African Journal of Food Science Vol. 6(12), pp. 335-343, 29 June, 2012
Available online at http://www.academicjournals.org/AJFS
DOI: 10.5897/AJFS12.013
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Structure and functional properties of resistant starch from butyrylated arenga starches

Abdul Rahim[1]*, Haryadi[2], Muh. Nur Cahyanto[2] and Yudi Pranoto[2]

[1]Departement of Food Science, Faculty of Agricultural, Tadulako University, Jl. Soekarno Hatta km 8 No. 32 Palu, Central Sulawesi, 94118 Indonesia.
[2]Departement of Food Science, Faculty of Agricultural Technology, Gadjah Mada University, Jl. Flora No. 1, Bulaksumur, Yogyakarta, 55281 Indonesia.

Accepted 4 June, 2012

Butyrylated arenga starches (BAS) with different degree of substitution (DS 0.074, DS 0.151, DS 0.226) were synthesized by the reaction of arenga starch with butyric anhydride under varying pH and butyric anhydride concentration. The products were characterized for resistant starch (RS), chemical structure of the RS by nuclear magnetic resonance (NMR) spectroscopy and functional properties including water and oil holding capacity (WHC and OHC), bile acid/salts and cholesterol binding. The results indicated that RS increased with increase in the DS of BAS. Analysis of ^{1}H NMR spectra of RS from BAS revealed the presence of methylene (CH_2) and methyl (CH_3) protons, while the ^{13}C NMR spectra gave the signals of methylene, methyl and carbonyl carbon of butyryl groups. Compared to the ^{13}C NMR spectrum of the native starch, the modified starches gave additional signals broadening of the C1 and C6 signals. Those results further suggested that the butyryl groups were substituted at the C2 and C6 positions. The WHC and OHC of RS from BAS increased with increase in the DS, indicating that butyrylation increased both hydrophilicity and hydrophobicity of the starches. The bile acid/salts of RS from BAS increased with the increase of DS and the total cholesterol decreased with the increasing DS.

Key words: Resistant starch, butyrylated arenga starch, degree of substitution.

INTRODUCTION

Resistant starch (RS) plays important physiological roles and has the potential to improve human health and lower the risk of many diet-related diseases. RS having a physiological effect similar to that of dietary fiber, is defined as the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals and enters the colon where it is fermented by bacteria to short chain fatty acids (SCFA) and gases (Englyst et al., 2000; Topping and Clifton, 2001). Acetate, propionate and butyrate are the major SCFA and they are attracting considerable interest for their potential ability to maintain the normal physiological function of the large bowel through modulation of colonic muscular activity, stimulation of electrolyte and fluid uptake, enhancement of blood flow and prevention of the overgrowth of pathogenic microorganisms (Bajka et al., 2006, 2010). The SCFA, particularly butyrate, have been implicated in promoting good colonic health and preventing the incidence of colorectal cancer. Butyrate was reported to increase the level of glutathione, an antioxidant, in colonic mucus, improving colonic resistance to toxic agents in the diet (Hamer et al., 2009). A significant decrease in bile acids (cholic acid, chenodeoxycholic acid, deoxycholic acid, lithocholic acid, isolithocholic acid and ursodeoxycholic acid) and total neutral sterols (coprostanol, cholesterol, 4- cholesten-3-one, campesterol, stigmasterol and β-sitosterol) in the fecal matter of healthy adults was reported after consuming experimental foods rich in RS for 4 weeks (Brouns et al., 2002; Hylla et al., 1998). Animal studies have shown that they pass into the large bowel where the esterified acids are released by bacterial enzyme activity (Annison et al., 2003). It has been suggested that RS promotes a higher

*Corresponding author. E-mail: a_pahira@yahoo.com. Tel: +62274 544716. Fax: +62274 589797.

proportion of butyric acid than other indigestible carbohydrates.

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). Acylated starch with low DS is commonly obtained by esterification of native starch in an aqueous medium in the presence of an alkaline catalyst. Acetylation can be performed with relative ease to significantly improve the physicochemical and functional properties of the starch, even at low DS (Xu et al., 2004). During acetylation, three free hydroxyl (OH) groups on C2, C3 and C6 of the anhydroglucose unit of the starch molecule can be replaced with acetyl groups in a kinetically controlled reaction; therefore, the theoretic maximum DS is three. In fact, these three free –OH groups have different reactivities (Bai et al., 2011). The primary –OH on C6 is more reactive and is acetylated more readily than the secondary ones on C2 and C3 due to steric hindrance (Xu et al., 2004). Between the other two –OH groups, the OH on C2 is more reactive than is the one on C3, mainly because the former is close to the hemi-acetal and more acidic than the latter.

