NUEJ
Naresuan University
Engineering Journal

Characterization of Native and Oxidized Cassava Starch Prepared Using a Solution Plasma Process

Nattha Klanarong[1], Samorn Hirunpraditkoon[1], Nagahiro Saito[2], Isarawut Prasertsung[3*]

[1]Department of Industrial Engineering, Faculty of Engineering, Naresuan University, Phitsanulok 65000, Thailand.
[2]Department of Molecular Design and Engineering, Nagoya University, Chikusa-ku, Nagoya 464-8603, Japan.
[3]Department of Biotechnology, Faculty of Technology, Mahasarakham University, Mahasarakham 44150, Thailand.

*Corresponding author e-mail: isarawut_nui@hotmail.com

(Received: 25 February 2020, Revised: 16 April 2020, Accepted: 16 June 2020)

Abstract

In this work, a solution plasma process (SPP) was used to treat cassava starch suspensions in order to prepare oxidized cassava starch. The plasma treatment time and pulsed frequency were varied from 0 to 300 min and from 15 to 30 kHz, respectively. Oxidation levels were determined by carbonyl and carboxyl content which formed on the cassava starch granules after oxidation with SPP. The amylose content of untreated and plasma treated cassava starch was then determined. FT-IR was employed to analyze the chemical structural change of cassava starch plasma treated samples. Oxidation levels tended to increase as time and pulsed frequency of plasma treatment increased. The amylose contents decreased due to depolymerization of the cassava starch by oxidation with SPP. FT-IR spectra exhibited important peaks occurring on plasma treated cassava starch from 1737-1742 cm^{-1}, which were identified as carbonyl and carboxyl groups formed during the oxidation process.

Keywords: Solution plasma process, Oxidized cassava starch, Hydroxyl radical, Carbonyl, Carboxyl.

1. INTRODUCTION

Starch is typically the primary food ingredient in almost every country around the world where it is often used as thickener and gelling agent (Singh et al., 2017). It is a natural polysaccharide that can be found in various parts of plants likes roots (sweet potatoes, cassava), tubers (cassava), stems (sago), grains (corn, rice, wheat, barley, oats and sorghum) and legume seeds (Gurunathan et al., 2015). The main chemicals composing starch are amylose and amylopectin. The structural characteristics of starch are mainly semi-crystalline and crystalline, which have been reported at between 20 to 45% of the total composition (Breuninger et al., 2009; Tadini, 2017).

Cassava starch or *Manihot esculenta* is used as rotational crop in Thailand. During 2017-2018, the average yield of cassava was 28.67 million tons. It has a high molecular weight and purity (Colivet & Carvalho, 2017). The physicochemical, thermal, and pasting properties of cassava starch are useful for various industries, such as the food, pharmaceutical, textile, paper and cosmetic industries. However, the main drawbacks of native cassava starch are its low solubility, thermal stability and physical properties (Jayakody, & Hoover, 2008). Therefore, modified starch has been employed to improve these properties for various applications (Kaur et al., 2012).

Oxidized starch is one modification method derived from the degradation of its polymer chain by an oxidation reaction. The chemical modification of starch is done by adding carbonyl and carboxyl groups on amylose chains. Oxidation of cassava starch enhances some of its properties by reducing retrogradation and viscosity, increasing thermal resistant and decreasing bacterial contamination (Thunwall et al., 2008). Oxidized starch is extensively used in many industries, especially the paper, textile, decoration, laundry and coating industries (Dias et al., 2011). An interesting application for oxidized starch is as a biodegradable film. Previously, many researchers have studied biodegradable films from oxidized starch. For example, Biduski *et al.* and Pauli *et al.* prepared biodegradable films from oxidized sorghum starch (Biduski et al., 2017) and cassava starch (Pauli et al., 2011) with sodium hypochlorite for biodegradable food packaging. They found that the tensile strength and solubility of oxidized starch were improved, thus demonstrating a potential for food packaging applications.

Based on available literature, there have been several methods to prepare oxidized starch, including chemical and physical methods. The chemical methods have widely used vinyl acetate (Colivet & Carvalho, 2017); acid hydrogen peroxide (Dias et al., 2011); sodium hypochlorite (Fonseca et al., 2015) and combinations of an acid and sodium hypochlorite (Biduski et al., 2017) to synthesize modified starch. This oxidation is a method that requires a short treatment time, however, chemical contamination of the starch may occur which renders it unsuitable for use in the food and pharmaceutical industries. Therefore, starch modification is presently performed using physical methods such as heat-moisture treatment (Rosa Zavareze et al., 2012); ozone treatment (Castanha et al., 2017) and microwave treatment (Kaasova et al., 2002) to avoid the above contamination from chemical residues (Tomasik & Zaranyika, 1995). However, all of these physical processes require high temperatures which induce deformation of starch granules (Ashogbon, 2018).

