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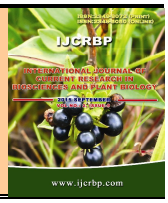

Original Research Article

Chemical and Functional Properties of Acetylated Arenga Starches Prepared at Different Reaction Time

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Abstract

Arenga starch obtained from the pith of palm *Arenga* (*Arenga pinnata*) and subjected to chemical modification by acetylation at different reaction time. In this study, acetylation of arenga starch was carried out. Arenga starch was reacted with acetic anhydride at 5% (starch basis, sb) in aqueous slurry at pH 8.0 ± 8.2for 15, 30, 45, 60, 75 and 90 min. The native and modified starches were characterized for acetyl percentage (Ac%), degree of substitution (DS), fourier transform infrared spectroscopy *(*FT-IR)spectra, crystallinity, water and oil holding capacity (WHC and OHC), swelling power and solubility. The results indicated that Ac% and DS of acetylated arenga starchesprepared with 60 min was the highest 2,715% and 0.105 respectively. The modification resulted in the acetyl group incorporation with the starch molecule as shown by absorption of the ester carbonyl group at band 1720 cm^{-1} of the fourier transform infrared spectra. X-ray diffraction revealed that increasing reaction time was related to decreasing crystallinity. The WHC and OHC of acetylated arenga starches increased until 60 min, indicating that acetylation increased both hydrophilicity and hydrophobicity of the modified starches. Swelling power tended to increase and solubility to decrease along with the increase reaction time until 60 min of acetylated arenga starches.

Keywords

Acetylated arenga starches

Arenga pinnata

Chemical properties

Functional properties

Reaction time

Introduction

Chemical modifications of starch including esterification are efficacious methods to improve the properties of starch. The chemical and functional properties achieved, depending on by chemical substitution, on starch source, reaction conditions, type of substituent, extent of degree of substitution (DS), and the distribution of the substituent in the starch molecule (Hirsch and Kokini, 2002). Acetylated starch exhibits increased stability in food applications compared to its native counterpart; therefore, it has been used to increase the stability and resistance of food products to retrogradation (Singh et al., 2004). Chemical modification of native starches is

often required to improve their properties, as well as to overcome the undesirable changes in product texture and appearance caused by retrogradation or breakdown of starch during storage and processing (Hung and Morita, 2005; Miladinov and Hanna, 2000). Starch acetates are prepared commercially with a low (< 0.1) degree of substitution (DS) through the reaction of an aqueous suspension of starch granules with acetic anhydride (Biswas et al., 2008; Shogren, 2003; Shogren and Biswas, 2006).

Acetylated starch is a starch ester that has been extensively studied over the last two decades (Gonzalez and Perez, 2002; Singh et al., 2007). In modified starch, part of hydroxyl groups in anhydroglucose units have been converted to acyl groups. Acylated starch with low DS is commonly obtained by esterification of native starch in an aqueous medium in the presence of an alkaline catalyst. A low DS with (0.01-0.20) acylated starch has been applied in many areas, such as film forming, binding, adhesion, thickening, stabilizing, texturing (Boutboul et al., 2002; Matti et al., 2004; Singh et al., 2004; Sodhi and Singh, 2005).

In the current study, acetylated starches were prepared by reacting arenga starch with acetic anhydride in aqueous slurry to determine the optimum reaction time and to give the chemical and functional of properties such as acetyl percentage (Ac%), degree of substitution (DS), fourier transform infrared (FT-IR) spectra, cristallinity, water and oil holding capacity (WHC and OHC), swelling power and solubility.

Materials and methods

Arenga starch (*Arenga pinnata* Merr.) used for this study was obtained from Sigi, Central Sulawesi Provinsi, Indonesia. High-purity acetic anhydride 98% was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The Sodium hydroxide (NaOH) and hydrochloric acid (HCl) were purchased from Merck. The chemicals for analysis used in the study were of analytical grade purchased at local agent.