Acetylation of corn starch contribute in raising the amount of RS content and also found that substitution with acetyl groups reduced the enzyme susceptibility in gelatinized starches (Chung et al., 2008). Acetylation of starch leads to a product with an increased hydrophobicity. Depending on the DS, some of the highly hydrophilic hydroxyl groups in the anhydroglucose units of starch are converted to more hydrophobic acetyl groups which become more easily to bind with bile acid/salt to reduce cholesterol level (Li et al., 2011). Butyrylation must lead to more hydrophobic groups than acetylation since butyryl groups contains a longer hydrophobic carbon chain. The objective of the present study was to obtain butyrylation method for arenga starches using butyric anhydride and characteristics of RS, chemical structure of the RS and functional properties including water and oil holding capacity, bile acid/salts and cholesterol binding capacities.

MATERIALS AND METHODS

Materials

Arenga starch (*Arenga pinnata* Merr.) used for this study was obtained from Klaten, Central Java Province, Indonesia, having the following proximate composition: amylose 31%, protein 0.82%, fat 0.13%, and moisture content 11%. High-purity butyric anhydride 98% was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Enzymes: heat-stable α-amylase and amyloglucosidase (given by PT. Rejo Madu Sari, Pati, Central Java Province). Cholic acid, sodium deoxycholate, sodium taurocholate, lechitin and cholesterol were obtained from Wako Pure Chemical Industries (Osaka, Japan) and other standard reagents for analysis used in the study were obtained from Merck, Darmstadt.

Preparation of butyrylated arenga starch

Butyrylated arenga starch (BAS) was prepared by a modified procedure of Phillips et al. (1999), and DS was determined by a titration method. Starch (100 g) was dispersed in 225 ml of distilled water and stirred for 60 min at 25°C. The suspension was adjusted and controlled at pH (9, 10) with 3.0% NaOH solution. Butyric anhydride of (10, 15% starch base, sb) was added drop-wise to the stirred slurry. The reaction was allowed to proceed for 40 min after the completion of butyric anhydride addition. The slurry was then adjusted to pH 4.5 with 0.5 N HCl. After sedimentation, it was washed twice with distilled water and once with 95% ethanol, and then oven-dried at 40°C for 48 h, and ground on an analytical mill. Butyrylation of starch with 15% (sb) butyric anhydride for 40 min at pH 9 gave the BAS with DS 0.074, with 10% (sb) butyric anhydride at pH 10 gave that with DS 0.15, and with 15% (sb) butyric anhydride at pH 10 gave that with DS 0.226.

Isolation of RS from BAS

RS from native arenga starch and that from BAS were isolated according to the enzymatic-gravimetric method (AOAC, 1990) with slightly modified. A total of 4 g BAS was suspended in 160 ml of 0.08 M phosphate buffer pH 5.5. Suspension BAS was gelatinized in boiling water until the suspension became more viscous. Gelatinized starch suspension was cooled at room temperature until it reached at approximately 85°C, then added with 40 µl of α-amylase and incubated in shaking water bath for 75 min. After incubation, pH of the digested starch suspension was adjusted to 4.5 by adding HCl 1N solution. The suspension was added with 80 µl of amyloglucosidase and incubated for 45 min at 60°C in shaking water bath. After incubation, the solution was heated at 100°C for 15 min, then centrifuged at 3400 g for 10 min. The supernatant obtained was added with 96% alcohol to obtain the final alcohol concentration of 80% then centrifuged at 3400 g for 10 min to remove the simple sugars dissolved in the supernatant. The RS precipitate was washed using 80% alcohol and subsequently centrifuged at 3400 g for 10 min, and then freeze-dried.

Determination of RS

The RS determined as a dietary fiber according to the enzymatic-gravimetric method (Ebihara et al., 2006). To determine the level of RS, 4 g of sample was suspended in 160 ml of 0.08 M phosphate buffer (pH 5.5) before the addition of 40 µl of heat-stable α-amylase. The solution was incubated at 95°C for 75 min and then allowed to cool. The pH of the solution was adjusted to 4.5 by adding HCl 1 N, and the sample was incubated with amyloglucosidase at 60°C for 45 min. Aliquot (100 µl) was added with 10 ml of glucose oxidase reagent and the mixture was incubated at 20°C for 20 min. Absorbance was measured using a spectrophotometer at 510 nm. The concentration of RS was calculated as follow: RS (g/100 g) = (1 – G × 0.9 / wt. sample) × 100, where wt. sample was the initial weight (g), and G is the weight of glucose (g).

NMR spectroscopy

Chemical structure of the RS was elucidated using nuclear magnetic resonance (NMR) spectroscopy recorded using an NMR