Takai (2008) developed a new physical process and called it the solution plasma process (SPP). Previously, the solution plasma process has been used in many applications such as the preparation of low molecular weight chitosan (Tantiplapol et al., 2015), conversion of cellulose to reducing sugar (Prasertsung et al., 2017) and preparation of reducing sugar from cassava starch waste (Prasertsung et al., 2019). This SPP method has been found to produce the hydroxyl radical (OH·). The hydroxyl radical induced oxidation reaction has introduced new functional groups in the starch granular structure, resulting in improved characteristics of starch granules. In addition, SPP does not require strong chemical and high temperatures. Therefore, the objective of this study was the novel modification of cassava starch using the SPP process. The influences of pulse frequency and plasma treatment time on the properties of starch were explored and reported.

2. MATERIALS AND METHODS

2.1 Materials

High purity cassava starch was purchased commercially from a market. It was produced by the Thai Flour Industry Co., Ltd., Thailand. In order to preserve the properties of cassava starch, it was kept in a desiccator at 50% relative humidity and 30 ºC. All chemicals used in the work were of analytical grades.

2.2 Solution plasma experiment

The experimental setup of the solution plasma process was from Prasertsung et al. (2019) as shown in Figure 1. The equipment consisted of a glass reactor with 1 mm-diameter tungsten electrodes (99.95%, CaHc Co., Ltd., Thailand) installed on both sides of the reactor. The distance between the electrodes was adjusted to 0.2 mm. Before plasma treatment, the glass reactor was connected to a DC pulsed power supply. The cassava starch colloid at a concentration of 3% (w/v) was poured into the reactor. The colloid was then oxidized using the SPP at a voltage of 16 kV and a pulse width of 3 µs. The plasma treatment time and applied pulsed frequency were varied from 0-300 minutes and 20-30 kHz, respectively. In order to prevent gelatinization of starch, the mixture temperature was controlled at 45 ºC using a coolant.

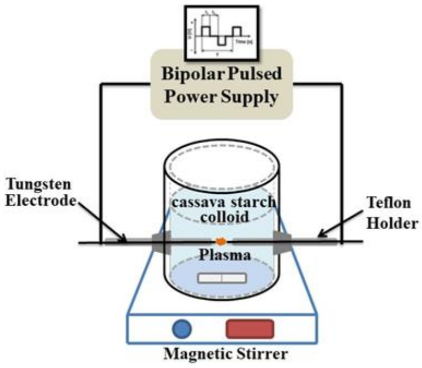

Figure 1 Experimental SPP setup

2.3 Characterization of oxidized cassava starch

2.3.1 Determination of hydroxyl radicals content

During the SPP, highly active species, especially hydroxyl radicals, were produced. In order to determine the hydroxyl radical production during plasma treatment, modified salicylic acid (SA) method was used (Prasertsung et al., 2017). In brief, 1 mL of SA (1×10^{-3} mol/L) was mixed with 1 mL of treated and untreated plasma samples. These suspensions were centrifuged for 8 min and the filtrates were further characterized for hydroxyl radicals using UV–vis Spectrophotometer (Shimadzu UV-9100, Japan) at 303 nm. The calibration curve was performed using SA as a standard reagent. Therefore, the concentration of hydroxyl radical could be calculated.

2.3.2 Carbonyl and carboxyl contents

Carbonyl and carboxyl contents can indicate the degree of depolymerization by oxidation within the SPP. Therefore, carbonyl content was determined according to Dias et al. (2011). The oxidized cassava starch was completely gelatinized at 95 ºC for 30 min and then cooled to 40 ºC. Next, the solution pH was adjusted to 3.2 using 0.1 mol/L HCl solution and 15 mL of hydroxylamine chloride was added (prepared by dissolving 25 g of reagent grade hydroxylamine chloride in water, adding 100 mL of 0.5 mol/L NaOH and sufficient distilled water to provide a final volume 500 mL). Finally, the mixed solution was kept in an oven at 40 ºC for 4 h and then rapidly titrated with 0.1 M of HCl until a pH of 3.2 was reached. The carbonyl content was calculated using Eq. (1).

$$CO = ((V_b - V_s) \times M \times 0.028 \times 100) / W \quad (1)$$

where V_b is the volume of HCl used for the blank (mL), V_s is the volume of HCl required for the sample (mL), M is the molarity of HCl and W is the sample weight (dry basis).

