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Value Addition and Influence of Succinylation Levels on the Quality of Wild Yam (*Discorea villosa*) Starch

Akeem Olayemi Raji[1], Sofiat Damilola Ismael[1], Maimuna Sani[1], Monsurat Oyewale Raji[2] and Olubumi Florence Adebayo[1]

1. Department of Food Science and Technology, College of Agriculture and Veterinary Medicine, Kwara State University, Malete, Ilorin, Nigeria.

E-mail: akeem.raji@kwasu.edu.ng

2. Department of Food Technology, Faculty of Technology, University of Ibadan, Nigeria.

E-mail: rajimonsurat78@gmail.com

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Abstract

Background: Recent increase in demand for starch in food applications has called for research into other new sources. *Dioscorea villosa* is an underutilized wild yam starch source and its starch was isolated and succinylated at 0, 3, 6, 9 and 12% (0-12% succinic anhydride: 88-100% starch) using standard procedures. The degree of substitution, chemical composition, functional properties, anti-nutritional factors, pasting properties, atomic spectra and α- amylase and α-glucosidase activities of the samples were determined using standard methods. Data obtained were analysed using ANOVA.

Results: The percentage succinyl and the degree of substitution increased from 0 - 10.45% and 0 - 0.19% respectively. The amylose, amylopectin, α- amylase and α- glucosidase activities varied from 49.88-59.70%, 40.29-50.11%, 0.17-0.96 and 0.62-10.07 respectively. The phytate, tannin, oxalate and saponin ranged from 0.08 - 0.13 mg/g, 0.04 - 0.08mg/g, 0.21 - 0.31mg/g and 0.18 - 0.26mg/g respectively. The peak viscosity, trough, break down, final viscosity, set back, peak time and pasting temperature values ranged from 94.32 - 274.69 RVU, 83.50-197.50 RVU, 7.94-81.28 RVU, 113.69 - 367.39 RVU, 30.14 -173.89 RVU, 5.30 - 6.94 mins, and 84.78 - 94.70 ^{0}C respectively.

Conclusion: Isolation and subsequent succinylation improved the wild yam starch quality and reduced its anti-nutritional factors. Succinylation at 9% had the best functional and pasting properties, therefore making it suitable as a potential emulsifier, fat replacer to prevent obesity and stabilizer in food applications. Its limited enzyme activities make it desirable in the production of slow digestible food products, such as snacks for diabetic.

Keywords: Wild yam starch, succinylation, functional properties, nutritional quality

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1.0 Introduction

Yam belongs to the family *Dioscorea spp* and is a semi-perishable class of food due to its relative high moisture content.[1] Yam is a tuber crop that is grown widely in most part of the world (China, Korea, Japan, Ghana, America and Nigeria). Yam is majorly grown in sub-Saharan Africa, with the production of more than 95% of the global yam cultivation.[2] *Dioscorea villosa L* (common name wild yam or atlantic yam) is a member of the *Dioscoreceae* family and it is a deciduous perennial herbaceous twine that grows counterclockwise over small and medium-size shrubs. It grows abundantly in Mexico, the eastern and central regions of United States, south of Canada and Central America.[3] It has also been found in north central and south western parts of Nigeria. Yam contains mainly carbohydrates with little amount of proteins, lipids and vitamins.[4]

Starch is one of the most abundant organic chemicals on earth and it is a renewable resource. It is the major raw material in the food industry because of its good thickening and gelling properties, which makes it an excellent ingredient for the manufacture of various food products.[5] It is a semi- crystalline carbohydrate synthesized in plastids and it is found in the leaves of green plants. It is also synthesized in the amyloplasts of seeds, grains, roots, rhizomes and tubers of most plants where it serves as the chemical storage form of energy.[6]

Native starches are mainly used as food, but irrespective of their sources they are undesirable for many applications because of their inability to withstand processing conditions.[7] Starch succinates provide a number of desirable properties which include high viscosity, high thickening power, low gelatinization temperature, high paste clarity, better paste stability and good film forming properties.[8] Resistant starch or modified starch is regarded as a starch that can resist amylase digestion in the small intestine to some varying degrees, and are degraded to short-chain fatty acids by the action of microflora in the large intestine.[9] However, introduction of resistant starch to the diet also dilutes dietary energy density. This is desirable in the production of slow digestible food products, such as snacks for people suffering from diabetes.[9]

However, the food situation in most developing tropical countries is alarmingly worsening, this is because of the increasing population and shortage of fertile land.[10] This has also affected the demand for starch as a raw material for food, pharmaceutical and allied industries, thereby

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preventing African attainment of self-sufficiency in starch production. Some lesser known and unconventional crops could be good sources of nutrients and starch, and even have the potential of broadening the present narrow food base of the human species.[11] *Dioscorea villosa* is one of the underutilized starch sources, and it is less explored. Therefore, research direction should be geared towards *Dioscorea villosa* starch succinylation and its potential application in food systems.

2.0 Materials and methods

2.1 Materials

Dioscorea villosa tubers were purchased at Malete Market, Ilorin, Kwara state, Nigeria, while the reagents used for succinylation were purchased from Showcrown laboratory, Ibadan, Oyo State, Nigeria.

2.2 Methods

2.2.1 Isolation and purification of native *dioscorea villosa* starch

The method of Emeje *et al.* [12] with slight modifications was used. *Dioscorea villosa* tubers were peeled, washed with clean water and chopped into smaller sizes (1.0 cm thick). The yam slices (500g each) were then dropped into a 0.2% (w/v) sodium metabisulphite solution to prevent enzymatic browning. The treated slices were blended in a conventional blender (MARSHAL hex CORONA, USA) for about 2-3 mins. The slurry obtained was sieved with a muslin cloth and further soaked in 1.5 liters of 0.03 M NaOH solution for 1 hour. The supernatant was decanted and the residue was neutralized with 0.5M HCL solution to a pH of 6.0. The neutralized residue was washed three times with distilled water and centrifuged at 1500 rpm for 5 mins. The wet starch obtained was thinly spread on trays, dried in a forced draught air oven drier (CARBOLITE PF 200, England) at 50^{o}C for 12 h.

2.2.2 Succinylation of *Dioscorea villosa* starch

The method of Awokoya *et al.*[13] was used for the succinylation of purified *Dioscorea villosa* starch at ambient temperature (30^{o}C). Starch (100g) was dispersed in 300 mls of distilled water and stirred magnetically for 1h, after which it was adjusted to a pH 11.0 using 0.03 M NaOH solutions. The samples were succilynated using succinic anhydride at 0, 3, 6, 9 and 12% and the

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mixture were stirred gently using a magnetic stirrer for 2 h. At the end of the reaction, the pH of each slurry was adjusted to pH 6.0 using 0.03M NaoH solution, filtered, washed with distilled water for six times and dried in a forced draught air oven drier (CARBOLITE PF 200, England) at 50^{o}C for 12 h, milled and kept in a air tight jar to prevent re-absorption of moisture.

2.2.3 Determination of percentage starch yield

The percentage yield of starch on dry basis was determined using the method of Moorthy [14] and was expressed as % dry weight of starch per unit dry matter of peeled yam flesh.

2.2.4 Determination of degree of substitution of succinylated starch

The alkali saponification method described by Jyothi *et al.*[15] was used. An accurately weighed quantity of 0.5 g of the starch sample was measured in a 100 mls conical flask, containing 25 mls of 75% ethanol. It was mixed thoroughly and 20mls of 0.5 M aqueous sodium hydroxide solution was then introduced into the mixture. Occasional swirling of the flask was done as it was kept for 72 h. Back titration of excess alkali was done with 0.5M hydrochloric acid). Percentage succinyl and degree of substitution (DS were determined using Equations 1 and 2 respectively.

$$\% \text{ succinyl} = \frac{(\text{Blank titre} - \text{sample titre}) \times 0.1 \times \text{molarity of acid} \times 100}{\text{Weight of sample}} \quad (1)$$

$$\text{Degree of substitution (DS)} = \frac{162 \times \% \text{ succinyl}}{10{,}000 - (99 \times \% \text{ succinyl})} \quad (2)$$

2.3 Analyses

2.3.1 Proximate Composition

The proximate composition which comprises of the moisture, protein, ash, fat, crude fibre and carbohydrate of the samples were evaluated using AOAC [16] methods.

2.3.2 Amylose and amylopectin

Total amylose content of each sample was determined iodimetrically following the standard procedure described by Morrison and Laignelet [17], while the percentage of amylopectin was then

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evaluated by subtracting the percentage amylase content from the amount of carbohydrate in the sample.

2.3.3 Physicochemical properties

2.3.3 .1 Determination of water absorption capacity (WAC)

Abbey and Ibeh [18] method was employed for the determination of water absorption capacity. One gram of each of sample was weighed separately and distilled water was added to it in order to make it up to 10 mls of dispersion. The mixture was then centrifuged at 3500 rpm for 15 mins. The supernatant obtained was discarded and the tube with its contents was reweighed as gram water absorbed per gram of sample. The gain in mass was expressed as the water absorption capacity of the starch sample.