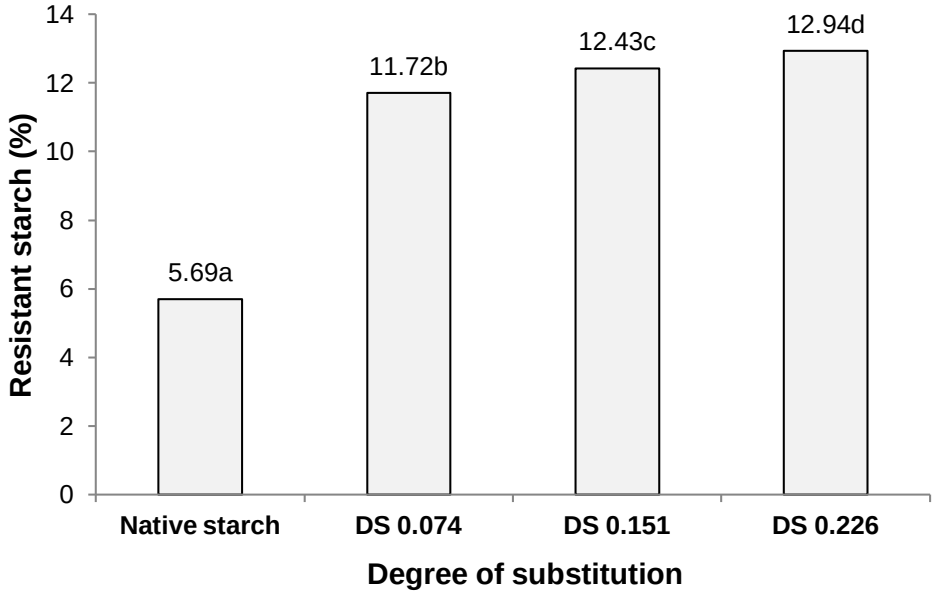

Figure 1. Effects of DS on the RS content of butyrylated arenga starches. Figures in the histograms followed by different letters indicate significant differences at ($p < 0.05$).

Bruker Avance II instrument at 600 MHz (field 14 T, operating at 600 MHz) for ^{1}H-NMR and at 525 MHz for ^{13}C-NMR, respectively. Each one of RS from the native starch and that of BAS (25 mg) were dissolved respectively in dimethyl sulphoxide-d_6 (0.75 ml) at 75°C to obtain clear solutions. They were then transferred into an NMR glass tube. All analyses were carried out at 25°C.

Water and oil holding capacity

Water and oil holding capacity (WHO, OHC) of the RS from native starch and BAS were measured using a method of Larrauri et al. (1996). 25 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 and WHC and OHC were calculated as g water or oil per g of dry sample, respectively.

The bile acid/salts binding

The binding of bile acid/salts (cholic acid, sodium taurocholic sodium deoxycholic) was measured by *in vitro* analysis (Barsby et al., 2000). The sample (100 mg) was admixed with 10 ml of solution of each bile acid. The solutions were prepared in 0.1 mol phosphate buffer pH 7.6 for each bile acid in 2 µmol/ml concentration. The samples and parallel blank samples were incubated at 37°C for 30 min. Centrifugation was performed at 2000 g for 5 min. The sample (50 µl) was combined with 5 ml 70% sulphuric acid and 1 ml of freshly prepared furfural solution (2.3 g/L) with careful mixing of the whole sample. Absorbance was measured at 510 nm after 80 min. The results were expressed as percent of bile acid absorption.

The cholesterol binding

The cholesterol binding was measured by *in vitro* analysis (Barsby et al., 2000). The sample (100 mg) was combined with 2 ml emulsion composed of 1% lecithin, 1.375% sodium salt of deoxycholic acid and 0.225% cholesterol prepared in 0.1 mol phosphate buffer of pH 6.8. Incubation for 1 h at 37°C was performed in a shaking water bath. Cholesterol absorption by 20 µl emulsion was analysed using reagent kits. The results were expressed as percent of cholesterol adsorbed by samples.

Statistical analysis

The differences between the mean values of multiple groups were analyzed by one-way analysis of variance (ANOVA) with Duncan's multiple range tests. ANOVA data with a $P < 0.05$ were classified as statistically significant. SPSS 13.0 software, Origin 75 and Microsoft Excel 2007 program were used to analyze and report the data. Mean values from the duplicated experiments were reported.

RESULTS AND DISCUSSION

RS content

RS and its yield of the BAS tended to increase with increasing DS (Figure 1). This was likely because of the introduction of ester groups to the starch molecules retarded partially the enzyme-substrate complex formation. Therefore, the RS content of the chemically modified starches increased. Earlier workers reported that RS content of octenyl succinylated corn starches (Wang et al., 2011) and that of octenyl succinylated high amylose maize starch (Zhang et al., 2011), increased with increasing the DS. All substituted arenga starches had a higher resistance to enzyme hydrolysis, compared to the native starch. The results suggested that BAS had more influence on resistance to enzyme than the native starch.