The carboxyl content was determined according to Biduski et al. (2017). The suspension was prepared by dispersing 3 g of oxidized cassava starch in 25 mL of HCl 0.1 mol/L and stirring for 1 h. Then, the oxidized cassava starch suspension was vacuum filtered using a Buchner funnel and washed with 400 mL of distilled water when the oxidized cassava starch was transferred to a beaker. Then, 300 mL of distilled water was added and heated to 95 ºC for 30 min under continuous agitation. The hot oxidized cassava starch solution was titrated with 0.01 mol/L of NaOH with constant stirring until a pH of 8.2 was reached. The native cassava starch was used as a blank test. The carboxyl content was calculated using Eq. (2).

$$COOH = ((V_s - V_b) \times M \times 0.045 \times 100) / W \quad (2)$$

where V_s is the volume of NaOH required for the sample (mL), V_b is the volume of NaOH used to test the blank (mL), M is the molarity of NaOH and W is the sample weight (dry basis).

2.3.3 Amylose content

The depolymerization of oxidized cassava starch granules was investigated to study the decrease in amylose content. The determination of amylose content of oxidized cassava starch using colorimetric method was conducted according to McGrance et al. (1998). 20 mg of oxidized cassava starch was mixed with 8 mL of dimethyl sulfoxide 90% (DMSO) and then heated to a temperature of 95 ºC for 15 min. The mixed solution was then cooled to 40 ºC and the volume adjusted to 25 mL. The sample solution was mixed with 5 ml of I_2/KI (2.5/6.5 mmol/L) and adjusted to a final volume of 50 mL. The mixed solution was kept for 15 min and then amylose content was determined using a UV–vis Spectrophotometer (Shimadzu UV-9100, Japan) at 600 nm. The calibration curve was performed using pure amylose from potato (SIGMA-ALDRICH Co., USA) as a standard reagent. The amylose content was then calculated.

2.3.4 Fourier Transform Infrared Spectrometer

FT-IR (Digilab, FTS 7000 Series, USA) was used to characterize the chemical compositions of native and oxidized cassava starch powder. The FT-IR spectra of the powder samples prepared with KBr were obtained over the range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1}.

3. RESULTS AND DISCUSSION

The carbonyl and carboxyl contents of oxidized cassava starch are shown in Figure 2. The results showed that carbonyl and carboxyl groups in oxidized starch after plasma treatment increased with increasing plasma treatment time and pulse frequency. This revealed that the oxidation reaction of cassava starch occurred during plasma treatment. The number of carbonyl and carboxyl groups in starch can be associated with depolymerization by oxidation, which increases the amount of carbon available for the reaction (Biduski et al., 2017). Hoover (2000) mentioned that higher levels of depolymerization in starch depended on different factors such as the oxidizing agent, the reaction time as well as the botanical origin of starch and most of the depolymerization occurs in the amylose fraction of the starch. During the oxidation reaction, the hydroxyl groups of glucose have oxidized to the carbonyl groups and thereafter to carboxyl groups (Zamudio-Flores et al., 2006). However, during the oxidation of cassava starch by SPP, hydroxyl radicals have been generated. These hydroxyl radicals have been considered as reactive oxygen species (ROS) and immediately reacted with the surrounding molecules (Halliwell, 1999)]. The generation of hydroxyl radicals was previously reported by Baroch et al. (2008). The generation of hydroxyl radicals during the plasma treatment has been caused by the reaction of oxygen radical with water as follows (Prasertsung et al., 2019):

$$e^- + O_2 \rightarrow O^\cdot + O^\cdot \quad (3)$$

$$O^\cdot + H_2O \rightarrow 2OH^\cdot \quad (4)$$

The production of hydroxyl radicals in SPP have been effective in depolymerization of the 1,4-glycosidic linkage and hydrogen bonds of the hydroxyl group within the starch structure. Carbonyl and carboxyl groups have been be formed (Prasertsung et al., 2019; Vanier et al., 2017). The mechanism of carbonyl and carboxyl groups formed during plasma treatment 1 to 4 is shown in Figure 3. The production of the hydroxyl radical in the system increases with increasing plasma treatment time, as shown in Figure 4. This may have enhanced the formation of carbonyl and carboxyl groups, as shown in Figure 5. In addition, an increase in pulsed frequency improved oxidation levels of oxidized cassava starch. Malik et al. (2001) reported that increasing of frequencies increased the average energy input in the system, which may have accelerated an inelastic collision of high energy electrons and the surrounding molecules, resulting in a high dissociation of the target molecules. This may have increased the amount of hydroxyl radicals in the plasma system, resulting in high amounts of carbonyl and carboxyl groups.