Acetylation of arenga starch

Acetylated starch was prepared by a modified procedure of Phillips et al. (1999) with slight modification. Starch (100 g) was dispersed in 225 ml of distilled water and stirred for 60 min at 25^{o}C. The acetic anhydride of 5% (starch basis, sb) was added drop-wise to the stirred slurry, while maintaining the pH 8.0 ± 8.2 using 3.0% NaOH solution. The reaction was allowed to proceed for 15, 30, 45, 60, 75 and 90 min after the completion of acetic anhydride addition. The slurry was then adjusted to pH 4.5 with 0.5 N HCl. After sedimentation, it was washed free of acid, twice with distilled water and once with 95% ethanol, and then oven-dried at 40^{o}C.

Determination of acetyl percentage and degree of substitution

The percentage of acetyl groups (Ac%) and degree of substitution (DS) were determined titrimetrically, following the method by Sanchez Rovera et al. (2010). Acetylated starch (1.0 g) and 50 ml of 75% aqueous ethanol were placed in a 250 ml flask. The loosely stopper flask was agitated, warmed to 50 ◦C for 30 min, cooled and 40 ml of 0.5 M KOH were added. The excess alkali was back-titrated with 0.5 M HCl using phenolphthalein as an indicator. The solution was left to stand for 2 h, and then the alkali leached from the sample was titrated. A blank, using the original unmodified starch, was also used. Initially, the acetyl (%) was calculated as:

$$\text{Acetyl \%} = \frac{[(\text{Blank} - \text{Sample}) \times \text{Molarity of HCl} \times 0.043 \times 100]}{\text{Sample weight}}$$

Blank and sample were titration volumes in ml, sample weight was in g. DS is defined as the average number of sites per glucose unit that possess a substituent group.

$$DS = \frac{(162 \times \text{Acetyl \%})}{[4300 - (42 \times \text{Acetyl \%})]}$$

Fourier Transform Infrared Spectroscopy (FT-IR) spectra analysis

Sample preparation and analysis parameters were performed according to Diop et al. (2011). Tablets were prepared from the mixture of the sample with KBr at a ratio of 1:100 (sample: KBr). Infrared spectra of native and acetylated starches samples were obtained by a Fourier Transform Spectrometer (IR Prestige 21, Shimadzu) in the 5000–400 cm^{-1} region.

XRD analysis

X-ray diffraction of native and acetylated starches was measured using method of Miao et al. (2011). X-ray diffraction analysis was performed with an X' Pert PRO X-ray powder diffractometer (PANalytical, Almelo, The Netherlands) operating at 40 kV and 30 mA with Cu Kα

radiation (λ = 1.5406 Å). The starch powders were packed tightly in a rectangular glass cell (15 x 10 mm, thickness 0.15 cm) and scanned at a rate of 2°/min from the diffraction angle (2θ) 3° to 80° at room temperature. The crystallinity was calculated according to the equation below:

$$Xc = \frac{Ac}{(Aa + Ac)}$$

where Xc is the crystallinity, A_c is the crystalline area and Aa is the amorphous area on the X-ray diffractogram.

Water and oil holding capacity

Water and oil holding capacity (WHO, OHC) of native and acetylated starches was measured using method of Larrauri et al. (1996). Twenty-five ml of distilled water or commercial olive oil were added to 250 mg of dry sample, stirred and left at room temperature for 1 h. After centrifugation, the residue was weighed WHC and OHC were calculated as g water or oil per g of dry sample, respectively.

Swelling power and solubility

The method of Adebowale et al. (2009) was employed to determine the swelling power and solubility of the starch. Five hundred (500) mg of starch sample was weighed into a centrifuge tube and it was reweighed (W_1). The starch was then dispersed in 20 ml of water. It was then heated at temperature of 80°C for 30 min in a thermostated water bath.

The mixture was cooled to room temperature and centrifuged at 3000g for 15 min. Supernatant was decanted carefully and residue weighed for swelling power determination. The weight of dry centrifuge tube and the residue and the water retained was taken as W_2.

$$\text{Swelling power} = \frac{W_2 - W_1}{\text{Weight of starch}}$$

Aliquots (5 ml) of the supernatant were dried to a constant weight at 110°C. The residue obtained after drying of the supernatant represented the amount of starch solubilized in water. Solubility was calculated as g per 100 g of starch on dry weight basis.