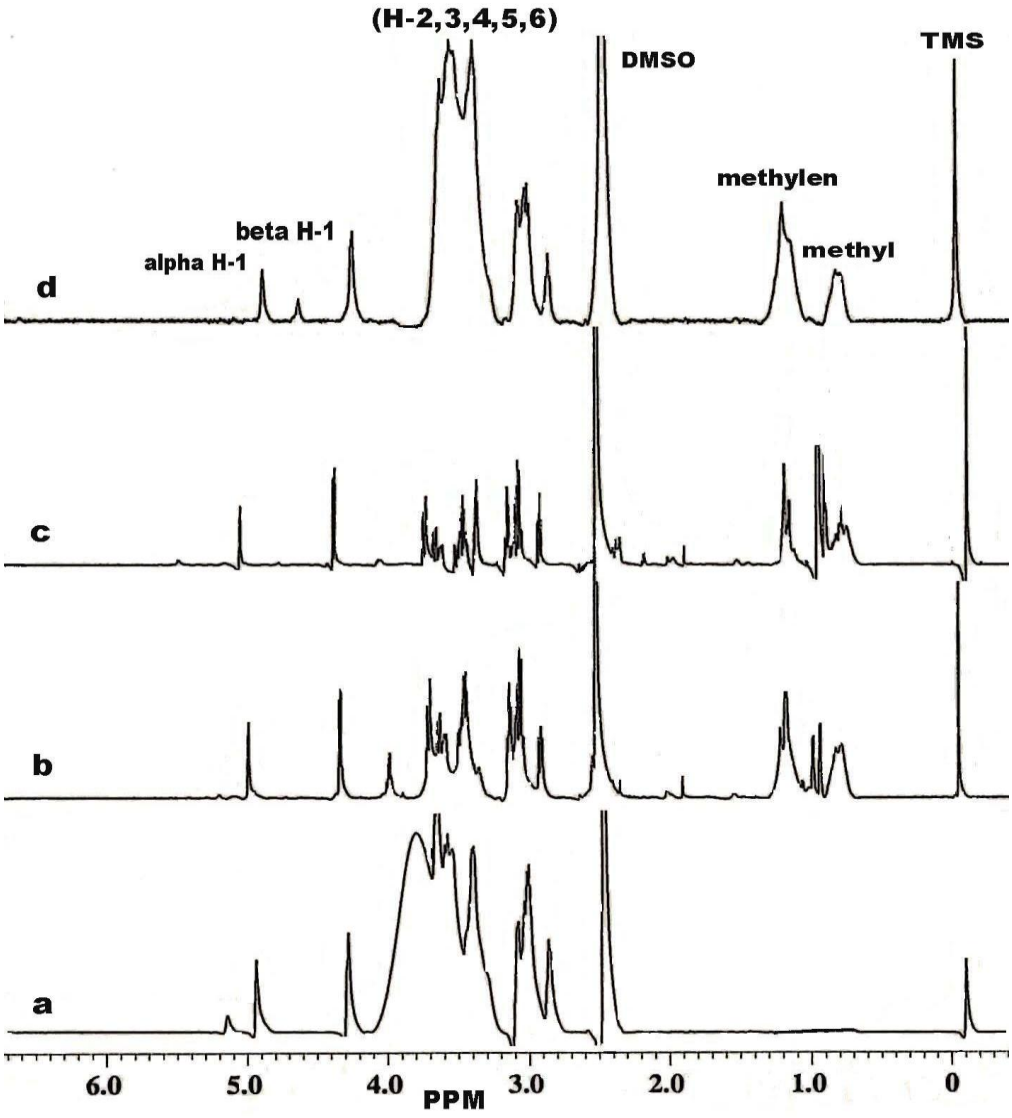

Figure 2. ^{1}H NMR spectra of RS from native arenga starch (a) and BAS with DS 0.074 (b), DS 0.151 (c) and DS 0.226 (d).

Structure of RS from native starch and BAS

^{1}H-NMR spectroscopy

^{1}H NMR spectra of RS from native arenga starch (a), and BAS with DS 0.074 (b), DS 0.151 (c) and DS 0.226 (d) are shown in Figure 2. Assignments are noted on each peak as reported by Bai et al. (2011). Compared to the native starch, BAS has several additional signals at 0.80 to 1.20 ppm, which are from the methylene (CH_2) and methyl (CH_3) protons of the butyryl group. Bai and Shi (2011) reported that octenyl succinic waxy maize starch (DS 0.088) had several additional signals at 0.7 to 3.0 ppm, which were from the octenyl succinic group. The broad peak at 0.8 to 1.0 ppm was from the methyl protons of the octenyl succinic group. ^{1}H NMR spectra also were helpful in determining the position of butyryl substitution on the starch molecule. Peak broadening and peak intensity decreasing at 5.06 ppm (H-1 of α-1,4-linked repeated units) were due to substitution of butyryl group at the C2 position. This was in accordance with the reported data by Bai et al. (2011), that peak broadening at 5.38 ppm (H-1 of α-1,4-linked repeated units) was due to substitution of octenyl succinic at the C2 position.

^{13}C-NMR spectroscopy

The ^{13}C NMR spectra of RS from native starch (a), and BAS with DS 0.074 (b), DS 0.151 (c) and DS 0.226 (d) are shown in Figure 3. It exhibits the peak characteristics of the anhydroglucose unit at 78.78, 73.30, 71.98 and