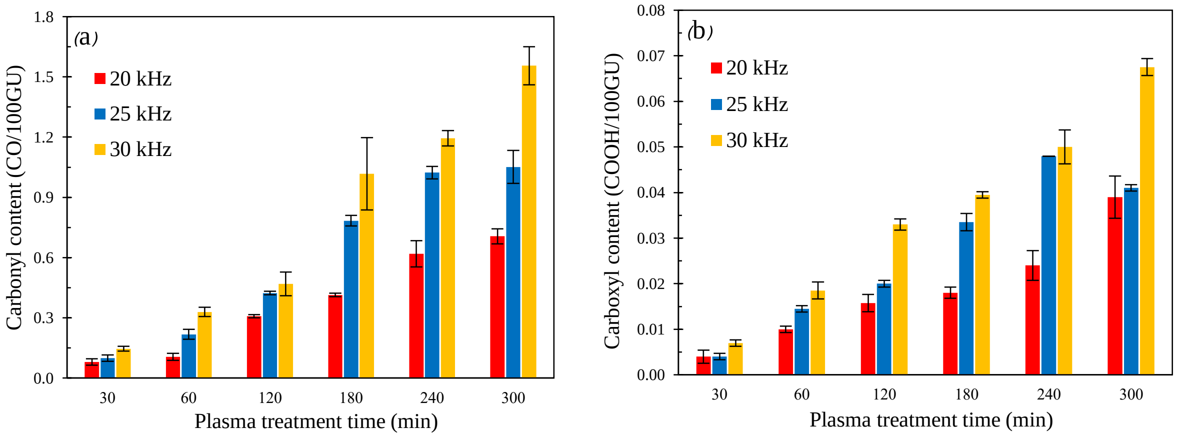

Figure 2 Formation of (a) carbonyl content and (b) carboxyl content of oxidized cassava starch.

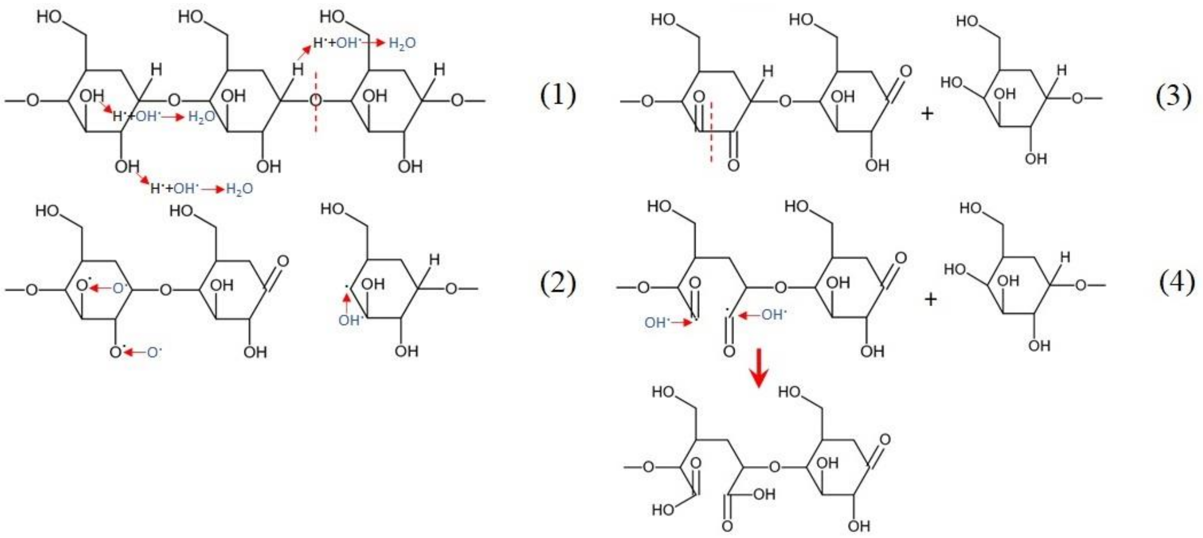

Figure 3 Mechanism of oxidized cassava starch by SPP.