Statistical analysis

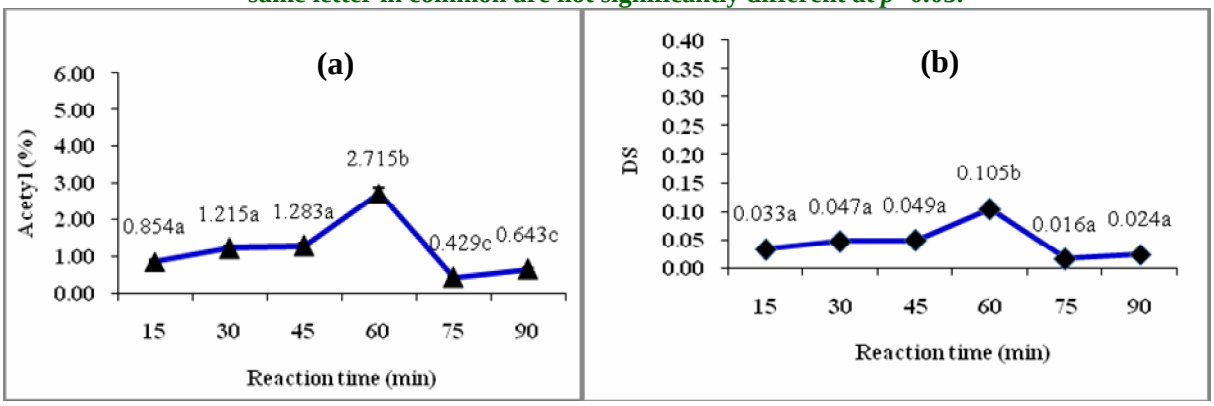
The data reported were the means of triplicate measurements. Statistical analysis was carried out with Duncan's multiple test (p<0.05) using SPSS version 18 software (SPSS Institute Inc., Cary, NC).

Results and discussion

Acetyl percentage and degree of substitution

Percentage of acetyl groups and degree of substitution of the acetylated arenga starch at different reaction time (15, 30, 45, 60, 75 and 90 min) are show in Fig.1. The acetylation of arenga starch promoted the incorporation of acetyl groups in the molecule, resulting in an acetyl percentage and degree of substitution between 0.429 to 2.715% (Fig.1a) and DS 0.016 to 0.105 (Fig.1b) respectively, allowing food application. The highest Ac% (2.715%) and DS (0.105) was reached by acetylation at reaction time 60 min. Increasing the reaction time from 15-60 min resulted in increase in Ac% and DS, but longer reaction (75 – 90 min) resulted in a decrease in Ac% and DS. An explanation was that, as the reaction progressed, the concentration of acetic anhydride was depleted, due to esterification and hydrolysis reactions similar to that of (Ruan et al., 2009).

Fig. 1: Effect of reaction time with 5% acetic anhydride on the acetyl (a) and DS (b). Numbers in the graph followed by a same letter in common are not significantly different at p<0.05.

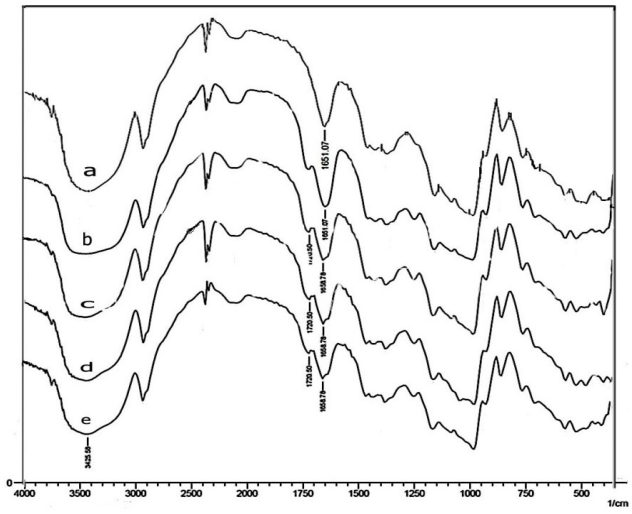


Mbougueng et al. (2012) evaluated the effect of at different reaction time in potato and cassava starches. Acetyl percentage and DS indicated the difference ($p < 0.05$) in the acetylation reaction time. The DS for cassava starches was 0.10 and 0.26 at 10 min and 20 min of reaction, respectively; in the case of potato starches DS was 0.10 and 0.18.