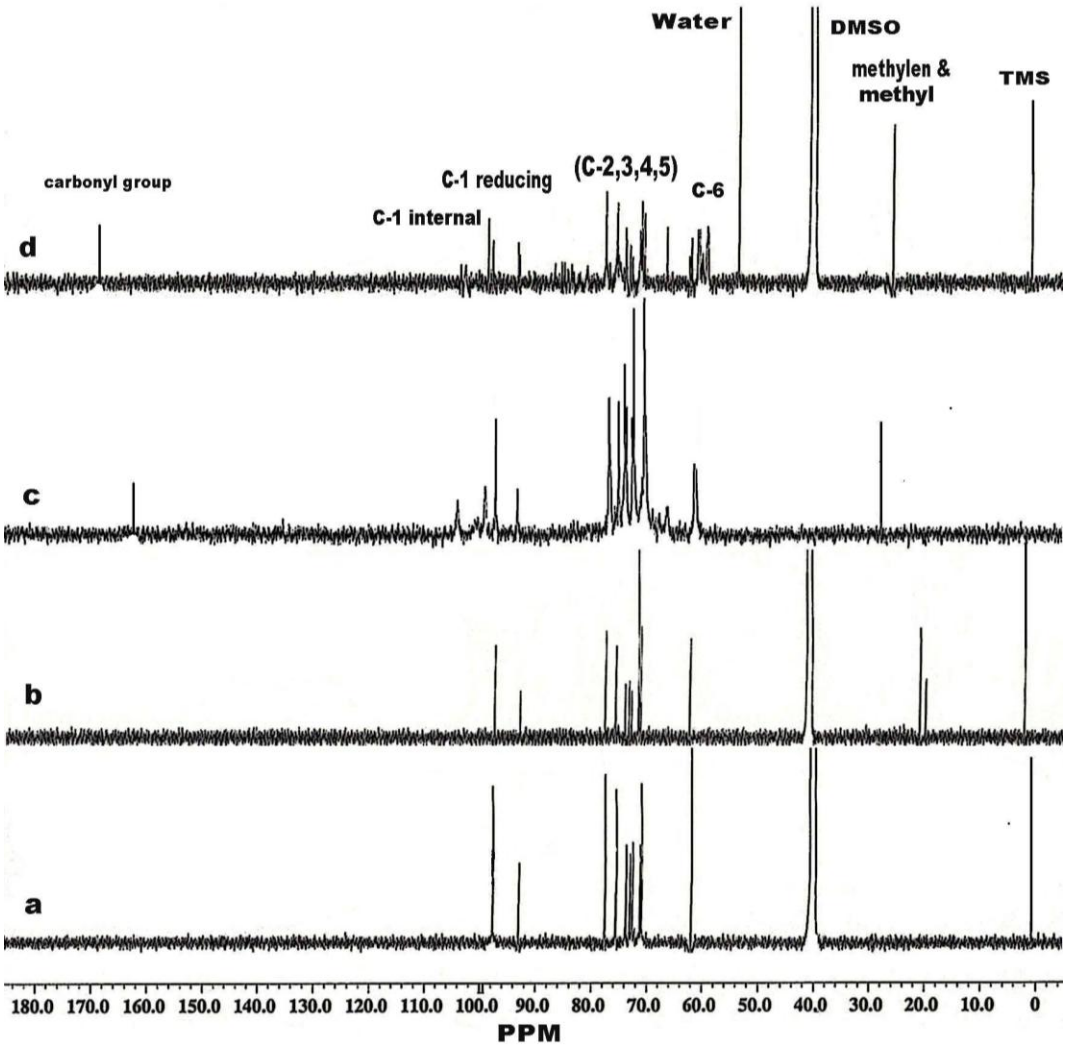

Figure 3. ^{13}C NMR spectra of RS from native arenga starch (a), and BAS with DS 0.074 (b), DS 0.151 (c) and DS 0.226 (d).

71.64 ppm, which correspond to C4, C3, C2 and C5, which are in accordance with the reported spectra of the acetylated starch (Chi et al. (2008). Further, they also reported that other two signals were observed at 100.85 and 100.27 due to C1 (1, 6) and C1 (1, 4) of the glucosidic link in amylopectin and amylose, respectively; while the signals of C6 (1,6) and C6 (1,4) appeared at 60.89 and 60.54 ppm. Based on the spectra identification aforementioned, the signal at 61.12 ppm of the ^{13}C NMR spectrum of RS from native starch and BAS were assigned as C-6 of starch anhydroglucose unit. The resonance at 76.65 ppm was assigned to C-4 at non-reducing end; the other signals at at 72.97, 71.84 and 70.19 ppm were C-2, 3 and 5. Because of α and β forms of reducing ends, C-2, 3 and 5 positions showed additional signals. They overlapped each other, and were not well resolved in 1-D NMR spectra. The carbons of C-1 α- and β-forms of reducing ends were well resolved, and gave signals at 96.78 and 92.39 ppm, respectively.

