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Isolation and determination of chemical composition of sweet potato starch using Viscozyme cassava C

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Abstract
Sweet potato was one of most important food crop in the world which has a great source of dietary energy, in the form of carbohydrates. This study was carried out to investigate the appropriate conditions to produce high yield of sweet potato starch using commercial cellulase and determine chemical composition of the isolated starch. The optimum enzymatic conditions for improving sweet potato starch extraction were found to be 3 U/g of concentration of cellulase, ratio of water and sample 150ml/100 g sweet potato at 4 h of incubation time. Chemical composition of isolated starch with enzyme-assisted isolation was not significantly different from starch using without enzyme. The results show that sweet potato starch treated with cellulase only enhanced yield of isolated starch with high purification.

Keywords: sweet potato, starch, cellulase, starch isolation

1. Introduction
Sweet potato (*Ipomoea batatas, L.*), one of the most important food crops over the world, planted mainly in tropical countries. In Vietnam, sweet potato is the fourth one of the most important food crops and the second one of the largest producers in the world [1]. Based on statistic data of Ministry of Agriculture and Rural Development of Vietnam, the production of sweet potato in Vietnam was estimated for 1.45 million of tons for the year of 2015. Chemically, sweet potato has a great source of starch accounting for around 50 – 80% of the root dry matter [2]. Starch granules in the sweet potato are imbedded in cellulosic fibers and held together by pectin substrates. Therefore, starch isolation from sweet potato tuber requires high levels of water during technological processing [3]. The starch extraction process from roots and tubers involves of grating the raw material, in order to break vegetal cells and release the starch. The success of starch extraction from tubers depends on complete rupture of the cell walls and thereby releasing the starch granules [4]. Recently, new methods for starch isolation have been developed to avoid the limitation of the traditional sedimentation method. The sweet potato starches in industrial scale are isolated by ultrasound pretreatment [5], mechanical disintegration of the cell wall and then utilization of water to wash starch granules out, or enzyme-assisted extraction method which applied enzymatic treatments to enhance the recovery of starch from roots and tubers [6, 7].
Cellulase is hydrolytic enzyme capable of hydrolyzing the most abundant organic polymer. Cellulase has potential in industries and is used in food, beverages, textile, laundry, paper and pulp industries etc. Enzymatic hydrolysis of cell wall component such as cellulase can release better of starch granules [8]. Isolation of starch from potato by using cellulase from *Penicillium funiculosum* was also investigated by Gayal and Hadge [7]. Cellulase is used on potato tubes and release of starch at various enzyme concentrations with different incubation time. Starch was recovered about 68% for 6 hours and increased to 90% for 2 hours by adding pectinase.
There have been several researches on application of enzymatic methods to increase the recovery of starch from roots and tubers [7, 9, 10]. However, to the best of our knowledge, little investigation on the use of cellulase for isolation of starch of sweet potato and determination of appropriate condition for isolation have been reported. Therefore, the objective of this study was to investigate the isolation conditions of starch from sweet potato using cellulase and to determine chemical composition of starches extracted by enzymatic method to that of the conventional method.

2. Materials and Methods
2.1. Materials
Sweet potato (*Ipomoea batatas*) was collected in Vinh Long province, Vietnam. Sweet potato had white flesh color. All the tubers in this experiment was in the uniformity of shape and size and did not contain any contamination including insects, smelly and rotten parts. After collecting, sweet potatoes were washed carefully and stored at 8 to 10ºC for further experiments.
The enzyme used in this study was a commercial product named Viscozyme Cassava C of Novozyme, Denmark. The optimal conditions for this enzyme were pH of 4.5–6.0, and temperature of 40–60°C. Generally, a storage temperature of enzyme was 25 °C.
All chemicals used for chemical compositions analysis were purchased from Merck Co.

2.2. Isolation of starch

2.2.1 Isolation of starch using different cellulase concentrations

Extraction of starch was described by Benesi et al. [11] with modification. Sweet potatoes were washed under tap water to remove dirt and sorted. The sweet potatoes after washing with water was peeled and sliced. The sweet potato tubers (100 g) was weighted and grinded in high speed with 100 ml of distilled water in a Philips blender for 2 min. The ground sweet potato was transferred to a beaker. The mixture was controlled at pH 5.5 – 6 before enzyme cellulase was added with different concentrations (1, 2, 3, 4 U/g). The beaker was covered by aluminum paper and kept in incubator for 2 h at 40 °C with a shaking at the speed of 125 rpm. Mixture after incubating was added with 400 ml of distilled water and then filtered in a 250-µm sieve. Mixture was filtered again in a 105-µm sieve and centrifuged at 3500 rpm for 10 min. Sediment was taken and dried in an oven at 40 °C for 24 h to the moisture content of 10-11%. Enzyme concentration which produced the highest yield was used for next experiment.

2.2.2 Isolation of starch at different incubation time

The sweet potato tubers (100 g) after washing was peeled, sliced and grounded with 100 ml of distilled water in a Philip bender for 2 min. The ground sweet potato was transferred to a beaker and the mixture was adjusted to pH 5.5 – 6 before cellulase was added with optimal concentration as the first experiment. The beaker was covered by aluminum paper and kept in incubator at different time (1, 2, 3, 4 h) at 40 °C with a shaking at the speed of 125 rpm. Mixture after incubating was added with 400 ml of distilled water and then filtered in a 250-µm sieve. Mixture was filtered again in a 105-µm sieve and centrifuged at 3500 rpm for 10 min. Sediment was taken and dried in an oven at 40 °C for 24 h to the moisture content of 10-11%. An appropriate incubation time which produced the highest yield was used for next experiment.

2.2.3 Isolation of starch using different ratios of sample and water

The sweet potato after washing with tap water was peeled and sliced. The sweet potato tubers (100 g) was weighted and grinding at high speed in Philips bender for 2 min with distilled water (50 ml, 100 ml, 150 ml, 200 ml). The ground sweet potato was transferred to a beaker and the mixture was adjusted to pH 5.5 – 6 before cellulase was added with optimal concentration as the first experiment. The beaker was covered by aluminum paper and kept for incubation at incubation time from second experiment in incubator at 40 °C at the speed of 125 rpm. Mixture after incubating was added with 400 ml of distilled water and then filtered in a 250-µm sieve. Mixture was filtered again in a 105-µm sieve and centrifuged at 3500 rpm for 10 min. Sediment was taken and dried in an oven at 40 °C for 24 h to the moisture content of 10-11%. The isolated starch had the highest yield was used for next experiment.

2.2.4 Isolation of starch using different sieving times

The isolation procedure was carried out as the same as in section 2.2.3 with optimal enzymatic concentration, incubation time and ratio of sample and water. Mixture after incubating was added with 400 ml of distilled water and then filtered in a 250-µm sieve. The washing and filtering step was repeated at different times (1, 2, 3, 4 times). Then, the mixture was filtered again in a 105-µm sieve and centrifuged at 3500 rpm for 10 min. Sediment was taken and dried in an oven at 40 °C for 24 h to the moisture content of 10-11%. An appropriate sieving times which produced high yield was choose to isolate starches from sweet potato tubers and chemical composition of the isolated starch was determined.

2.2.5 Determination of chemical composition of starch

Moisture content of sweet potato starches was determined using Moisture Balance Analyzer. The AACC approved methods 46-10, 30-10, and 08-01 [12] were used to analyze protein, lipid, and ash contents of sweet potato starches, respectively. Total carbohydrate content was calculated from the subtraction of protein, lipid and ash contents.

2.2.4 Statistical analysis

All experiments were done at least in duplicate and the results were presented as the average value. Analysis of variance (ANOVA) was used to analyze data by SPSS and Excel software.

3 Results and Discussion

3.1 Yield of isolated starch using by different cellulase concentrations

Table 1 shows the change in yield of sweet potato starch using different concentrations of cellulase. Increase in enzyme concentration resulted in increased starch yield. Average yield of starch increased from 10.94 g to 12.65 g when enzyme concentration increased from 1 U/g to 3 U/g. The yield was not significant difference between samples treated with 3U/g and 4U/g. The yield of starch increased when concentration of enzyme increased. As a result, the appropriate concentration of enzyme was chosen as 3U/g. The yield of starch extracted when using different cellulase concentration was the same as reported by Nandan [5].

Table 1. Yield of sweet potato starch using different cellulase concentrations[1]

Enzyme concentration (U/g sample)	Yield (g)
0	10.50 ± 0.02^{a}
1	10.94 ± 0.02^{a}
2	11.64 ± 0.02^{b}
3	12.65 ± 0.04^{c}
4	12.74 ± 0.02^{c}

[1]Means by the same letter in the same column is not significant difference (P <0.05), n = 3.

3.2. Isolation of starch at different incubation time

Table 2 shows the change in yield of starch when incubated at different time. Average yield of isolated starches increased from 11.54 g to 13.14 g when time of incubation increased from 1 hour to 3 hours. There were significant differences between sample incubated at 1 h, 2 h and 3 h ($P<0.05$) and not significant difference among sample incubated at 3 h and 4 h. Yield of starch extracted dependent on the incubation time. The longer time of incubation, yield of isolation starch was increased. As a result, the appropriate of incubation time to isolation of starch was 3 h.

Table 2. Yield of sweet potato starch at different incubation time[1]

Incubation time (h)	Yield (g)
1	11.54 ± 0.02^{a}
2	12.65 ± 0.04^{b}
3	13.14 ± 0.04^{c}
4	13.18 ± 0.04^{c}

[1]Means by the same letter in the same column is not significant difference (P <0.05), n = 3.

3.3. Isolation of starch using different ratios of sample and water

Table 3 shows the change in yield of sweet potato starch when using different ratios of sample and water. Average isolated starch increased from 12.68 g to 20.86 g when amount of water increased from 50 ml to 150 ml. There were significant differences between sample had level of water 50 ml, 100 ml and 150 ml (P<0.05) but not significant differences among sample used amount of water of 150 ml and 200 ml. As a result, the appropriate level of water was 150 ml.

Table 3. Yield of sweet potato starch at different ratios of sample and water[1]

Ratios of sample and water (ml)	Yield (g)
1:0.5	12.68 ± 0.11^{a}
1:1	13.14 ± 0.04^{b}
1:1.5	20.86 ± 0.08^{c}
1:2	21.15 ± 0.08^{c}

[1]Means by the same letter in the same column is not significant difference (P <0.05), n = 3.

3.4. Isolation of starch with different sieving times

Table 4 shows the different yields of sweet potato starch isolated with different sieving times. The amount of water was added to wash the biomass to release more starch from the residue. There were significant differences in starch yields with different washing and sieving times with water (P<0.05). The yield of starch increased when the amount of water for washing increased. Average isolated starch increased from 15.0 g to 22.5 g when washing times increased from one time to four times. Although the starch yield increased with increasing washing times, the amount of water used also increased. In addition, washing more times might affect the purification of starch because the fiber was filtrated together. Even though the yield of starch with 3-time washing was lower than that with 4-time washing, the 3-time washing used less amount of water resulted in high beneficial economy. Therefore, the 3-time washing was considered as an appropriate times to isolate sweet potato starch.

Table 4. Yield of sweet potato starch with different sieving times[1]

Sieving times	Yield (g)
1	15.0 ± 0.21^{a}
2	19.0 ± 0.12^{b}
3	21.0 ± 0.09^{c}
4	22.5 ± 0.22^{d}

[1]Means by the same letter in the same column is not significant difference (P <0.05), n = 3.

3.5. Chemical composition of starch

Table 5 shows the chemical composition of the isolated starch with and without enzyme-assisted isolation. The protein, ash and fat contents of the isolated starch without enzyme (control) was not significantly different from those of the starch isolated using enzyme treatment. The total carbohydrate content of sweet potato starch extracted with and without enzyme were not significant different (P>0.05). It meant that some of extraneous matter still remained in sweet potato starch product which was extracted by enzyme.

Table 5. Chemical composition of starch with and without enzyme-assisted isolation[1]

Composition (%, dry basis)	Starch isolated with water	Starch isolated with enzyme
Protein	0.31 ± 0.03^{a}	0.25 ± 0.02^{a}
Lipid	0.08 ± 0.01^{a}	0.10 ± 0.01^{a}
Ash	0.13 ± 0.01^{b}	0.08 ± 0.01^{a}
Total carbohydrate	99.37 ± 0.02^{a}	99.43 ± 0.02^{a}

[1]Means by the same letter in the same row is not significant difference (P <0.05), n = 3.

4. Conclusion

The isolation of starch with cellulose treatment was effective to improve the starch yield of sweet potato. The optimum enzymatic conditions for improving sweet potato starch extraction were found to be 3 U/g of concentration of cellulase, ratio of water and sample 150ml/100 g sweet potato at 4 h of incubation time Chemical composition of isolated starch with enzyme-assisted isolation was not significantly different from starch using without enzyme.

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