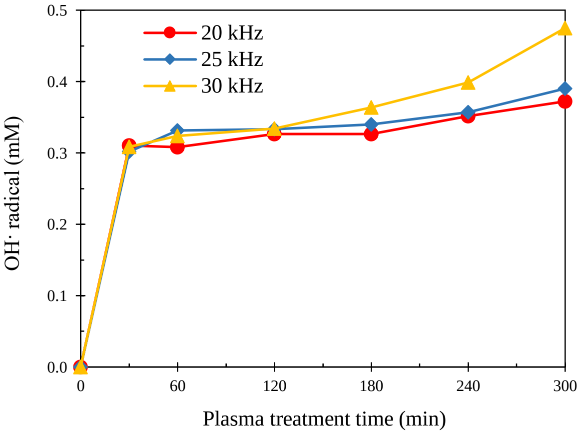

Figure 4 Hydroxyl radical concentration of plasma treated oxidized cassava starch colloid.

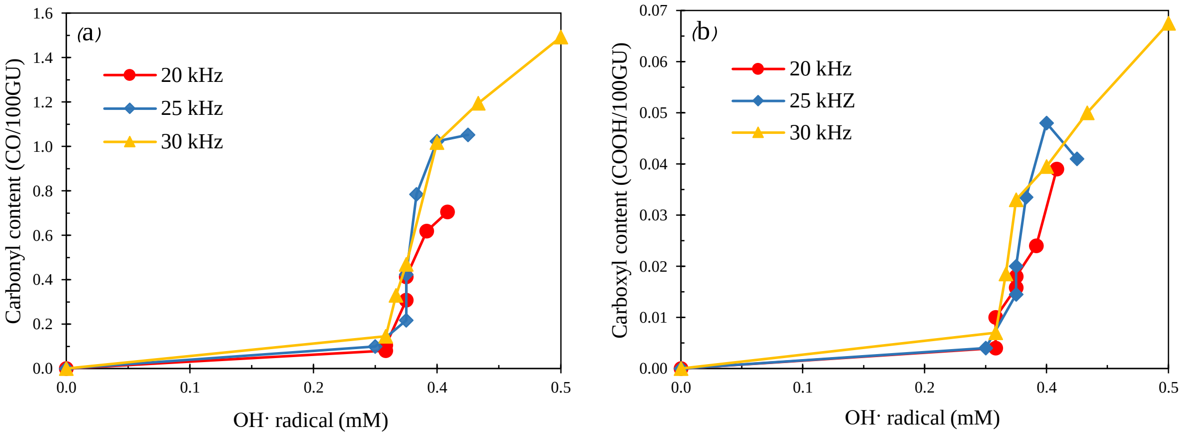

Figure 5 Formation of hydroxyl radical (a) carbonyl content and (b) carboxyl content.

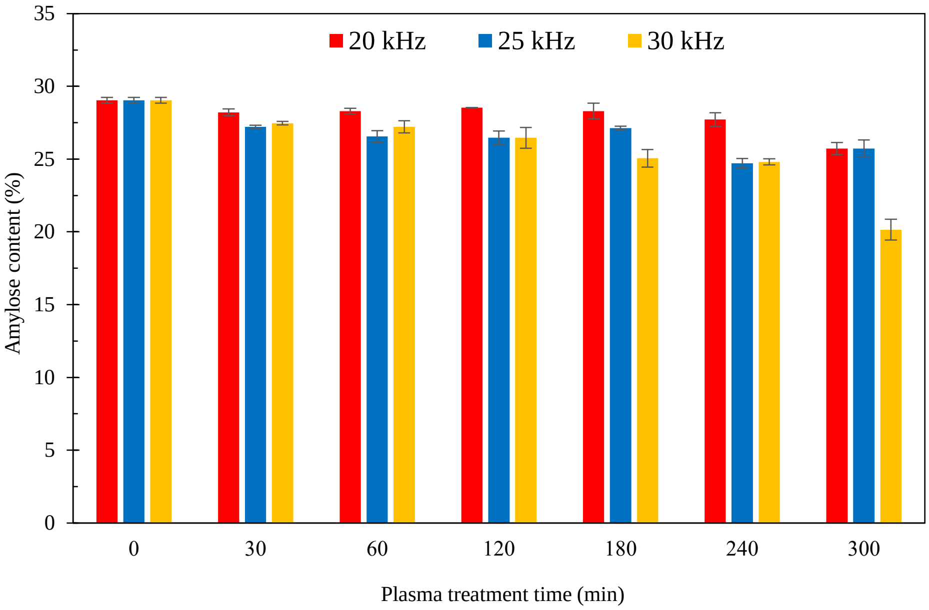

Figure 6 Amylose content of untreated and plasma treated cassava starch.