FTIR spectra

The FTIR spectra of native and acetylated arenga starches with different reaction time are shown in Fig.2. The peak at 1720 cm^{-1} is because of the introduction of ester group which is present in the acetylated arenga straches, which otherwise is absent in native arenga starch. Similarly, the reduction of the intermolecular hydrogen bonding between 3700-3000 cm^{-1} confirms the introduction of the acetyl groups in the arenga starch structure thus replacing the hydroxyl groups. Compared to that of the native starch curve (Fig.2a), new absorption band at 1720 cm^{-1} appeared in acetylated arenga starches curves (Fig.2b; 2c; 2d; 2e) is C=O stretching vibration of an acetyl group.

Fig. 2: FTIR spectra of native arenga starch (a) and acetylated arenga starches at different reaction time: 30 min (b), 45 min (c), 60 min (d) and 75 min (e).



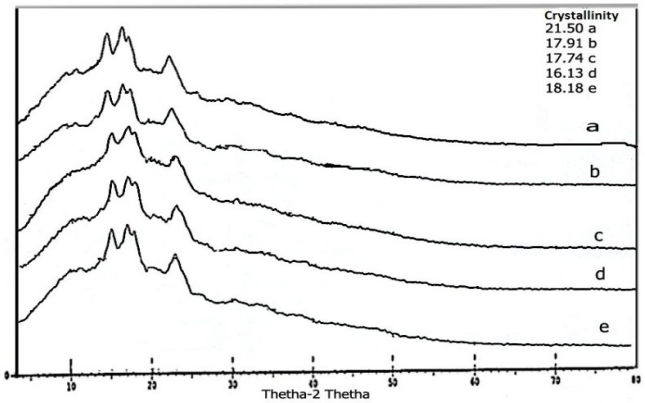
Acetylated barley starches had strong absorption bands at 1735–1740 cm^{-1} (C=O stretching of acetyl group) with evidence of acetylation. On the other hand, since the intensity of the hydroxyl group peak at 3000–3600 cm^{-1} decreased, it has been suggested that the hydroxyl groups in the starch molecules were converted into acetyl groups (Halal et al., 2015). The structures of butyrylated arenga starch at band of 1728 cm^{-1} (Rahim et al., 2012).

Crystallinity

The X-ray diffraction patterns of native and acetylated arenga starch samples are shown in Fig.3. The peaks observed in present study showed that native and acetylated arenga starches displayed typical A-type pattern with main peaks at $2\theta = 15^o$, 17^o, 18^o and 23^o. Chi et al. (2008) and Miao et al. (2011) described that A-type pattern of starches exhibited sharp peaks at $2\theta = 15^o$, 17^o, 18^o and 23^o. These results suggested that esterification did not change the crystalline pattern of acetylated arenga starches up to DS 0.105. These were agreed with the findings of Song et al. (2006), who mentioned that the esterification occurred primarily in the amorphous regions and did not change the crystalline pattern of starches.

Degree of crystallinity of the acetylated arenga starch granules was lower than that of the native starch. This indicated that the granules of acetylated arenga starches had been damaged to some extent by the modification processes. Intra and intermolecular hydrogen bonds were responsible for the highly ordered crystalline structure. The results were in accordance that report of Halal et al. (2015), that showed lower crystallinity when compared to that of native barley starch and by Lopez et al. (2010), that degree of crystallinity of the acetylated corn starch granules was lower than that of the native starch.

Fig. 3: X-ray diffractograms and cristallinity of native arenga starch (a) and acetylated arenga starches at different reaction time: 30 min (b), 45 min (c), 60 min (d) and 75 min (e).



Water and oil holding capacity

WHC and OHC of the native and acetylated arenga starches increased with the increase reaction time until 60 min, then decrease for longer time are show in Fig. 4.