2.3.3 .2 Oil absorption capacity (OAC)

Two grams of each starch sample was mixed with 20 mls of oil in a blender operating at a high speed for 30s. The mixture was allowed to stand at 30^{o}C for 30min, and was later centrifuged at 1000 rpm for 30 mins. The supernatant volume in the graduated cylinder was noted. Densities of water and oil were assumed to be 1 g/ml and 0.93 g/ml. Results of the means of triplicate determinations were reported.[18]

2.3.3 .3 Determination of Bulk Density

The bulk densities of the samples were determined using Okezie and Bello [18] method. A known gram of flour sample was weighed into measuring cylinder (W_1) and tapped gently to eliminate spaces within the flour particles. After tapping, volume occupied by the sample in the cylinder was noted and then weighed (W_2).

$$\text{Bulk density (g/cm3)} = \frac{W_2 - W_1}{\text{Volume of sample}} \quad (3)$$

2.3.3 .4 Swelling power and solubility index

Solubility and swelling power were determined using the modified methods of Adebowale *et al.*[19]. A gram of yam starch was poured into a 50 ml graduated centrifuge tube containing 40ml

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of distilled water. The suspension was stirred gently to avoid fragmentation of the starch granules. The suspension was heated in the tube at 85°C with constant gentle stirring, in a water bath with thermostatic regulated temperature for 30 mins. After heating, the tube was removed from the water bath, wiped to dryness on the outside, cooled to room temperature and centrifuged at 2200 rpm for 15 mins. The swelling power was calculated as the weight of paste sediment per gram of starch used. The supernatant was poured into a pre-weighed moisture can and evaporated in a thermostatically controlled drying oven at 105°C.The residue left behind was weighed and the solubility index was determined. Swelling power (SP) and solubility index (SI) were calculated by:

$$\text{SP} = \frac{\text{Weight of wet mass of sediment}}{\text{Weight of dry matter in the gel}} \quad (4)$$

$$\text{SI}\ (\%) = \text{Weight of dry solids after drying x } \frac{100}{2.5} \quad (5)$$

2.3.3 .5 Gelatinization temperature

Gelatinization temperature was determined according to the method of Chavan *et al*.[20]. One gram of each starch of sample was weighed accurately in replicate and poured into 20 mls screw capped tubes. Distilled water (10 mls) was added to each sample. The samples were gently heated in a water bath until solid gels were formed. The respective temperature was determined and taken as gelatinization temperature at complete gel formation.

2.3.4 Pasting Property Determination

The method of IITA[21] was used for the determination of pasting properties (peak viscosity, trough, breakdown viscosity, final viscosity, set back viscosity, peak time and pasting temperature). The pasting characteristics of the samples were measured using a Rapid Visco Analyzer (Model RVA-4C, Newport Scientific, Warriewood, Australia), which had an interface with a personal computer that was equipped with the thermocline software supplied by same manufacturer.

2.3.5 Anti-nutritional factors

Anti-nutritional factors such as tannin, saponin and oxalate were determined using AOAC[16] methods.

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2.3.6 FT-IR spectroscopy

The IR spectra of each starch sample was run as KBr pellets on FTIR System (Spectrum BX PerkinElmer, England) in the frequency range 350–40000 c/m or more in accordance with the slightly modified method of Gbenga *et al.*[22]. Two milligrams of each sample was mixed uniformly with pure KBr powder. The mixture was kept in an evacuable KBr die with a 13 mm clear disk and it was hydraulic pressed to form a KBr pellet using the pressure in range of 10,000–15,000 lbs for all samples to prevent polymorphic changes. The pellet of each sample was placed in a cell holder (Universal Demountable Cell), the holder containing the pellet was inserted into the FTIR equipment and scanned at a range of 350–4000nm or more. The spectrum of the suspected compound was recorded and it was visually displayed on the computer screen.

2.3.7 Enzyme digestibility

2.3.7.1 α-amylase

The procedure described by Kwon *et al.*[23] was used for the determination of alpha amylase inhibition assay with slight modification. *Dioscorea villosa* starch (15 g) was introduced into 10 ml of distilled water and homogenized for 1 min with a waring laboratory blender (Winsted, CN) placed on ‘‘high’’. The homogenate was subjected to centrifugation at 5000 rpm for 10 min. The supernatant obtained was filtered under vacuum through a whatman no 2 filter paper. The crude extract obtained was then used for in vitro assays. Porcine pancreas α amylase (No.A3176) was purchased from Sigma, Cat., Chemical Co., St. Louis, MO, USA. A 500 µ L of starch extract and 500 µ L of 0.02 mol/L sodium phosphate buffer (pH 6.9 with 0.006 mol/L sodium chloride) which possessed 0.5 mg/mL α amylase solution were pre incubated at 37°C for 10 min. Thereafter, 500 µL of 1% *Dioscorea villosa* starch solution in 0.02 mol/L sodium phosphate buffer was introduced. The mixture was eventually incubated at 37°C for 15 min, and the reaction was stopped with 1.0 mL dinitrosalicylic acid (DNSA) color reagent. The reaction mixture was further incubated at 100°C in a water bath for 5 min and cooled to ambient temperature. The cooled reactant mixture was diluted with 10 mL distilled water. The reference test (absence of starch extract) was also determined. The absorbance was measured at 540 nm and the percentage α amylase inhibition was expressed using the formula stated below:

$$\%\ \text{Inhibition} = \frac{\text{Abs}_{540}(\text{control}) - \text{Abs}_{540}(\text{extract}) * 100}{\text{Abs}_{540}(\text{control})} \quad (6)$$

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Where Abs_{540} (control) is the absorbance of the reference test and Abs_{540} (extract) is the absorbance of the test containing the extract.

The plots of percentage inhibition versus log inhibitor concentration were used to determine the IC_{50} values, and were measured by non linear regression analysis from the mean inhibitory values. Acarbose was the reference inhibitor that was used and each sample was analysed in replicate.

2.3.7.2 α-glucosidase

The modified method of Kim *et al.*[24] was used to determine the inhibitory effect of the extracts against α-glucosidase, using α-glucosidase from *B. stearo thermophillus* (Sigma, Cat. No. G 3651). Five units of α-glucosidase was incubated with different extracts dilutions (0-200 µ L) for 15 mins. After pre incubation, 3 mol/L para nitrophenyl glucopyranoside (PNPG) dispersed into 20 mmol/L phosphate buffer (pH 6.9) was added to initiate the reaction. The reaction mixture was then incubated at 37°C for 20 min. Thereafter, 2 mL of 0.1 mol/L Na CO was added to terminate the reaction. The absorbance (yellow colour) from PNPG was read at 400 nm. The reference test (absence of starch extract) was also determined. The percentage α glucosidase inhibition was expressed using the formula stated below:

$$\%\ \text{Inhibition} = \frac{\text{Abs}_{400}(\text{control}) - \text{Abs}_{40}0(\text{extract}) * 100}{\text{Abs}_{400}(\text{control})} \quad (7)$$

Where Abs_{400} (control) is the absorbance of the reference test and Abs_{400} (extract) is the absorbance of the test containing the extract.

The plots of percentage inhibition versus log inhibitor concentration were used to determine the IC_{50} values, and were measured by non linear regression analysis from the mean inhibitory values. Acarbose was used as the reference inhibitor and each sample was analysed in replicate.

2.4 Statistical analysis

Data obtained in replicate were analysed using statistical package for social science (SPSS) version 16.0 package software ($p < 0.05$).