The ^{13}C NMR spectra of RS from BAS showed several additional signals that were clearly resolved. The signals at 18.06 to 19.11 ppm were assigned as the carbon with three protons attached to it. So, it was from the methyl carbon of butyryl groups. Signals from 24.93 to 27.58 ppm were from the carbons with two protons attached to them. They were assigned to the methylene carbons of the butyryl side chain. The resonances at 165.61 and 175.11 ppm were the carbons without proton, and they should be from the carbonyl of butyryl groups. All the additional signals compared with native starch were from butyryl group, providing the evidence of butyric anhydride modification on arenga starch. Vargha and Truter (2005) showed that acetylated wheat starch having several additional signals at 20.49 to 30.68 ppm was assigned to the methyl, and at 168.93 to 205.95 ppm were assigned to the carbonyl of acetyl group. The^{13}C-NMR spectra of RS from BAS were analyzed to study the substitution position of butyryl groups on starch molecules. Figure 3

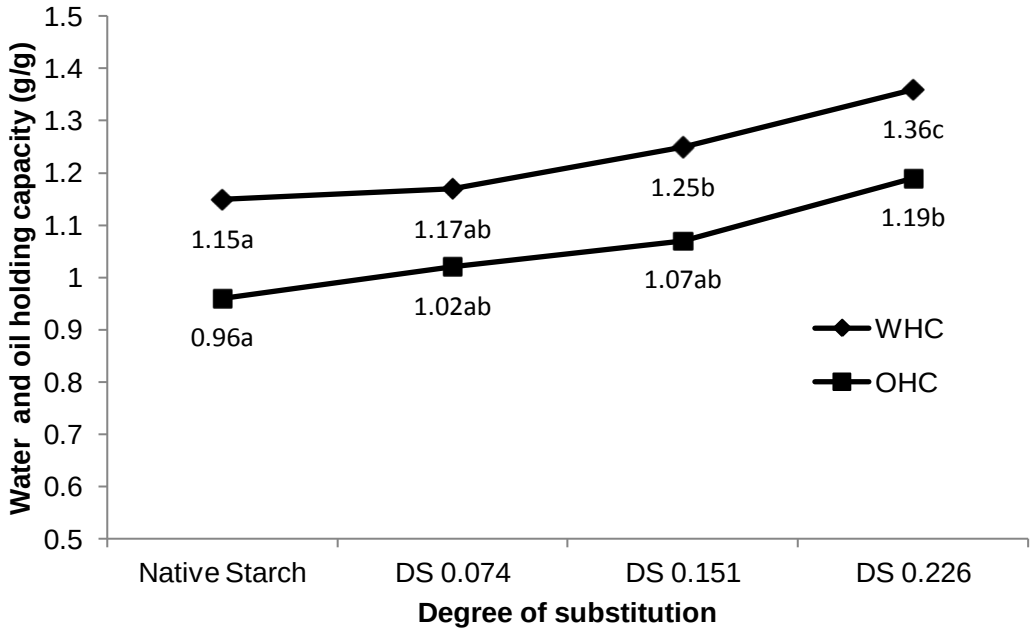

Figure 4. Effects of DS of RS from BAS on the water and oil holding capacities. Figures in the graph followed by different letters indicate significant differences at $p < 0.05$.

shows that the internal C-1 of RS from BAS had an additional shoulder or decreased intensity at 97.73 ppm compared to native arenga starch. The shoulder became more resolved or decreased as DS increased from 0.074 to 0.226, suggesting that the substitution at C2 position increased. In addition, the peak at 165.61 and 175.11 ppm which corresponds to the carbonyl of butyryl group evidences the modification on C-6. Apparently, the intensity of the signal for C2 and C6 significantly decreased with increase of DS, which indicated that the butyrylation reaction at C6 and C2 positions does occur. The significant decrease of the signal for C-6 indicated that the reactivity of C6 is higher than that of C2. Li et al. (2009) reported that the hydroxyl groups at C2 and C6 are more active than C-3 because of their steric property. Substitution with the hydroxyl at C2 position can change the chemical shift of internal C1, whereas substitution at C6 can change the chemical shift of C6. This result is similar to that observed in methylation of cassava starch with monochloroacetic acid (Bello et al., 2010). The carboxymethylation on C2 (C1) may also be identified by the appearance of a signal at 97.6 ppm. In addition, the peak at 178 ppm, which corresponds to the carbonyl C8 group, shows evidence of the modification at C6.

Water and oil holding capacity

WHC and OHC of RS from the native arenga starch and BAS increased with the increase of DS (Figure 4). These data indicate that either hydrophilicity or hydrophobicity tend to increase after butyrylation. Increase in water and oil absorption was a result of introduction of functional groups to the starch molecules, which facilitated a more enhanced holding capacity. This behavior of RS from BAS was ascribed to its particular structure with both hydrophobicity of butyryl hydrocarbon chains and hydrophilicity of carboxyl groups. At low level of DS, the butyryl groups were not sufficient to change the behavior of hydroxyl groups. There was weakening of inter-molecular hydrogen bonds in starch with the introduction of butyryl groups. Das et al. (2010) reported that the water and oil binding capacity increased with increasing DS (0.018 to 0.058).

The bile acid or salts binding

The data on the bile acid/salts absorption are presented in Figure 5. This data indicated the affinity to cholic acid, sodium taurocholate and sodium deoxycholate of RS from BAS tended to increase with increasing DS, compared to that of RS of native starch. The binding of cholic acid by the RS from BAS were from 3.15 to 8.04%, sodium taurocholate at 4.67 to 8.83% and the sodium deoxycholate at 5.00 to 9.88% (sb). The bile acid/salts binding capacity of RS from BAS significantly increased compared to that of the RS of native arenga starch. The interaction of RS from BAS with bile acid/salts were possibly chemical binding and entrapment in a gel matrix resulting from hydrophobic or hydrophilic interactions, since RS from BAS might have those properties (hydrophobic, hydrophilic) after butyrylation (Figure 4).