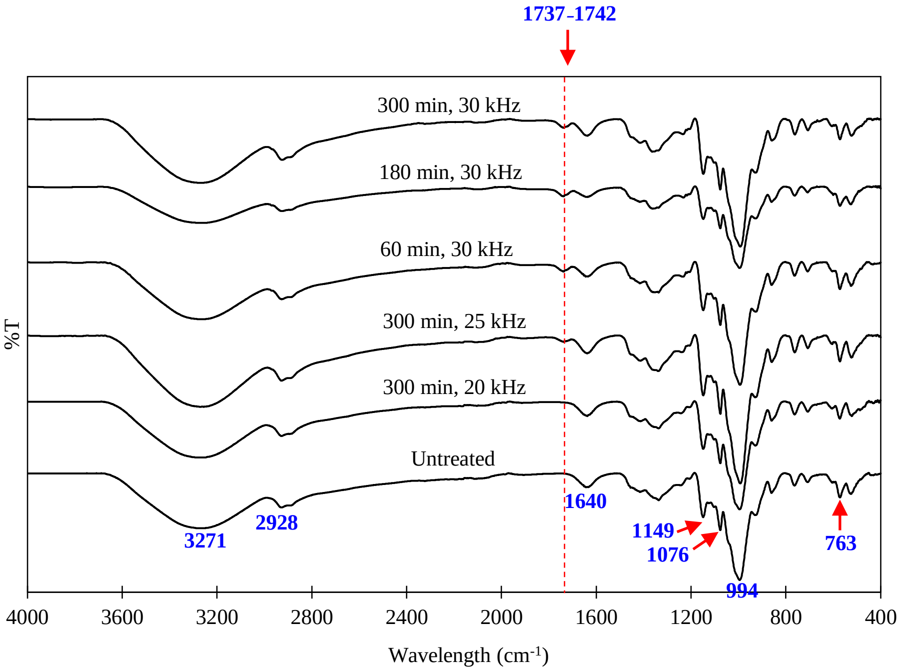

Figure 7 FT-IR spectra of untreated and plasma treated cassava starch.

The amylose content of starch is shown in Figure 6. The amylose content of oxidized cassava starch decreased with increasing plasma treatment time and pulse frequency. The reduction of amylose content indicated depolymerization by the oxidation reaction in the starch. The starch granules consist of amylose and amylopectin which represented amorphous and crystal regions, respectively (Breuninger et al., 2009). Chávez-Murillo et al. (2008) mentioned that depolymerization of starch components (amylose and amylopectin) during the oxidation process mainly occurred in the amylose component. During depolymerization, the starch chain was degraded to become shortened and unable to form amylose-iodine complexes. Amylose-iodine complexes are formed only when the chain length is greater than 100 glucose units (Kuakpetoon & Wang, 2001). Therefore, molecules were not detected as amylose molecules.

To confirm the results described above, FT-IR was employed to analyze the chemical structural change of oxidized cassava starch prepared by SPP. The FT-IR spectra of untreated and plasma treated samples are shown in Figure 7. The peaks of untreated sample displayed at 3271 cm^{-1} and 2928 cm^{-1} corresponded to O–H and C–H stretching, respectively (Prachayawarakorn & Tamseekhram, 2019). The peak at 1634 cm^{-1} and 1149 cm^{-1} were defined as the O-H bending of water and asymmetric stretching of the C–O–C glycosidic linkage, respectively (Mina et al., 2011; Klein et al., 2014). The other apparent peaks at 1076 cm^{-1}, 994 cm^{-1} and 763 cm^{-1} were identified as C–O–H vibration, C–O stretching and C–H_2 rocking, respectively (Bower & Maddams, 1992). After plasma treatment, the new peak at 1737-1742 cm^{-1} corresponded to carbonyl and carboxyl groups. This confirmed that the SPP treatment could generate the oxidation reaction of cassava starch.