The results indicate that with increase in time from 15 min to 60 min, the WHC and OHC of acetylated arenga starches was found to increase, however further decrease to 90 min, it did not reflect increase in WHC and OHC leveling-off in accessibility of arenga starch for the reactants indicating near stagnancy in level of acetylation. These data indicate that either hydrophilicity or hydrophobicity tend to improve after acetylation. Improvement in water and oil absorption was a result of introduction of functional groups to the starch molecules, which facilitated a more enhanced holding capacity (Rahim et al., 2012).

Fig. 4: Effect of reaction time with 5% acetic anhydride on the WHC and OHC of native and acetylated arenga starches. Numbers in the graph followed by a same letter in common are not significantly different at $p<0.05$.

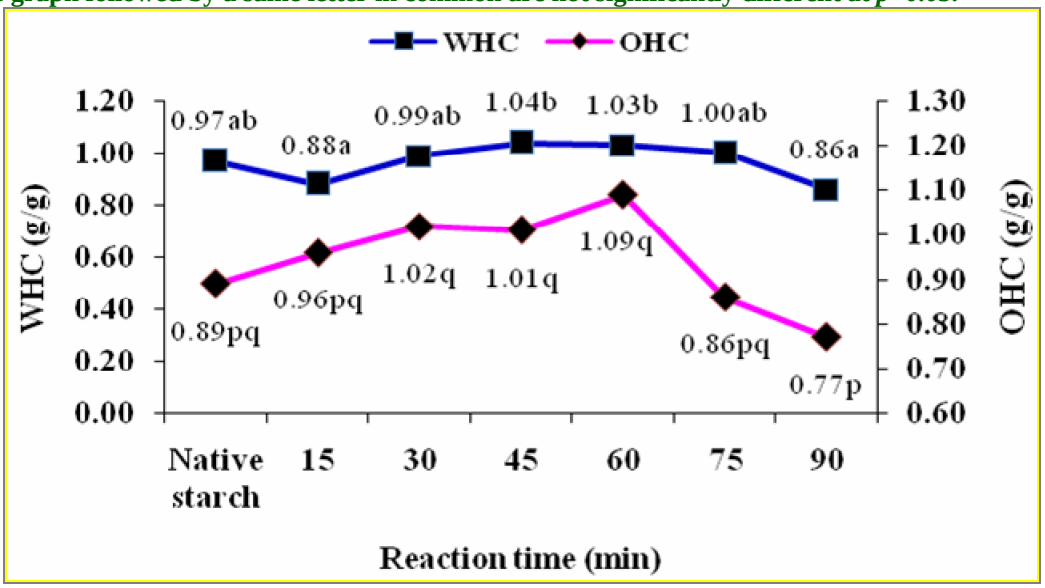
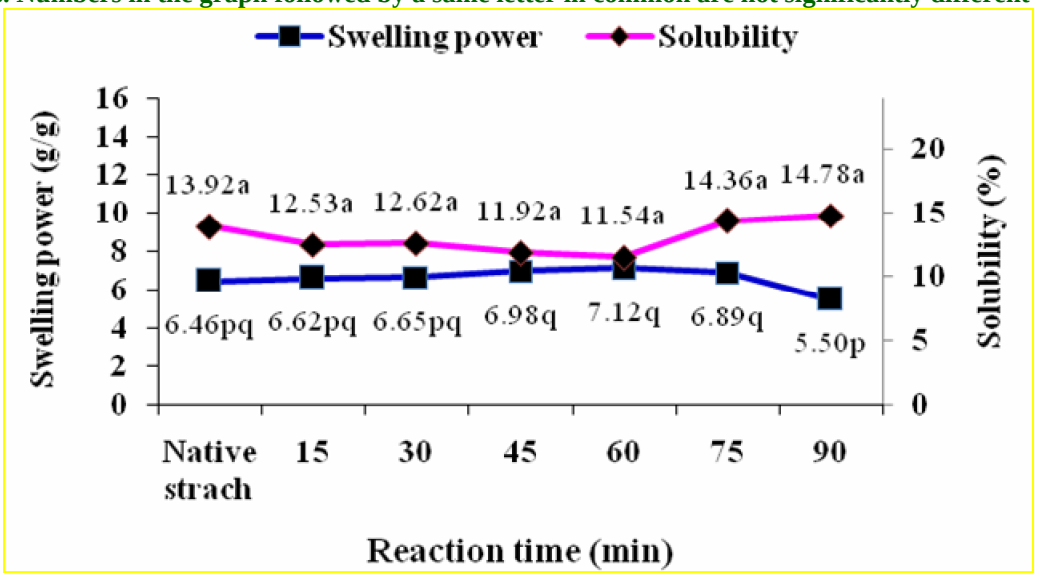


Fig. 5: Effect of reaction time with 5% acetic anhydride on the swelling power and solubility of native and acetylated arenga starches. Numbers in the graph followed by a same letter in common are not significantly different at $p<0.05$.



Adebowale et al. (2006) found that WHC of the acetylated sword bean starch at DS 0.14 was higher than that of the native starch, while Teli and Valia (2013) reported that the OHC of the acetylated banana was higher than that of the raw banana.

Swelling power and solubility

Swelling power and solubility of native and acetylated arenga starch samples are shown in Fig.5. Swelling power of the acetylated arenga starches increased with

the increase reaction time until 60 min, then decrease for longer time, while solubility of the acetylated arenga starches to tend constant for longer reaction time.This phenomenon was probably caused by a weakening of intermolecular association force due to introduction of acetyl groups that reduced the hydroxyl groups after acetylation. These were similar to the results of Souza et al. (2015) that swelling power of the acetylated oat β-glucans increased with increasing time from 10 min to 20 min.

Conclusion

The acetylation of arenga starch promoted the incorporation of acetyl groups in the molecule at reaction time from 15 min to 90 min, resulting in a degree of substitution between 0.016 and 0.105, allowing food application. Acetylated of arenga starch with 5% acetic anhydride at reaction time from 15 min to 90 min to improve the chemical and functional properties of the modified starches. This report will be useful to promote the use of the acetylated arenga starch for industrial applications in food production.

Acknowledgement

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References

Adebowale, K.O., Afolabi, T.A., Olu-Owolabi, B.I., 2006. Functional, physicochemical and retrogradation properties of sword bean (*Canavalia gladiata*) acetylated and oxidized starches. Carbohydr. Polym. 65, 93–101.

Adebowale, K.O., Henle, T., Schwarzenbolz, U., Doert, T., 2009. Modification and properties of African yam bean (*Sphenostylis stenocarpa Hochst. Ex A. Rich*.) Harms starch I: Heat moisture treatments and annealing. Food Hydroc. 23, 1947–1957.

Biswas, A., Shogren, R.L., Selling, G., Salch, J., Willett, J.L., Buchanan, M., 2008. Rapid and environmentally friendly preparation of starch esters. Carbohydr. Polym. 74, 137–141.

Boutboul, A., Giampaoli, P., Feigenbaum, A., Ducruet, V., 2002. Influence of the nature and treatment of starch on aroma retention. Carbohydr. Polym. 47, 73–82.

Chi, H., Xu, K., Wu, X., Chen, Q., Xue, D., Song, C., Zhang, W., dan Wang, P., 2008. Effect of acetylation on the properties of corn starch. Food Chem. 106, 923–928.

Diop, C., Li, H.L., Xie, B.J., Shi, J. 2011. Effects of acetic acid/acetic anhydride ratios on the properties of corn starch acetates. Food Chem. 126, 1662–1669.

Gonzalez, Z., Perez, E., 2002. Effect of acetylation on some properties of rice starch. Starch/Starke 54, 148–154.

Halal, S.M.L.E., Colussi, R., Pinto, V.Z., Bartz, J., Radunz, M., Carreno, N.L.V., Dias, A.R.G., Zavareze, E.R., 2015. Structure, morphology and functionality of acetylated and oxidized barley starches. Food Chem. 168, 247–256.

Hirsch, J.B., Kokini, J.L., 2002. Understanding the mechanism of cross-linking agents ($POCl_3$, STMP, and EPI) through swelling behavior and pasting properties of cross-linked waxy maize starches. Cereal Chem. 79, 102–107.

Hung, P. V., Morita, N., 2005. Effects of granule sizes on physicochemical properties of cross-linked and acetylated wheat starches. Starch/Starke 57, 413–420.

Larrauri, J.A., Ruperez, P., Borroto, B., Saura-Calixto, S., 1996. Mango Peels as a New Tropical Fibre: Preparation and Characterization. Lebensm. Wiss. u. Technol. 29, 729–733.

Lopez, O.V., Zaritzky, N.E., Garcia, M.A., 2010. Physicochemical characterization of chemically modified corn starches related to rheological behavior, retrogradation and film forming capacity. J. Food Engg. 100, 160–168.

Matti, E., Tomas, A., Pasi, S., Reino, L., Soili, P., Sari, H. 2004. Determination of the degree of substitution of acetylated starch by hydrolysis, 1H NMR and TGA/IR. Carbohydr. Polym. 57, 261–267.

Mbougueng, P. D., Tenin, D., Scher, J., Tchiegang, C., 2012. Influence of acetylation on physicochemical, functional and thermal properties of potato and cassava starches. J. Food Engg. 108, 320–326.

Miao, M., Zhang, T., Mu, W., Jiang, B., 2011. Structural characterizations of waxy maize starch residue following in vitro pancreatin and amyloglucosidase synergistic hydrolysis. Food Hydrocol. 25, 214–220.

Miladinov, V.D., Hanna, M.A., 2000. Starch esterification by reactive extrusion. Ind. Crops Prod. 11, 51–57.

Phillips, D. L., Huijum, L., Duohai, P., Harold, C., 1999. General application of Raman spectroscopy for the

determination of level of acetylation in modified starches. Cereal Chem. 76, 439–443.

Rahim, A., Haryadi, Cahyanto, M.N., Pranoto, Y., 2012. Characteristics of butyrylated arenga starch prepared at different reaction time and butyric anhydride concentration. Int. Food Res. J. 19(4), 1655-1660.

Ruan, H., Chen, Q. H., Fu, M. L., Xu, Q., He, G.Q., 2009. Preparation and properties of octenyl succinic anhydride modified potato starch. Food Chem. 114, 81–86.

Sánchez-Rivera, M. M., Flores-Ramírez, I., Zamudio-Flores, P. B., González-Soto, R., Rodríguez-Ambríz, S. L., Bello-Pérez, L. A., 2010. Acetylation of banana (*Musa paradisiaca* L.) and maize (*Zea mays* L.) starches using a microwave heating procedure and iodine as catalyst: Partial characterization. Starch/Stärke 62, 155–164.

Shogren, R.L., 2003. Rapid preparation of starch esters by high temperature/pressure reaction. Carbohydr. Polym. 52, 319–326.

Shogren, R.L., Biswas, A., 2006. Preparation of water soluble and water swellable starch acetates using microwave heating. Carbohydr. Polym. 64, 16–21.

Singh, J., Kaur, L., McCarthy, O.J., 2007. Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications-a review. Food Hydrocol. 21, 1–22.

Singh, N., Chawla, D., Singh, J., 2004. Influence of acetic anhidrida on physicochemical, morphological and thermal properties of corn and potato starch. Food Chem. 86, 601-608.

Sodhi, N. S., Singh, N., 2005. Characteristics of acetylated starches prepared using starches separated from different rice cultivars. J. Food Engg. 70, 117–127.

Song, X. Y., He, G. Q., Ruan, H., and Chen, Q. H., 2006. Preparation and properties of octenyl succinic anhydride modified early indica rice starch. Starch/Starke. 58, 109–117.

Souza, N.L., Bartz, J., Zavareze, E.R., Oliveira, P.D., Silva, W.S.V., Alves, G.H., Dias, A.R.G., 2015. Functional, thermal and rheological properties of oat β-glucan modified by acetylation. Food Chem. 178, 243–250.

Teli, M.D., Valia, S.V., 2013. Acetylation of banana fibre to improve oil absorbency. Carbohydr. Polym. 92, 328– 333.