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3. Results and Discussion

The results obtained from various analyses carried out on the succinylated wild yam starch are discussed below:

3.1 Starch yield

The yield of the native starch was 81.44±3.22% (dry weight basis). The result obtained fell within the general range of 60 to 82% (dry weight basis) reported for yam species by Otegbayo *et al*. (2011).[25]

3.2 Effect of succinylation levels on the % succinyl content and degree of substitution (DS)

The results obtained for the % succinyl content and degree of substitution (DS) ranged from 5.32-10.45% and 0.10-0.20 respectively. Linear models were the best to describe the relationship with succcinylation levels of the starch (Figures 1-2). The coefficient of determinations R^2 of their linear models (0.93-0.95) suggested that they might fit well for the above data. It was observed that the % succinyl content and degree of substitution (DS) of the succinylated yam starch samples increased as the levels of succinylation (inclusion of succinic anhydride) increased. Degree of substitution (DS) is expressed as the average substitution number per anhydroglucose unit (AGU), which ranges from 0 to 3.[26] However, the values obtained in this study for degree of substitution fell within the standard recommended range for DS values (0-3). However, increase in the degree of substitution value could be due to swelling power of the *Dioscorea villosa* starch granules, diffusion and absorption processes of the reactants, and enhanced contact between the esterifying agent and starch. [13]

3.3 Effect of succinylation levels on the proximate composition of wild yam starch

Table 1 shows the proximate composition of both native and succinylated starch samples. The moisture, carbohydrate, protein, ash, crude fat and crude fibre contents ranged from 7.22 - 11.22%, 85.53- 86.28%, 2.30 - 6.11%, 0.16 - 0.40%, 0.18 - 0.45% and 0.09 - 0.23% respectively. However, succinylation levels had significant influence on the proximate composition of the

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dried wild yam starch succinates. The values obtained for moisture contents were within the 10-20% range recommended for commercial starches.[27] This implies that the dried starch samples might have better storage stability. High carbohydrate contents (> 85%) observed in all the yams samples implied that *Dioscorea villosa* yam is a good carbohydrate source. Succinylation decreased the values of non-starch components (protein, ash and fat content) of native starch and make it useful in some industrial applications.[8,13, 26] However, it was discovered that the protein content of the native starch decreased by 54.65 - 62.36% after succinylation at various levels (3.0 -12.0%). Lowest protein content was obtained from the sample that was succinated at 9%. The protein content of the native starch was higher than the values Ihegwuagu *et al.* [28] reported for native sweet potato starch (0.17%) and Olayinka *et al.* [26] reported for native red and white varieties of sorghum starches (1.54 - 2.27%), but lower than the values Arueya and Oyewale [9] established for acha starch (7.44%). The low values of protein observed in the *Dioscorea villosa* starch succinates depicted the level of purity of the modified starch samples as shown by low levels of nitrogen and ash. The ash contents obtained for both native and modified starch samples were not above the recommended limit (0.5%) for grade A industrial starches, [13] implying that the starch samples might have good functional properties. It was observed that the starch fat contents decreased up till 6% succinylation level and increased slightly thereafter. However, reduction in fat content observed after succinylation might be due to washing of degraded starch molecule. Similar findings were reported by Olayinka *et al.* [26] who worked on effect of succinylation on the properties of red and white sorghum starches and Olu-Owolabi *et al.* [29] who researched on chemical modification of starch from acha grains. Similarly, it was noticed that there was a significant decrease in the crude fiber contents of samples up till 6% succinylation level and changed inversely thereafter. Decline in the crude fibre contents observed after succinylation of native *Dioscorea villosa* starch sample is agreement with the previous reports.[27]

3.4 Effect of succinylation levels on the amylose and amylopectin contents of wild yam starch

The amylose and amylopectin contents of the starch samples varied from 49.89-59.70% and 40.28-50.11% respectively. Sample succinated at 3% succinic anhydride inclusion had the highest amylose content, but the lowest amylopectin content. Sample with 6% succinylation level had the lowest amylose content, but the highest amylopectin content. Variations in the

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amylose and amylopectin contents of the starch succinates depend on the quantity of incorporated succinyl groups in modified starch carbon chains. [30] Moderate amylose contents observed in the succinylated *Dioscorea villosa* showed that if they are used in food systems, their swelling ability will be enhanced. [31]

3.5 Effect of succinylation levels on the functional properties of wild yam starch

Table 2 shows the result of the functional properties of native and succinated wild yam starch samples. The bulk densities, water absorption capacities, oil absorption capacities, swelling capacities, solubility indices and gelatinization temperatures of the samples varied from 0.71-0.82 g/ml, 78.67- 109.33%, 78.00 – 107.33%, 9.37 - 10.07 %, 2.67 – 5.33% and 57.67- 68.67^{o}C respectively. It was observed that the bulk density increased as the succinylation level increased. Bulk density is a parameter that can be used as an indicator for food product porosity, as it influences packaging design and choice of packaging materials. [32] The outcome of this finding might provide an insight into better packaging of D. *villosa* starch succinate, using bulk density as a reference parameter. The water absorption capacities of the starch samples decreased up till 6% succinylation level and increased moderately thereafter. Water absorption capacities in the succinylated samples were lower than the value obtained for the native starch sample. This might be due to rise in starch crystallinity which prevented water to migrate into the granules of the succinylated starch. This result is in accordance with the findings reported on water absorption capacities of chemically modified acha starch. [29] Higher oil absorption capacities observed in this study reveals that the starch might possess good emulsion stability. However, chemical modification of starch improves its oil absorption capacity, due to incorporation of functional groups in the molecules of starch, making it to have a high binding capacity than the native starch. [29] There was slight variation in the swelling capacities of the samples. The variation might be due to weakening of intermolecular hydrogen bonds, which is caused by the introduction of the succinyl group. Swelling capacity increased at some succinylation levels of the native starch, and this might be due to the increase in the long chains of amylopectin with the concurrent decrease in amylose content.[31] It was revealed that the solubility index of the starch samples decreased up till 3% succinylation level and increased moderately thereafter. Interestingly, starch succinates exhibited less water solubility than the native starch. However,

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316 such behaviour, might be linked to starch structural reorganization, which tends to weaken the
317 granules of starch during succinylation. Similar finding was established by Arueya and Oyewale
318 [8] who worked on influence of degree of succinylation on acha starch. Notably, there was a
319 significant increase in the gelatinization temperature up till of samples up till 9% succinylation
320 level and changed inversely thereafter. However, the gelatinization temperatures obtained in this
321 study fell within the range reported by Segura-Campos *et al.*[30] for octenylsuccinic starch from
322 *Phaseolus lunatus*. Lower gelatinization temperatures (57.67- 68.67^{o}C) observed in this study is
323 advantageous, as such starch samples could be used in processes where thickeners must gel at
324 lower temperatures.[30]. This might also conserve cooking energy.

325

326 **3.6 Effect of succinylation levels on the pasting properties of wild yam starch**

327

328 Table 3 shows the pasting properties of both native and succinylated *D. villosa* starch samples.
329 The peak viscosity, trough, breakdown viscosity, final viscosity, Set back viscosity, peak time
330 and pasting temperature values ranged from 94.36 - 274.69 RVU, 83.50 - 197.50 RVU, 7.94 -
331 81.28 RVU, 113.69 - 367 RVU, 30.14 - 173.89 RVU, 5.30 - 6.94 mins and 84.78 - 94.70 °C
332 respectively. The level of succinylation significantly influenced the pasting properties of the
333 samples at 95% confidence level. High peak viscosity indicates high starch content and it is
334 directly related to the water binding capacity of starch.[19] The peak viscosities observed in this
335 study for the modified starch samples were lower than the values (195.17-399.58 RVU) reported
336 by Ezeocha and Okafor [34] for D. *dumetorum*, but the native *D. villosa* starch peak viscosity fell
337 within the range stated above. The lower peak viscosities of the succinylated starch samples
338 implied that the succinylated granules were less rigid or more elastic, becoming progressively
339 fragile and disintegrated due to shearing effect. The reduction in the peak viscosity that was
340 observed in this study was in line with the finding of Arueya and Oyewale [8] who researched on
341 influence of degree of succinylation on acha starch. All the trough values obtained in this study
342 fell within the range (133.45 - 206.60 RVU) reported by Arueya and Oyewale [8] for acha starch
343 succinates, except for the starch sample that was succinylated at 6% which had the least trough
344 value. The values were also in the range of values (46.67 to 221.67 RVU) reported by Harijono
345 *et al.* [35], who researched on effect of blanching on properties of water yam (*Dioscorea alata*)
346 flour. High trough values noticed in native starch and starch succinates produced at 3, 9 and 12%

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succinylation levels imply that their paste might possess strong ability to withstand breakdown during cooling. Worthily, *D. villosa* starch succinates significantly exhibited lower breakdown viscosities when compared with the native starch. This finding was in line with the findings of Zainal *et al.*[36] who researched on carboxymethylation of sago starches and Arueya and Oyewale [8] worked on succinylated acha starch. However, lower breakdown viscosities observed in *D. villosa* starch succinates implies that they might have hot paste advantage over the native starch. Considerably high final viscosities observed in this study shows that they might possess good pasting quality. It is noteworthy that the relatively low setback observed in the succinylated starch samples, shows that the starch succinates possess low tendency to retrograde. This implies that *D. villosa* starch succinates might be useful in food processing where staling, quick precipitation of sols and loss of viscosity as a result of high retrogradation are their major concerns.[37] Sample whose succinylation level was 9% had the highest peak time value, while the native starch sample (with no succinylation) had the lowest peak time value. Lower pasting time observed in this study make the *D. villosa* starch samples suitable for the production of foods where shorter processing time is needed. The observed values were also far below the values established for aerial yam flour and starch (24.30 and 19.25 mins) by Sanful and Engmann [37], but were within the range (5.23 to 6.33 mins) reported by Arueya and Oyewale [9] for acha starch succinates. The pasting temperature values observed in this study were moderately higher than the values Arueya and Oyewale [8] reported for acha starch succinates and Amao *et al*. [38] reported for yam flours (75.1 to 77.3°C). Worthily, samples succinylated at 3% and 9% had the pasting temperatures (85.22°C and 86.25°C) that were closed to the value reported by Sanful and Engmann [37] for aerial yam flour (85.50°C).