4. CONCLUSIONS

The carbonyl and carboxyl groups of oxidized cassava starch prepared by SPP compared to the untreated cassava starch sample were found to be increased. An increase of plasma treatment time and pulsed frequency in the plasma process increased the amount of hydroxyl radicals, which enhanced the depolymerization reaction of starch. The results also showed that the amylose content of oxidized cassava starch was reduced. The chemical compositions of oxidized cassava starch granules prepared by SPP displayed an important peak at 1737-1742 cm^{-1}, which could be identified as the formation of carbonyl and carboxyl groups.

5. ACKNOWLEDGEMENT

The solution plasma equipment was supported from EcoTopia Science Institute and Technology and Department of Materials Engineering, Nagoya University. This research was funded by the Faculty of Engineering, Naresuan University, Thailand. Editing was performed by Thomas Elliott at the Graduate school, Naresuan University, Thailand.

6. REFERENCE

Ashogbon, A. O. (2018). Current Research Addressing Physical Modification of Starch from Various Botanical Sources. *Global Nutrition and Dietetics, 1*(1), 1-7.

Baroch, P., Anita, V., Saito, N., & Takai, O. (2008). Bipolar pulsed electrical discharge for decomposition of organic compounds in water. *Journal of Electrostatics, 66*(5-6), 294-299.

Biduski, B., Silva, F. T., Silva, W. M., El Halal, S. L. D. M., Pinto, V. Z., Dias, A. R. G., & Rosa Zavareze, E. (2017). Impact of acid and oxidative modifications, single or dual, of sorghum starch on biodegradable films. *Food Chemistry, 214,* 53-60.

Bower, D. I., & Maddams, W. F. (1992). *The vibrational pectroscopy of polymers.* Cambridge University Press.

Breuninger, W. F., Piyachomkwan, K., & Sriroth, K. (2009). *Tapioca/cassava starch: production and use.* Academic Press.

Castanha, N., Matta Junior, M. D., & Augusto, P. E. D. (2017). Potato starch modification using the ozone technology. *Food Hydrocolloids, 66,* 343-356.

Chávez-Murillo, C. E., Wang, Y. J., & Bello-Pérez, L. A. (2008). Morphological, physicochemical and structural characteristics of oxidized barley and corn starches. *Starch-Stärke, 60*(11), 634-645.

Colivet, J., & Carvalho, R. A. (2017). Hydrophilicity and physicochemical properties of chemically modified cassava starch films. *Industrial Crops and Products, 95,* 599-607.

Dias, A. R. G., Rosa Zavareze, E., Helbig, E., Moura, F. A., Vargas, C. G., & Ciacco, C. F. (2011). Oxidation of fermented cassava starch using hydrogen peroxide. *Carbohydrate Polymers, 86*(1), 185-191.

Fonseca, L. M., Gonçalves, J. R., El Halal, S. L. M., Pinto, V. Z., Dias, A. R. G., Jacques, A. C., & Rosa Zavareze, E. (2015). Oxidation of potato starch with different sodium hypochlorite concentrations and its effect on biodegradable films. *LWT-Food Science and Technology, 60*(2), 714-720.

Gurunathan, T., Mohanty, S., & Nayak, S. K. (2015). A review of the recent developments in biocomposites based on natural fibres and their application perspectives. *Composites Part A: Applied Science and Manufacturing, 77,* 1-25.

Halliwell, B. (1999). Establishing the significance and optimal intake of dietary antioxidants: the biomarker concept. *Nutrition Reviews, 57*(4), 104-113.

Hoover, R. (2000). Acid-treated starches. *Food Reviews International, 16*(3), 369-392.

Jayakody, L., & Hoover, R. (2008). Effect of annealing on the molecular structure and physicochemical properties of starches from different botanical origins-A review. *Carbohydrate Polymers, 74*(3), 691-703.

Kaasova, J., Hubackova, B., Kadlec, P., Prihoda, J., & Bubnik, Z. (2002). Chemical and biochemical changes during microwave treatment of wheat. *Czech Journal of Food Sciences, 20*(2), 74-78.

Kaur, B., Ariffin, F., Bhat, R., & Karim, A. A. (2012). Progress in starch modification in the last decade. *Food Hydrocolloids, 26*(2), 398-404.

Klein, B., Vanier, N. L., Moomand, K., Pinto, V. Z., Colussi, R., Rosa Zavareze, E., & Dias, A. R. G. (2014). Ozone oxidation of cassava starch in aqueous solution at different pH. *Food Chemistry, 155,* 167-173.