Thus, it makes sense that removing bile acid/salts

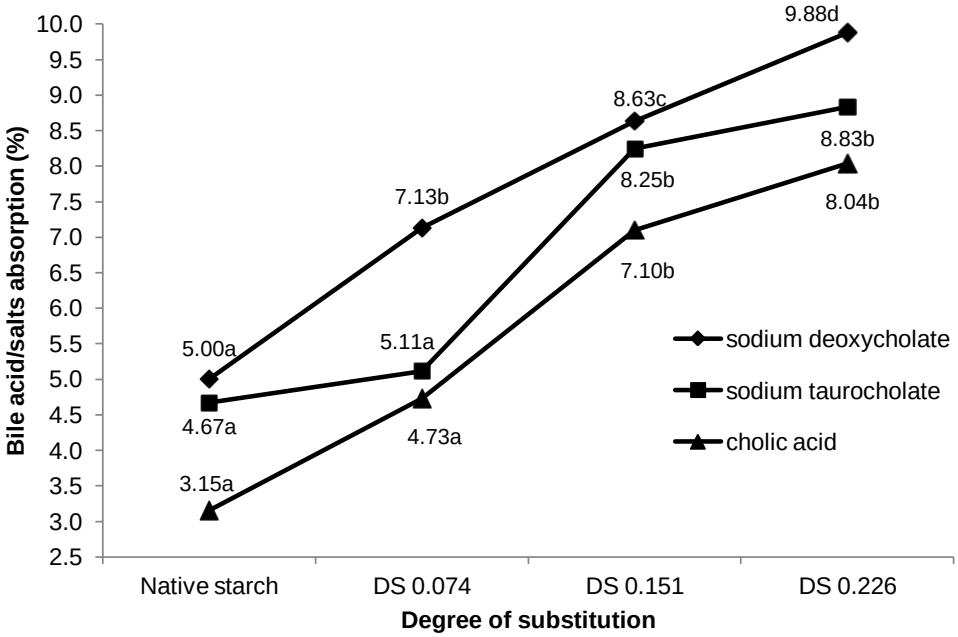

Figure 5. Binding of the bile acids/salts by RS from native and BAS with different DS. Figures in the graph followed by different letters indicate significant differences at $p < 0.05$.

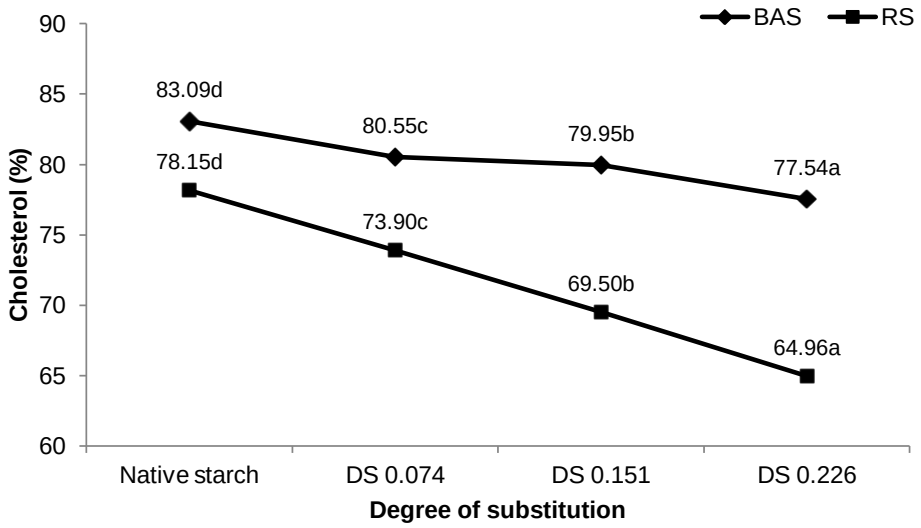

Figure 6. The decreased of total cholesterol by BAS and its RS with different DS. Figures in the graph followed by different letters indicate significant differences at $p < 0.05$.

directly from circulation or reducing the reabsorption of bile acid/salts, either by forming a complex with RS of butyrylated arenga starches or entrapping bile acid/salts in the viscous matrix, would have health benefits.

These were similar to the results of Thongngam and Clements (2005) which support the binding of bile salt (sodium taurocholate) with chitosan by hydrophobic interactions. Cornfine et al. (2010) reported the binding of bile acids with dietary fiber of lupin acetate by hydrophobic interactions.

The total cholesterol

The total cholesterol data are presented in Figure 6. The decreased of total cholesterol of BAS was 83.09% (native starch), 80.55% (DS 0.074), 79.95% (DS 0.151) and 77.54% (DS 0.226), whereas of RS from BAS was 78.15% (native starch), 73.90% (DS 0.074), 69.50% (DS 0.151) and 64.95% (DS 0.226). This data showed that the total cholesterol of BAS and its RS decreased with increase in DS, which is in accordance with the ability to

bind the bile acid/salts and increasing viscosity.