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3.7 Effect of succinylation levels on the anti-nutritional factors in wild yam starch

The anti-nutritional contents of the starch are shown in table 4. The saponin, tannin, oxalate and phytate contents observed in this study varied from 0.18- 0.26 mg/g, the contents of tannin in the samples varied from 0.04-0.0 mg/g, 0.04-0.0 mg/g, 0.21- 0.31mg/g and 0.08 - 0.13mg respectively. It is noteworthy to say that soaking, purification, succinylation and subsequent drying reduced the anti-nutritional contents of the starch samples to less harmful levels. This observation corroborates with the findings of Cemaluk *et al.*[39] who reported on the effect of soaking prior to oven-drying on some nutrient and anti-nutrient properties of bitter yam.

3.8 Effect of succinylation levels on the FT-IR spectral of wild yam starch samples

The FT-IR spectrum has proven to be a valuable tool to identify the functional group of the active components. The Fourier Transform Infrared (FT-IR) spectra of *Dioscorea villosa* starch showed typical absorption bands of a starch backbone. There was no strongly pronounced difference between the FT-IR spectrum profile of both native and succinylated starch (Figures 3-7). For the 0%, 3%, 6%, 9% and 12% succinylation levels, a characteristics bonds occurring at 3423.00cm^{-1}, 3425.00 cm^{-1}, 3425.00 cm^{-1}, 3422.00 cm^{-1} and 3426.00 cm^{-1} respectively corresponded to the O–H stretching vibration. Usually, absorption range for O–H vibration is 3700–3500 cm^{-1}, but the band in *Dioscorea villosa* starch was different, the O–H stretching peaks shifted to a lower wavelength. This might be as a result of intermolecular hydrogen bonds in the glycosidic ring weakening the O–H bond, thereby shifting the band absorption region to a lower frequency. Also, the O–H stretching showed the presence of carbohydrate. Similar result was observed by Arueya and Oyewale [8] who evaluated the FT-IR spectrum characteristics of succinylated acha starch.

3.9 Effect of succinylation levels on the enzymatic activities of wild yam starch

The values obtained for α- amylase and α- glucosidase activities of the starch samples ranged from 0.17-0.96 and 0.62-10.07 respectively. However, their plots against succilynation levels and their mathematical expressions are shown in figures 8-9 respectively. The graphs both showed a quadratic relationship and their coefficients of determination (R^2) were 1.00, suggesting that the models might fit well for the above data. It was observed that the α-amylase

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activity decreased as the succinylation levels increased up to 6% succinylation level, but increased thereafter. Contrarily, the α- glucosidase activity increased as the succinylation levels increased up to 6% succinylation level, but decreased thereafter. Reasonably, the succinyl groups' introduction altered the molecular associations of the starch molecules, thereby limiting the accessibility of the enzymes to the active sites for structural degradation. This is desirable in the production of slow digestible food products, such as snacks for people suffering from diabetes. Similar observation was reported by Marcazzan *et al*. [40] who reported on lower α-amylase activity towards succinylated starch, amylose and amylopectin. It was also in line with the findings of Jyothi *et al.* [41] who researched on the synthesis and characterization of low DS succinate derivatives of cassava (*Manihot esculenta* Crantz) starch, and Simsek *et al*.[42] who observed a decrease in starch digestibility upon succinylation.

4.0 Conclusion

Isolation and subsequent succinylation improved the wild yam starch quality, in relation to its functional, chemical composition, pasting properties and starch digestibility. It also significantly reduced its anti-nutritional factors. However, succilynation at 9% had the best functional and pasting properties, therefore making it suitable as a potential emulsifier and stabilizer in food applications.

Acknowledgement

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Table 1: Chemical composition of *Dioscorea villosa* starch

Level of succilynation (%)	Moisture content (%)	Protein content (%)	Fat content (%)	Ash content (%)	Crude fibre content (%)	Carohydrate content (%)	Amylose content (%)	Amylopectin content (%)
0.0	7.22±0.19^{d}	6.11±0.25^{a}	0.45±0.20^{a}	0.40±0.02^{a}	0.23±0.01^{a}	85.58±0.20^{a}	57.52±1.16^{b}	42.48±1.16^{c}
3.0	10.80±0.23^{a}	2.77±0.21^{a}	0.21±0.16^{c}	0.18±0.01^{c}	0.10±0.01^{c}	85.93±0.32^{a}	59.70±0.23^{a}	40.28±0.23^{d}
6.0	11.22±0.19^{a}	2.44±0.10^{bc}	0.18±0.01^{c}	0.16±0.01^{c}	0.09±0.00^{c}	85.90±0.17^{a}	49.89±0.11^{d}	50.11±0.11^{a}
9.0	9.40±0.64^{b}	2.30±0.25^{c}	0.32±0.19^{b}	0.28±0.02^{b}	0.16±0.01^{b}	85.53±0.87^{a}	53.30±0.42^{c}	46.70±0.42^{b}
12.0	8.62±0.50^{c}	2.33±0.30^{c}	0.32±0.22^{b}	0.28±0.02^{b}	0.16±0.01^{b}	86.28±0.19^{a}	54.37±0.84^{c}	45.63±0.84^{b}

Values are mean ± S.D (Values with the same superscript are not significantly different at ($P \leq 0.05$)

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Table 2: Functional properties of *Dioscorea villosa* starch

Level of Succilynation (%)	Water absorption capacity (%)	Oil absorption capacity (%)	Swelling capacity (%)	Bulk density (g/ml)	Solubility index (%)	Gelatinization temperature (^{0}C)
0.0	109.33±9.29^a	91.67±4.04^b	9.57±0.32^{ab}	0.71±0.01^d	5.33±1.15^a	57.67±0.58^d
3.0	94.67±6.66^b	78.00±1.73^c	10.07±0.28^a	0.76±0.01^b	2.67±1.15^b	62.67±1.15^c
6.0	78.67±7.02^c	85.67±6.43^{bc}	9.37±0.19^b	0.74±0.11^c	3.10±1.01^{ab}	63.00±1.00^c
9.0	91.33±3.06^b	87.33±6.03^{bc}	9.79±0.26^{ab}	0.81±0.01^a	3.30±1.15^{ab}	68.67±0.58^b
12.0	98.33±3.06^{ab}	107.33±7.57	10.03±0.23^a	0.82±0.01^a	4.00±2.00^{ab}	65.67±0.58^a

Values are mean ± S.D (Values with the same superscript are not significantly different at ($P \leq 0.05$)

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Table 3: Pasting properties of succinylated *Dioscorea villosa* starch

Succilynation Level (%)	Peak (RVU)	Trough (RVU)	Breakdown (RVU)	Final Viscosity (RVU)	Set Back (RVU)	Peak Time (mins)	Pasting Temperature (^{0}C)
0.0	274.69±0.17^a	197.50±0.17^a	81.28±0.21^a	367.36±0.71^a	173.89±0.34^a	5.30±0.10^b	84.78±0.15^c
3.0	187.86±1.62^b	179.97±1.56^b	7.94±0.13^e	222.47±5.97^b	42.30±4.24^b	6.92±0.08^a	85.22±1.09^{bc}
6.0	94.36±0.17^e	83.50±0.17^e	10.94±0.13^c	113.69±0.92^e	30.14±0.75^c	6.93±0.12^a	94.70±0.33^a
9.0	162.92±1.78^d	153.25±1.54^c	9.33±0.17^d	187.08±4.20^d	32.67±2.25^c	6.94±0.05^a	86.25±0.64^b
12.0	167.67±1.30^c	153.75±0.33^c	13.58±0.83^b	193.86±0.39^c	40.00±0.83^b	6.61±0.68^a	86.08±0.47^b

Values are mean ± S.D (Values with the same superscript are not significantly different at ($P \leq 0.05$)

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Table 4: Antinutritional factor of *Dioscorea villosa* starch

Level of Succilynation (%)	Phytate (mg/g)	Tannin (mg/g)	Oxalate (mg/g)	Saponin (mg/g)
0.0	0.13±0.02[a]	0.08±0.01[a]	0.26±0.01[b]	0.22±0.08[a]
3.0	0.08±0.01[b]	0.07±0.01[a]	0.21±0.01[c]	0.21±0.09[a]
6.0	0.09±0.00[b]	0.04±0.00[b]	0.22±0.02[c]	0.25±0.02[a]
9.0	0.08±0.00[b]	0.04±0.00[b]	0.30±0.01[a]	0.18±0.03[a]
12.0	0.08±0.01[b]	0.05±0.00[b]	0.31±0.01[a]	0.26±0.02[a]