Kuakpetoon, D., & Wang, Y. J. (2001). Characterization of different starches oxidized by hypochlorite. *Starch-Stärke, 53*(5), 211-218.

Malik, M. A., Ghaffar, A., & Malik, S. A. (2001). Water purification by electrical discharges. *Plasma Sources Science and Technology, 10*(1), 82-91.

McGrance, S. J., Cornell, H. J., & Rix, C. J. (1998). A simple and rapid colorimetric method for the determination of amylose in starch products. *Starch-Stärke, 50*(4), 158-163.

Mina, J., Valadez-Gonzalez, A., Herrera-Franco, P., Zuluaga, F., & Delvasto, S. (2011). Physicochemical characterization of natural and acetylated thermoplastic cassava starch. *Medellin, 78*(166), 174-182.

Pauli, R. B., Quast, L. B., Demiate, I. M., & Sakanaka, L. S. (2011). Production and characterization of oxidized cassava starch (Manihot esculenta Crantz) biodegradable films. *Starch-Stärke, 63*(10), 595-603.

Prachayawarakorn, J., & Tamseekhram, J. (2019). Chemical modification of biodegradable cassava starch films by natural mono-, di-and tri-carboxylic acids. *Songklanakarin Journal of Science & Technology, 41*(2), 355-362.

Prasertsung, I., Aroonraj, K., Kamwilaisak, K., Saito, N., & Damrongsakkul, S. (2019). Production of reducing sugar from cassava starch waste (CSW) using solution plasma process (SPP). *Carbohydrate Polymers, 205,* 472-479.

Prasertsung, I., Chutinate, P., Watthanaphanit, A., Saito, N., & Damrongsakkul, S. (2017). Conversion of cellulose into reducing sugar by solution plasma process (SPP). *Carbohydrate Polymers, 172,* 230-236.

Rosa Zavareze, E., Pinto, V. Z., Klein, B., El Halal, S. L. M., Elias, M. C., Prentice-Hernández, C., & Dias, A. R. G. (2012). Development of oxidised and heat-moisture treated potato starch film. *Food Chemistry, 132*(1), 344-350.

Singh, A., Geveke, D. J., & Yadav, M. P. (2017). Improvement of rheological, thermal and functional properties of tapioca starch by using gum arabic. *Lebensmittel-Wissenschaft & Technologie, 80,* 155-162.

Tadini, C. C. (2017). *Bio-based materials from traditional and nonconventional native and modified starches.* Academic Press.

Takai, O. (2008). Solution plasma processing (SPP). *Pure and Applied Chemistry, 80*(9), 2003-2011.

Tantiplapol, T., Singsawat, Y., Narongsil, N., Damrongsakkul, S., Saito, N., & Prasertsung, I. (2015). Influences of solution plasma conditions on degradation rate and properties of chitosan. *Innovative Food Science & Emerging Technologies, 32,* 116-120.

Thunwall, M., Kuthanova, V., Boldizar, A., & Rigdahl, M. (2008). Film blowing of thermoplastic starch. *Carbohydrate Polymers, 71*(4), 583-590.

Tomasik, P., & Zaranyika, M. F. (1995). Nonconventional methods of modification of starch. *Advances in Carbohydrate Chemistry and Biochemistry, 51,* 243-318.

Vanier, N. L., El Halal, S. L. M., Dias, A. R. G., & Rosa Zavareze, E. (2017). Molecular structure, functionality and applications of oxidized starches: A review. *Food Chemistry, 221,* 1546-1559.

Zamudio-Flores, P. B., Vargas-Torres, A., PérezGonzález, J., Bosquez-Molina, E., & Bello-Pérez, L. A. (2006). Films prepared with oxidized banana starch: mechanical and barrier properties. *Starch-Stärke, 58*(6), 274-282.

7. BIOGRAPHIES

Nattha Klanarong is Master's degree students in Chemical Engineering Program, Naresuan University Graduate School, Thailand.

Assoc. Prof. Samorn Hirunpraditkoon, Ph.D., is a lecturer in Chemical Engineering Program, Faculty of Engineering at Naresuan University, Thailand.

Asst. Prof. Isarawut Prasertsung, D.Eng., is a lecturer in Department of Biotechnology, Faculty of Technology at Mahasarakam University, Thailand.

Prof. Nagahiro Saito, Ph.D., is a developer solution plasma process. Currently works at the Department of Chemical Systems Engineering, Nagoya University, Japan.