According of Han et al. (2004), rats fed with RS from kintoki were able to increase mRNA hepatic cholesterol 7α hydroxylase and increase the bile acid and steroid that excreted with feces. Soluble dietary fibres were (1.3:1.4)-beta-D-glucan (βG) have been reported to lower plasma cholesterol levels in the human body, at least in part by preventing bile acid/salts from being reabsorbed into the enterohepatic circulation (Gunness et al., 2010).

Conclusion

This results showed that the butyrylation contribute in raising the amount of resistant starch (RS) content which tended to increase with the increase of DS. Butyrylation significantly decreased the susceptibility of starch to α-amylase and amyloglucosidase. Analysis NMR spectra of RS from BAS showed that the butyric groups were substituted at the C2 and C6 positions. WHC and OHC of RS from BAS increased with the increase in DS, indicating that butyrylation improved hydrophilic and hydrophobic properties of the starch. The bile acid/salts of RS from BAS increased with the increase of DS, whereas the total cholesterol decreased with the increasing DS. The results of this study have provided a more detailed understanding of the origin and characteristics of the interactions between bile salts and RS, which might show the use in the design of supplements, drugs or functional foods to reduce cholesterol levels.

ACKNOWLEDGEMENT

This research was funded by “Program Hibah Penelitian Doktor” with contract number LPPM-UGM/874/BID.I/2011 from the Ministry of National Education of Republic of Indonesia.

REFERENCES

Annison G, Illman RJ, Topping DL (2003). Acetylated, propionylated or butyrylated starches raise large bowel short chain fatty acids preferentially when fed to rats. J. Nutr., 133(11): 3523-3528.

AOAC (1990). Official Methods of Analysis, 15th edn. Association of Official Analytical Chemists, Virginia, USA.

Bai Y, Shi YC, Herrera A, Prakash OM (2011). Study of octenyl succinic anhydride-modified waxy maize starch by nuclear magnetic resonance spectroscopy. J. Carbohydr. Polym., 83: 407-413.

Bai Y, Shi YC (2011). Structure and preparation of octenyl succinic esters of granular starch, microporous starch and soluble maltodextrin. J. Carbohydr. Polym., 83: 520-527.

Bajka BH, Topping DL, Cobiac L, Clarke JM (2006). Butyrylated starch is less susceptible to enzymic hydrolysis and increases large-bowel butyrate more than high-amylose maize starch in the rat. Br. J. Nutr., 96(2): 276-282.

Bajka BH, Clarke JM, Topping DL, Cobiac L, Abeywardena MY, Patten GS (2010). Butyrylated starch increases large bowel butyrate levels and lowers colonic smooth muscle contractility in rats. J. Nutr. Res., 30: 427-434.

Barsby TL, Donald AM, Frazier PJ (2000). Starch advances in structure and function. Churchill College, Cambridge.

Bello M, Ochoa N, Balsamo V, Carrasquero FL, Coll S, Monsalve A, Ganzalez G (2010). Modified cassava starches as corrosion inhibitors of carbon steel: An electrochemical and morphological approach. J. Carbohydr. Polym., 82: 561-568.

Brouns F, Kettlitz B, Arrigoni E (2002). Resistant starch and the butyrate revolution. Trends Food Sci. Technol., 13: 251-261.

Chi H, Xu K, Wu X, Chen Q, Xue D, Song C (2008). Effect of acetylation on the properties of corn starch. J. Food Chem., 106: 923-928.

Chung HJ, Shin DH, Lim ST (2008). *In vitro* starch digestibility and estimated glycemic index of chemically modified corn starches. J. Food Res. Int., 41: 579-585.

Cornfine C, Hasenkopf K, Eisner P, Schweiggert U (2010). Influence of chemical and physical modification on the bile acid binding capacity of dietary fibre from lupins (*Lupinus angustifolius* L). J. Food Chem., 122: 638-644.

Das AB, Singh G, Singh S, Riar CS (2010). Effect of acetylation and dual modification on physico-chemical, rheological and morphological characteristics of sweet potato (*Ipomoea batatas*) starch. J. Carbohydr. Polym., 80: 725-732.

Ebihara K, Nakai Y, Kishida T (2006). Hydroxypropyl distarch phosphate from potato starch increases fecal output, but does not reduce zinc, iron, calcium and magnecium absorption in rats. J. Food Sci., 71: S163-S168.

Englyst HN, Kingman SM, Cummings JH (2000). Classification and measurement of nutritionally importance starch fraction. Eur. J. Clin. Nutr., 46: S33 - S50.

Gunness P, Flanagan BM, Gidley MJ (2010). Molecular interactions between cereal soluble dietary fibre polymers and a model bile salt deduced from ^{13}C-NMR titration. J. Cereal Sci., 52: 444-449.

Hamer HM, Jonkers D, Bast A, Vanhoutvin SALW, Fischer MAJG, Kodde A, