.2 and 7.37, respectively, and better at pH = 5.6 and 6.0 with average DP of 6.78 and 6.88, respectively. Thus, the α - amylase tended to perform better when the pH was higher. Statistical analysis showed that pH significantly affected the DP value of the hydrolysates, only at pH 5.6 and pH 6.0, there was no difference in DP of the hydrolysate. Thus, with the aim to hydrolyse sweet potato starch by Spezyme Alpha to obtain a low polymerization product, pH 5.8 was suitable for the branching reaction by transglucosidase enzyme. The composition of the hydrolysate was determined using HPLC. The effect of pH on the oligosaccharide composition in sweet potato hydrolysate by Spezyme Alpha is shown in Figure 4.

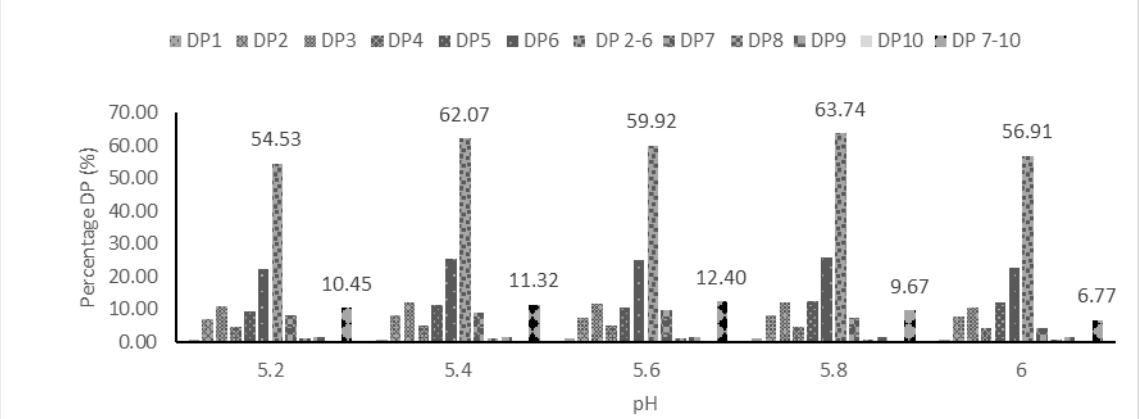

Figure 4. Effect of pH on the oligosaccharide composition in sweet potato starch hydrolysate by Spezyme Alpha.

Figure 4 indicates that at pH 5.8, the DP2-6 was released in the highest amount of 63.74 %, and at pH 5.2, that was 54.53 %. The DP7-10 content at pH 5.8 and pH 5.2 was 9.67 % and 10.45 %, respectively. Therefore, at pH 5.8, the enzyme was most active, the hydrolysis product had the shortest average DP. At pH = 5.4, 5.6 and 5.8, the content of DP2-6 was quite similar, ranging from 56.91 % to 62.07 %. Accordingly, choosing an appropriate hydrolysis pH of 5.8 was suitable for the hydrolysis reaction to create short chain oligosaccharides, facilitating the branching by transglucosidase enzyme to create IMO.

3.5. Effect of temperature on sweet potato starch hydrolysis by using Spezyme Alpha

The hydrolysis of starch was conducted at 70 ^{o}C – 90 ^{o}C, pH= 5.8, enzyme concentration of 0.03 % db, substrate concentration of 10 % db, for 60 minutes. The average DP and DE of the product after hydrolysis are given in Table 5.

Table 5. Effects of temperature on the degree of polymerization and dextrose equivalents of sweet potato starch hydrolysates by Spezyme Alpha.

Temperature (^{o}C)	Average DP	DE
70	7.80 ± 0.30 ^{a}	12.60 ± 0.50 ^{e}
75	7.32 ± 0.15 ^{b}	13.42 ± 0.27 ^{f}
80	5.65 ± 0.10 ^{c}	17.36 ± 0.32 ^{g}
85	4.77 ± 0.07 ^{d}	20.56 ± 0.28 ^{h}
90	5.86 ± 0.22 ^{c}	16.76 ± 0.63 ^{g}

Note: Values in the same column with different exponential values are different at significant p= 0.05.

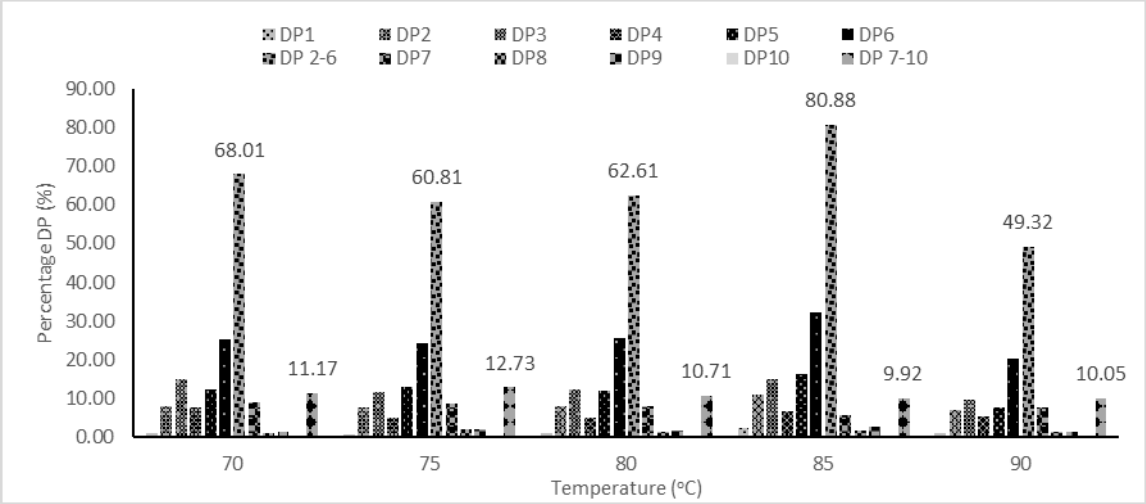

Figure 5. Effect of temperature on the composition of oligosaccharides in sweet potato starch hydrolysate by Spezyme Alpha.

The results in Table 5 show that when the hydrolysis temperature increased from 70 ^{o}C to 85 ^{o}C, the average DP decreased sharply from 7.8 to 4.77, the chain length decreased more than 200 times compared to the original sweet potato material, while the DE increased significantly from 12.60 to 20.56. At 85 ^{o}C, the best hydrolysis efficiency was obtained when the average DP was 4.77 and the DE was 20.56. This result was similar to the manufacturer's announcement and a previous study on cassava starch [19-20]. When the temperature continued to rise to 90 ^{o}C, the

DP increased to 5.86, higher than when hydrolysis was at 85 ^{o}C. This could be explained by the gradual decrease in enzyme activity when the temperature was too high. Statistical analysis showed significant differences between DP and DE values. At 80 ^{o}C and 90 ^{o}C, DP and DE were not different. Hydrolytic products of sweet potato starch were determined using HPLC. The effect of temperature on the oligosaccharide composition in sweet potato hydrolysate by Spezyme Alpha is described in Figure 5.

It is shown that temperature had a great impact on the oligosaccharide composition in the hydrolysis product. At 85 ^{o}C, the DP 2-6 content was the highest (80.88 %) and at 90 ^{0}C it was the lowest (49.32 %). In the range from 70 ^{o}C to 80 ^{o}C, the DP2-6 had a slight fluctuation from 60.81 % to 68.01 %. Thus, at a temperature of 85 ^{o}C, the *α*-amylase in Spezyme α had the best activity, making the content of oligosaccharides DP1-10 up to 90.8 %. DP1 (glucose) and DP2 (maltose) component's contents also increased significantly at 85 ^{o}C and those at other temperatures did not make a difference. The concentration of hydrolysates with DP7-10 did not change much at different temperatures, the highest was 12.73 % at 75 ^{o}C and the lowest was 9.92 % at 85 ^{o}C. In short, 85 ^{o}C was the best hydrolysis temperature for the highest amount of hydrolysate with DP2-6, which could promote the branching reaction for the next step.

Thus, the optimal parameters of the hydrolysis process by Spezyme Alpha to obtain hydrolysate with DP = 2 to 6 were as follows: hydrolysis temperature of 85 ^{o}C, hydrolysing time of 60 minutes, starch concentration of 10 %, enzyme concentration of 0.03 % db, and pH = 5.8.

3.6. Isomaltooligosaccharide (IMO) production

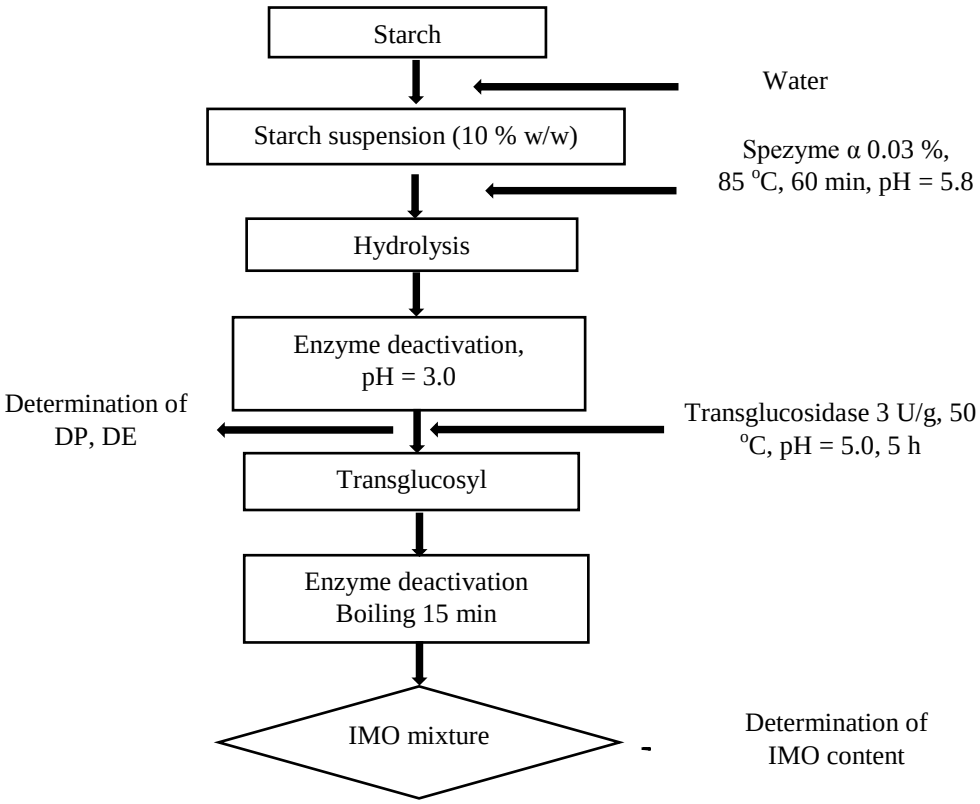

Figure 6. IMO production.

Table 6. DP and DE after hydrolysis and IMO content in product during IMO production.

DP of hydrolysis products by Spezyme Alpha	DE of hydrolysis products by Spezyme Alpha	IMO content after hydrolysis by Spezyme Alpha (% db)	IMO content after branching by transglucosidase (% db)
4.7 ± 0.065	21.12 ± 0.21	1.72 ± 0.07	61.83 ± 1.84

IMO was produced from sweet potato starch under hydrolysis conditions as described above, followed by branching by transglucosidase. DP and DE after hydrolysis and IMO content in product are presented in Table 6. The results showed that after the addition of transglucosidase enzyme, IMO content increased from 1.72 % to 61.83 %. Suwimol Chockchaisawasdee in 2012 [2] reached 68.55 % after 6 hours of banana starch branching. Anindya Basu *et al*. in 2016 obtained 74.73 % after 12 hours of reaction [12]. Effects of several factors on the activity of transglucosidase enzyme to improve the yield of IMO production will be mentioned in further study.

4. CONCLUSION

The effect of some factors on hydrolysis of sweet potato starch by Spezyme Alpha to form oligosaccharide with DP = 2 - 6 before being branched was investigated. The optimal conditions for hydrolysing sweet potato starch by Spezyme Alpha towards IMO production were as follows: hydrolysis temperature of 85 ^{0}C, hydrolysis time of 60 minutes, sweet potato concentration of 10 %, enzyme concentration of 0.03 %, and pH = 5.8. The branching process was carried out immediately afterwards by transglucosidase enzyme with concentration of 3 U/g, temperature of 50 ^{0}C, pH = 5.0, and reaction duration of 5 hours. The total IMO content was obtained at 61.83 ± 1.84 %.

Credit authorship contribution statement. Duong Hong Quan: Methodology, Data collection, Investigation, Drafting manuscript. Hoang Minh Tri: Data collection and analysis, Drafting manuscript, Formal analysis, Research summary. An Duy Tuyen: Sample preparation, Formal analysis. Vu Thu Trang: Supervision, Recommendation. Luong Hong Nga: Research design, Data analysis, Formal analysis. Supervision, Research summary and recommendation, Manuscript revision, Corresponding for manuscript revision.

Declaration of competing interest. The authors declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

1. Goffin D., Delzenne N., Blecker C., Hanon E., Deroanne C., and Paquot M. -Will Isomalto-Oligosaccharides, a Well-Established Functional Food in Asia, Break through the European and American Market? The Status of Knowledge on these Prebiotics, Crit. Rev. Food Sci. Nutr. **51** (5) (2011) 394-409. https://doi.org/10.1080/10408391003628955.
2. Chockchaisawasdee S and Poosaran N. - Production of isomaltooligosaccharides from banana flour, J. Sci. Food Agric. **93** (1) (2013) 180-186. https://doi.org/10.1002/jsfa.5747.
3. Kohmoto T., Fukui F., Takaku H., Machida Y., Arai M., and Mitsuoka T. - Effect of Isomalto-oligosaccharides on Human Fecal Flora, Bifidobacteria and Microflora **7** (2) (1988)

61-69. https://doi.org/10.12938/ bifidus1982.7.2_61.

4. Sorndech W., Tongta S., and Blennow A. - Slowly Digestible- and Non-Digestible α-Glucans: An Enzymatic Approach to Starch Modification and Nutritional Effects, Starch - Stärke **70** (9 - 10) (2018) 1700145. https://doi.org/10.1002/ star.201700145.
5. Chen H. L., Lu Y. H., Lin J. J., and Ko L. Y. - Effects of isomalto-oligosaccharides on bowel functions and indicators of nutritional status in constipated elderly men, J. Am. Coll. Nutr. **20** (2001) (1) 44-9. https://doi.org/10.1080/ 07315724.2001.10719013.
6. Wang H. F., Lim P. S., Kao M. D., Chan E. C., Lin L. C., and Wang N. P. - Use of isomalto-oligosaccharide in the treatment of lipid profiles and constipation in hemodialysis patients, J. Ren. Nutr. **11** (2) (2001) 73-79. https://doi.org/10.1016/S1051-2276(01)92591-9.
7. Sheng L., Dong-lian G. E., C. A. I., and Wan - Determination of glycemic index of xylitol and isooligosaccharide, Chin. J. Clin. Nutr. **14** (4) (2006) 235-237.
8. Tanaka K. - Japan Relies on Honey Imports, International Market News.
9. Hayakawa K et al. - Determination of saccharides in sake by high-performance liquid chromatography with polarized photometric detection, Biomed Chromatogr. **14** (2) (2000) 72-76. https://doi.org/10.1002/(SICI)1099-0801(200004)14:2%3C72::AID-BMC927%3 3.0. CO;2-S.
10. Tungland B. C and Meyer D. - Nondigestible Oligo- and Polysaccharides (Dietary Fiber): Their Physiology and Role in Human Health and Food, Compr. Rev. Food Sci. Food Saf. **1** (3) (2002) 90-109. https://doi.org/10.1111/j.1541-4337.2002.tb00009.x.
11. Basu A., Mutturi S., and Prapulla S. G. - Production of isomaltooligosaccharides (IMO) using simultaneous saccharification and transglucosylation from starch and sustainable sources, Process Biochem. **51** (10) (2016) 1464-1471. https://doi.org/10.1016/ j.procbio.2016.06.008.
12. Niu D., Qiao J., Li P., Tian K., Liu X., Singh., Lu F. - Highly efficient enzymatic preparation of isomalto-oligosaccharides from starch using an enzyme cocktail, Electron. J. Biotechnol. **26** (2017) 46-51. https://doi.org/10.1016/j.ejbt.2016.12.002.
13. Cui J., Li Y., Wang Q., Li J., Ou Y., Wang J.,Wang W. - Production, purification and analysis of the isomalto-oligosaccharides from Chinese chestnut (*Castanea mollissima* Blume) and the prebiotics effects of them on proliferation of *Lactobacillus*, Food Bioprod. Process **106** (2017) 75-81. https://doi.org/10.1016/j.fbp.2017.08.003.
14. Pan Y. C and Lee W. - Production of high-purity isomalto-oligosaccharides syrup by the enzymatic conversion of transglucosidase and fermentation of yeast cells, Biotechnol. Bioeng **89** (7) (2005) 797–804. https://doi.org/10.1002/bit.20402.
15. Lin Q., Xiao H., Zhao J., Li L., Yu F., Liu X., Cheng X. - Production of isomalto-oligosaccharide syrup from rice starch using an one-step conversion method, Int. J. Food Sci. Technol. **46** (6) (2011) 1194-1200. https://doi.org/10.1111/j.1365-2621. 2011.02623.x.
16. Kluncinec, J.D, Thompson D. B. - Fractionation of high-amylose maize starches by differential alcohol precipitation and chromatography of the fraction, Cereal Chem. **75** (6) (1998) 887-896 https://doi.org/10.1094/CCHEM.1998.75.6.887.
17. Le Ngoc Tu - Biomorphology of products from seeds, Science and Technology publisher, Hanoi, 2000, (in Vietnamese).

18. Hung D. T., Thuan V. T., Phi N. H., and Hoa L. T. N. - Determining suitable conditions for the hydrolysis of starch into raw materials for production of Isomalto Oligosaccharides (IMO), Science and Technology. (9) (2012) 39-41.
19. SPEZYME ® ALPHA - Alpha-Amylase for Dry Grind Ethanol Production, 2012.
20. Sakdaronnarong C., Srimarut N., Chantasod R., Sraphet S., Triwitayakorn K., and Jonglertjunya W. - Enzyme Matching Design Approach on Very High Gravity Liquefaction and Saccharification of Cassava Root and Cassava Starch for Ethanol Fermentation, BioResources **13** (2) (2018) 2304-2327. http://dx.doi.org/10.15376/biores.13.2.2304-2327.