Values are mean ± S.D (Values with the same superscript are not significantly different at ($P \leq 0.05$)

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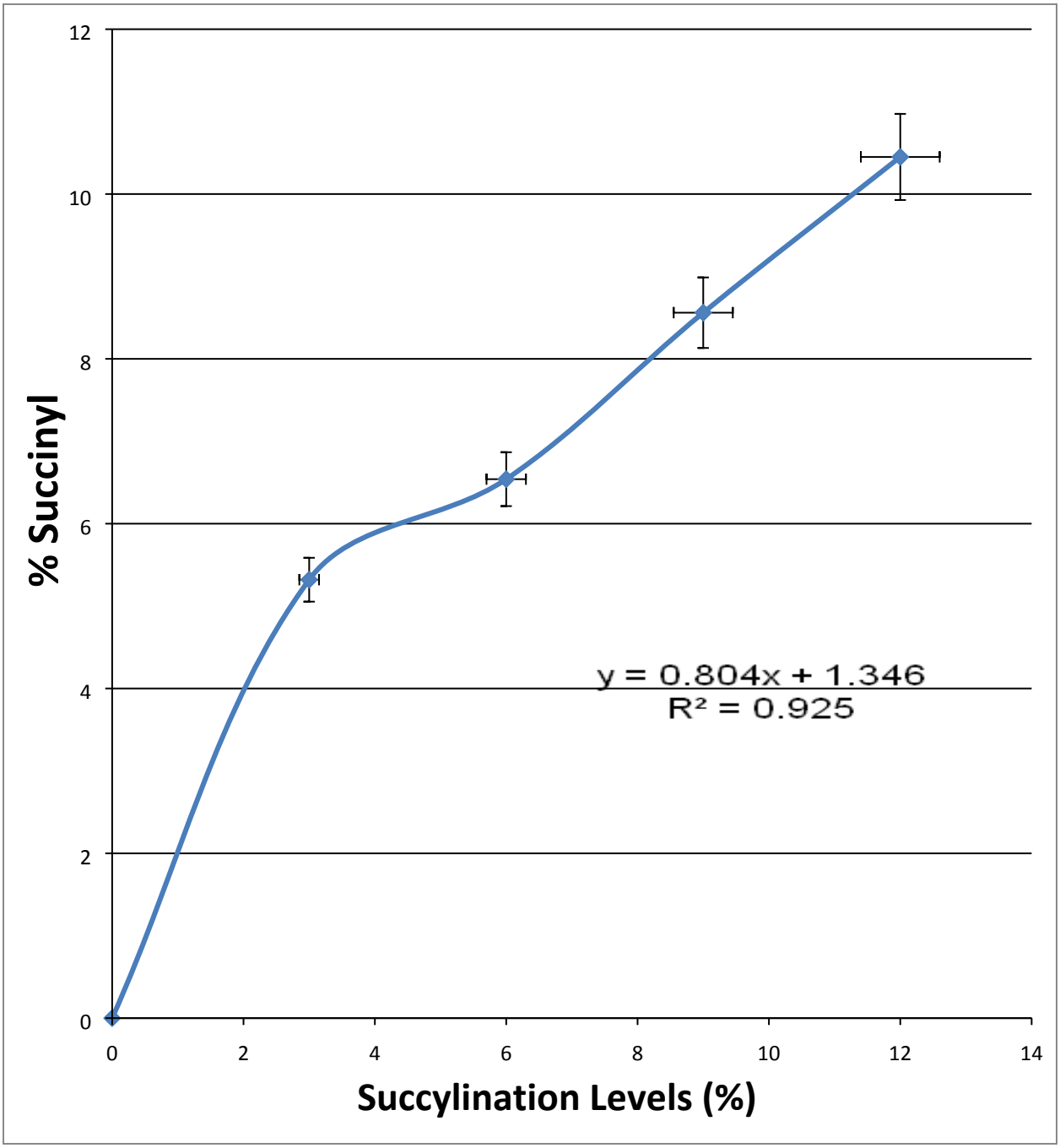


Figure 1: Percentage succinyl of Dioscorea villosa starch succinylated at different levels

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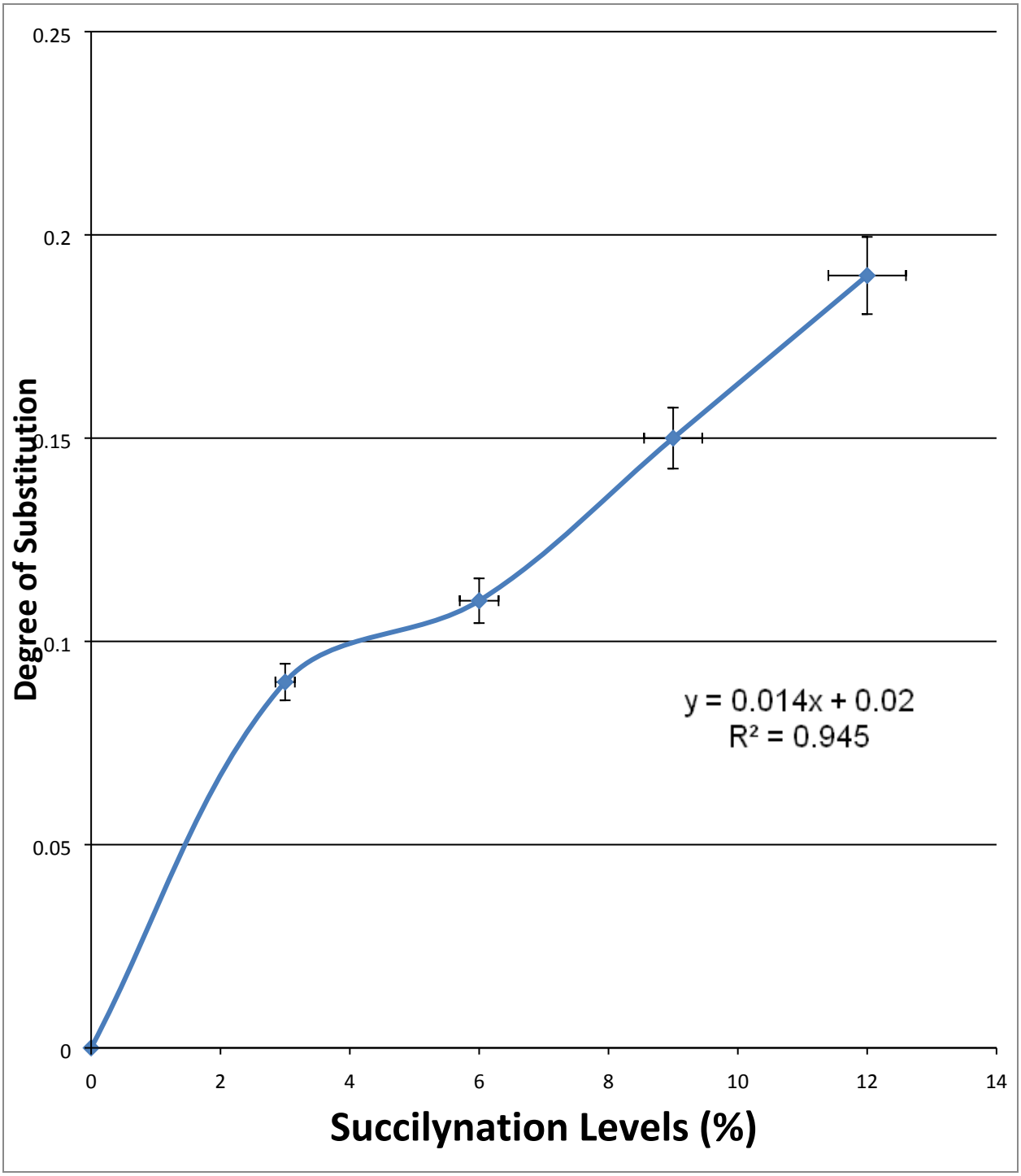


Figure 2: Degree of substation of *Dioscorea villosa* starch succinylated at different levels

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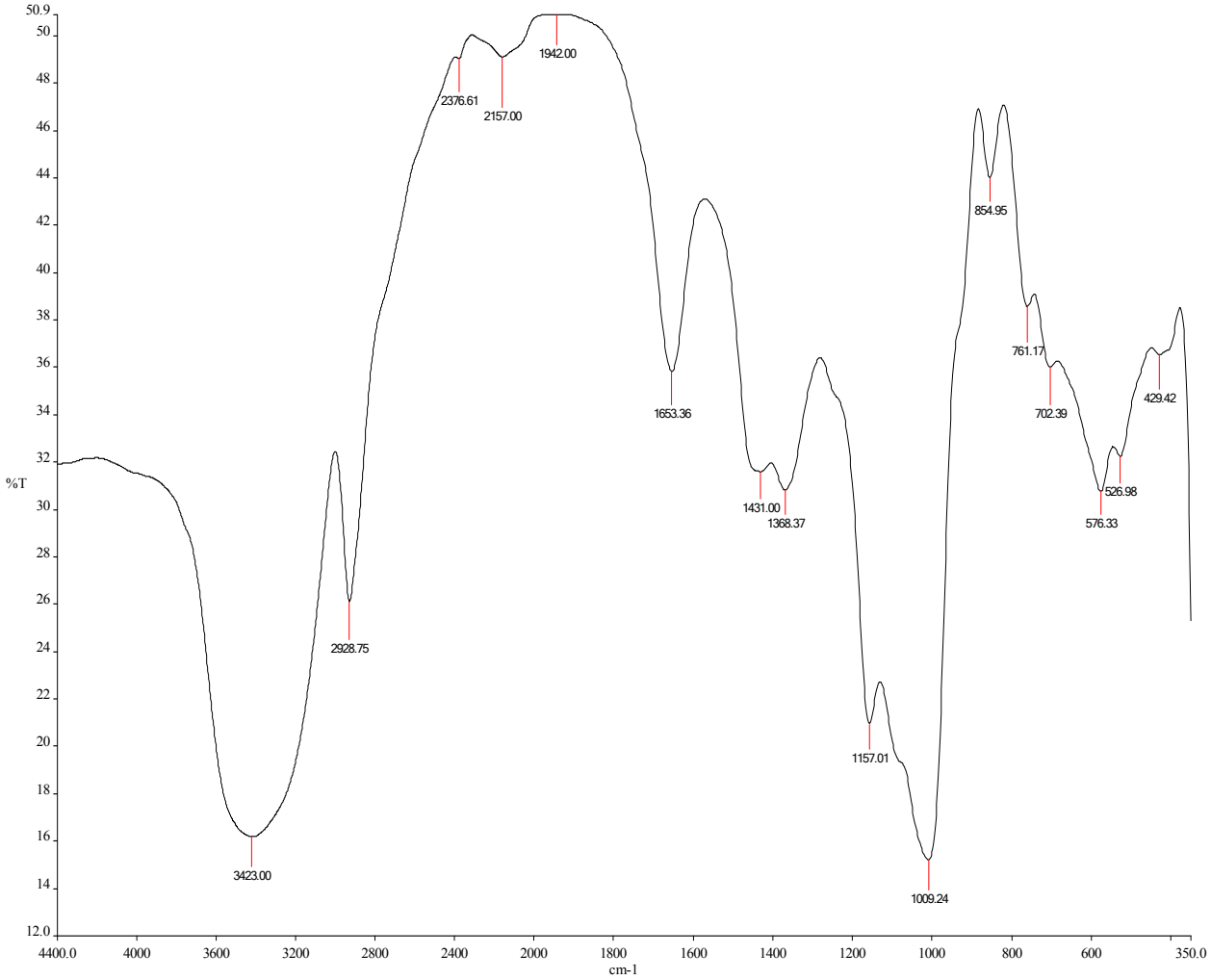


Figure 3: FTIR Graph for 0% Succinylation of *Discorea villosa* Starch

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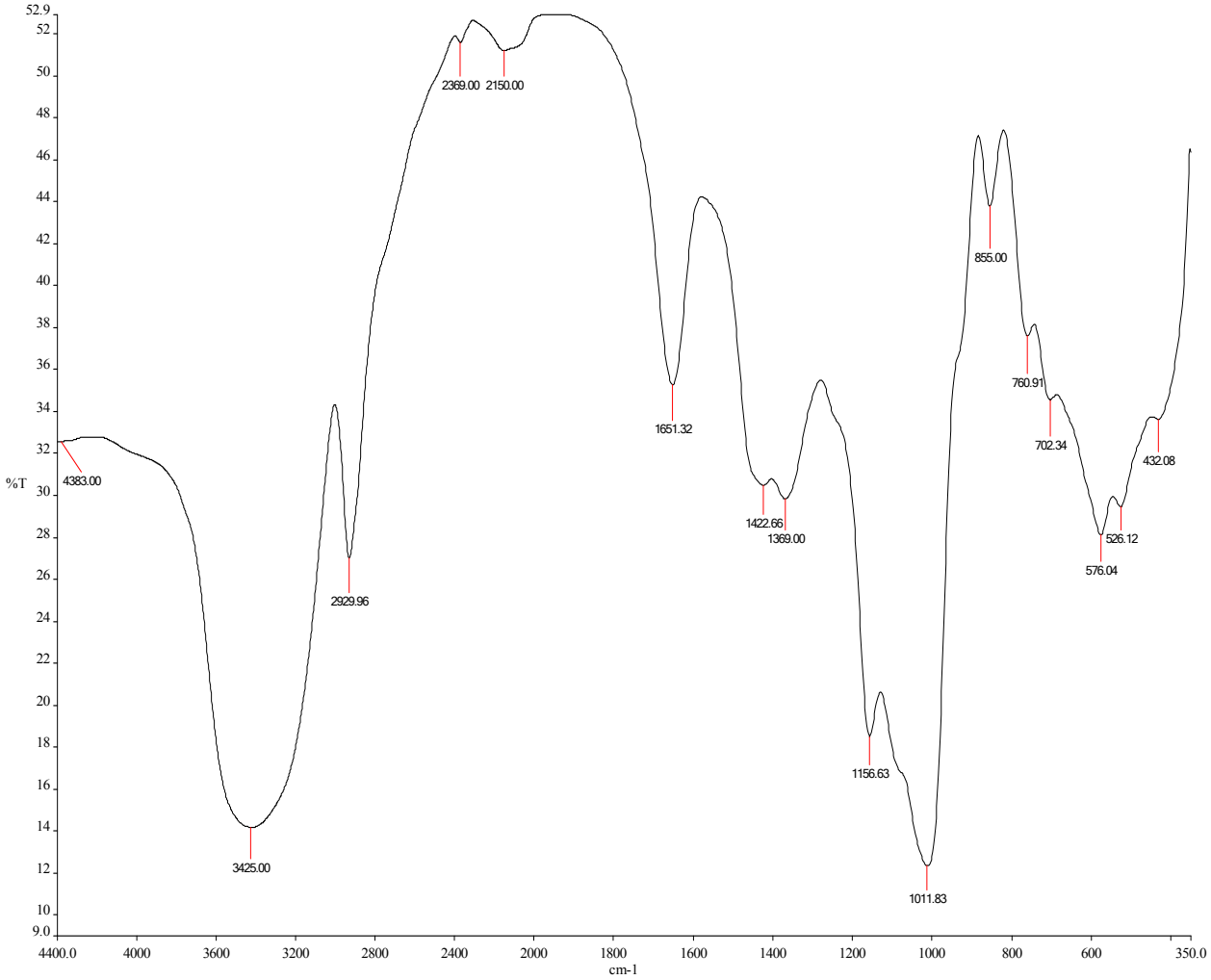


Figure 4: FTIR Graph for 3% Succinylation of *Discorea villosa* Starch

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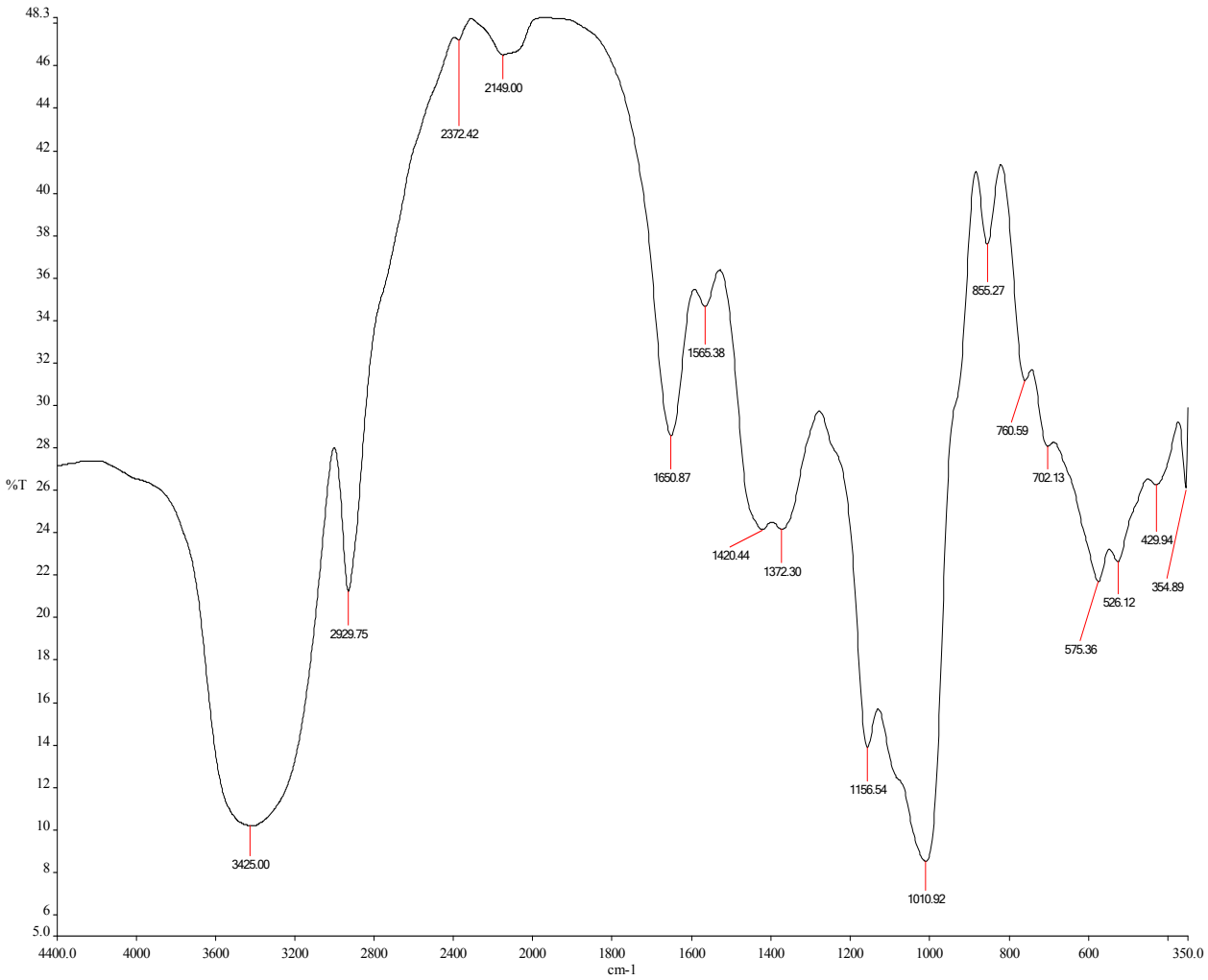


Figure 5: FTIR Graph for 6% Succinylation of *Discorea villosa* Starch

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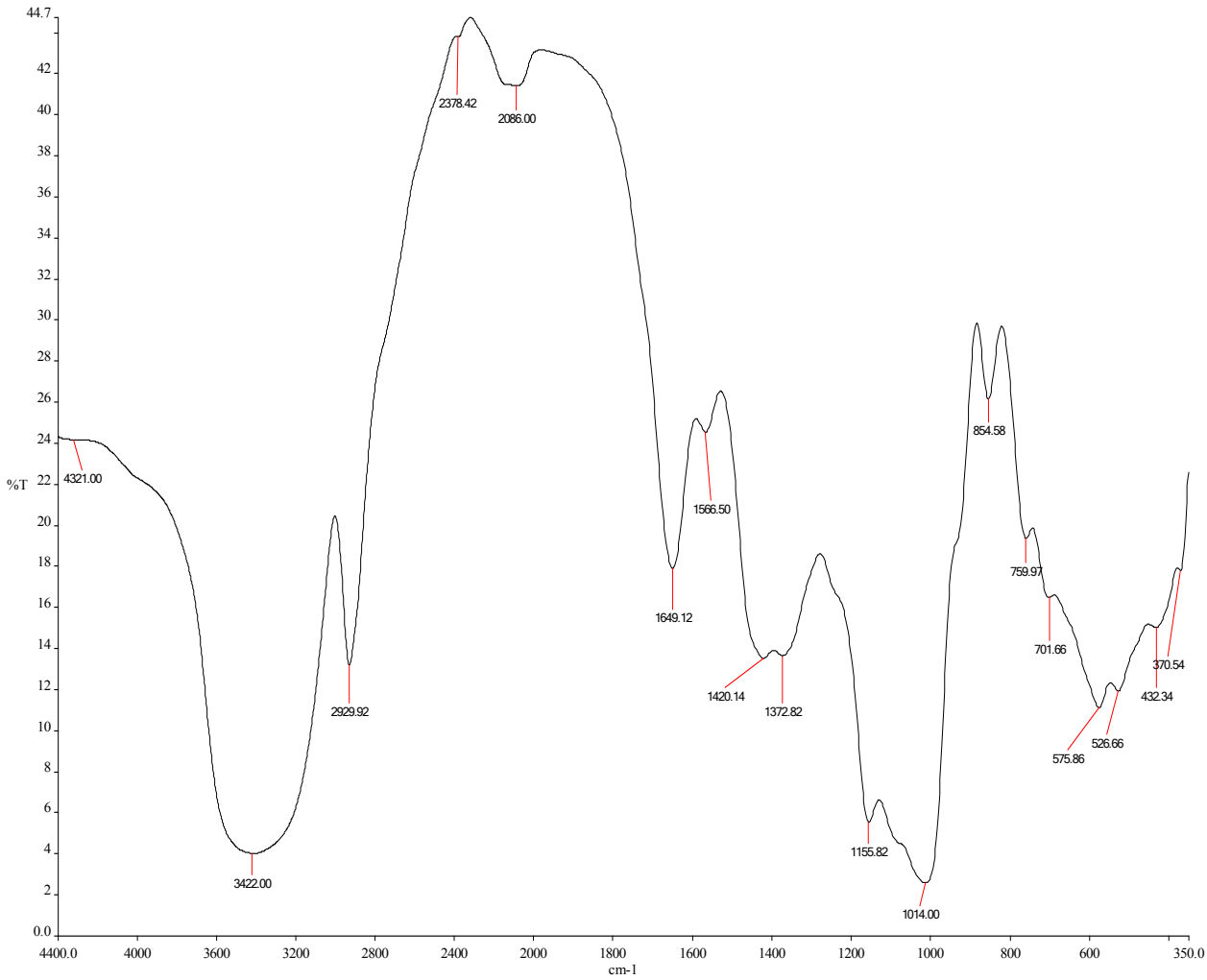


Figure 6: FTIR Graph for 9% Succinylation of *Discorea villosa* Starch

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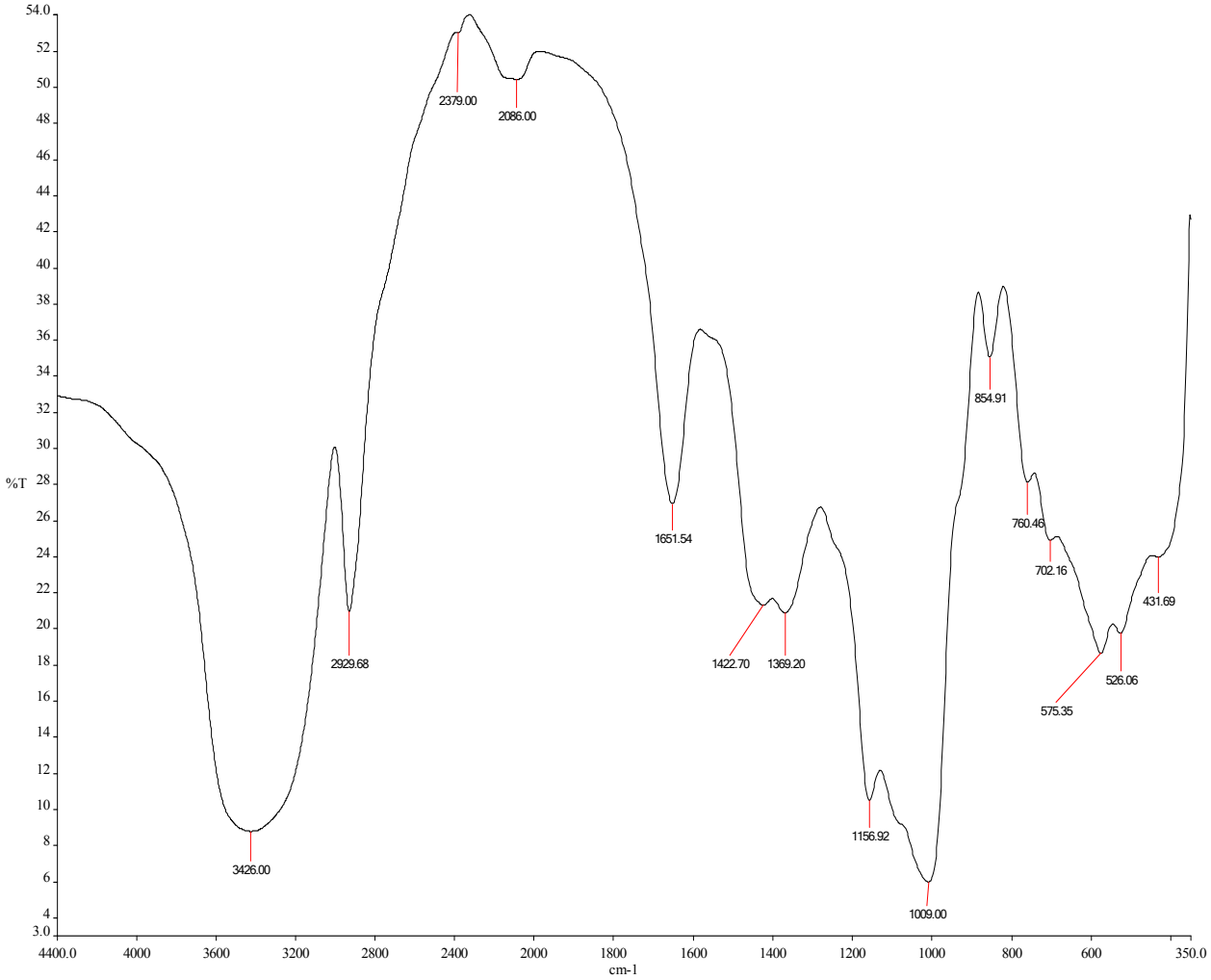


Figure 7: FTIR Graph for 12% Succinylation of *Discorea villosa* Starch

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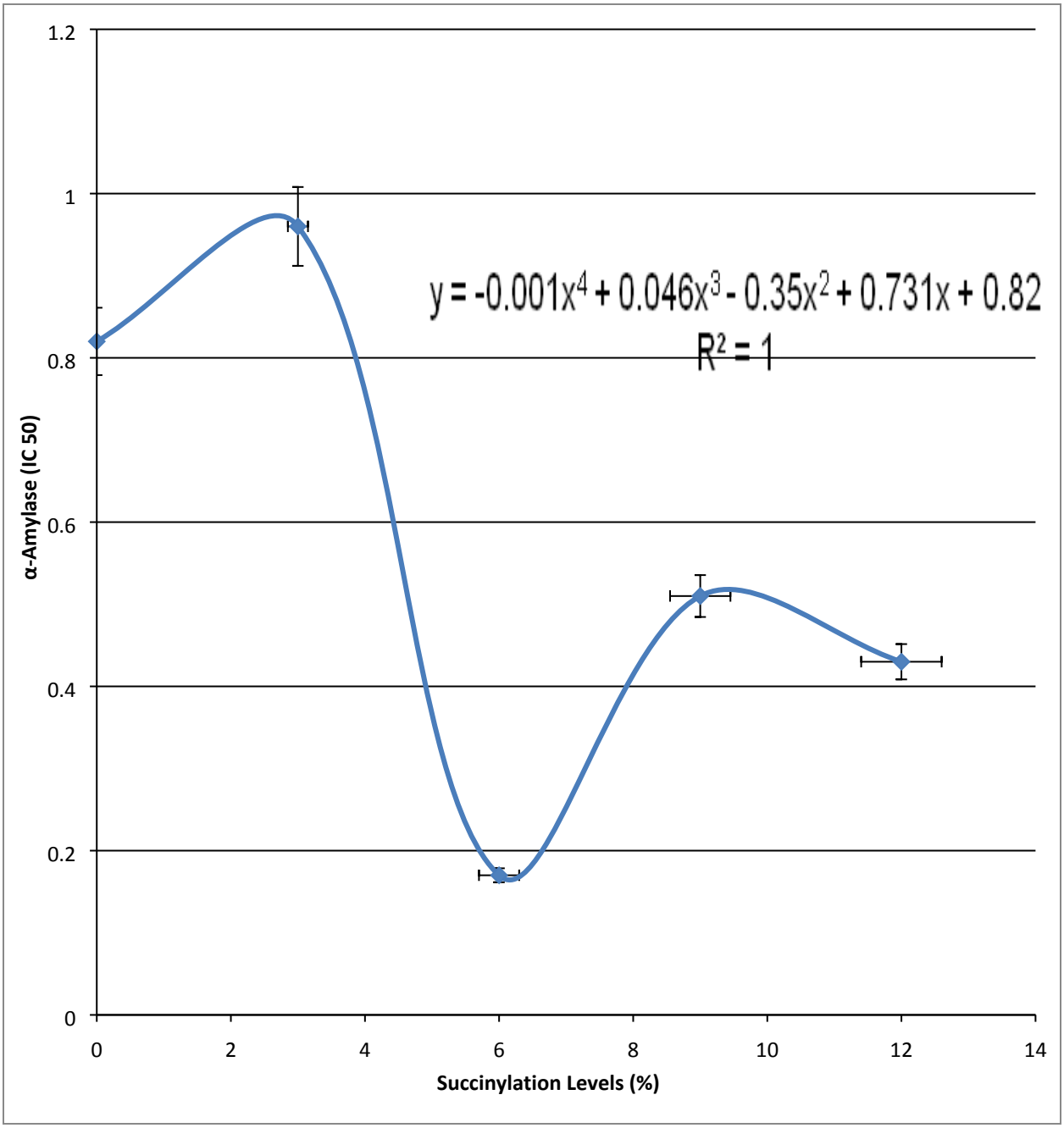


Figure 8: **α-amylase activity on *Dioscorea villosa* starch**

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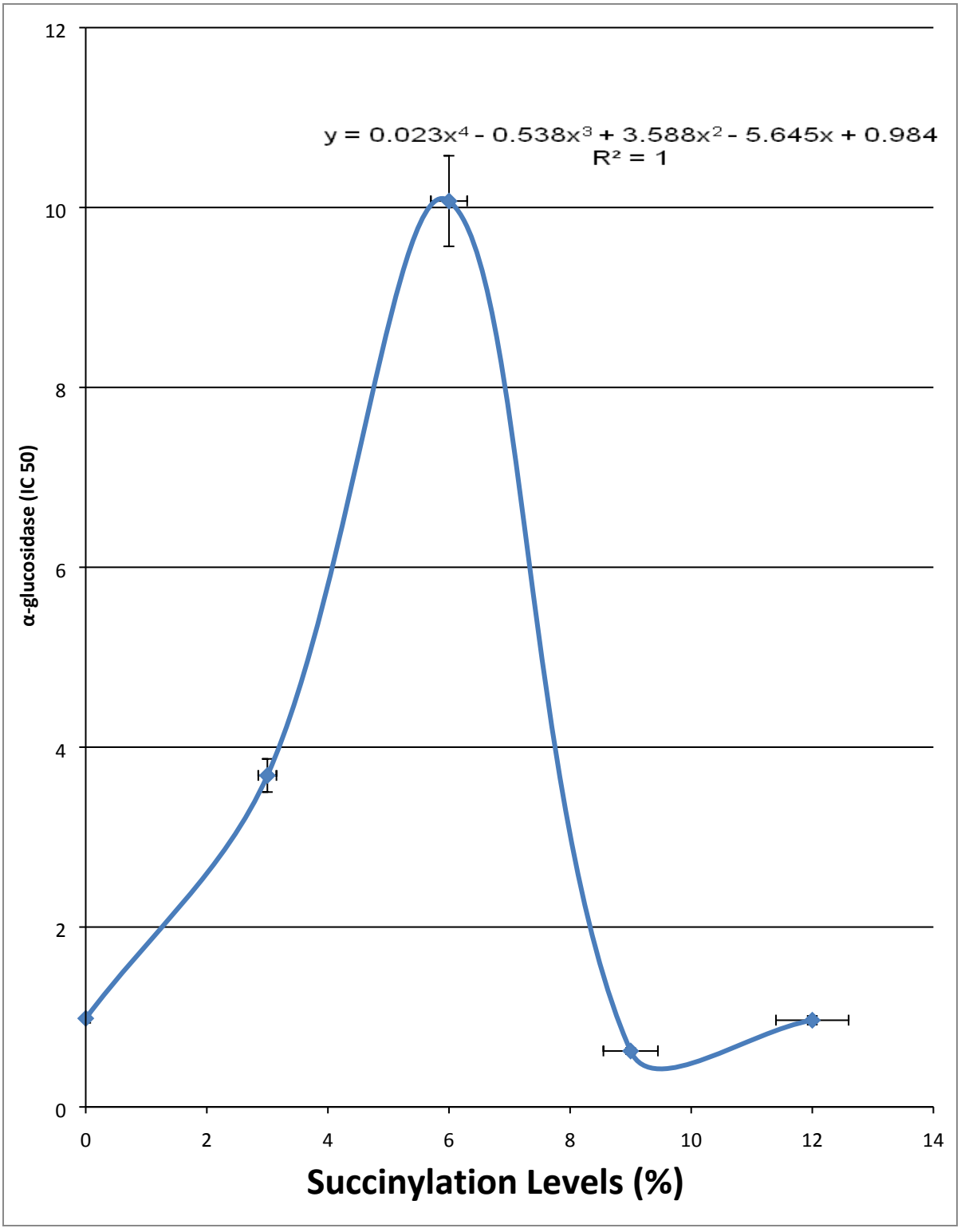


Figure 9: **α-glucosidase activity on *Dioscorea villosa* starch**

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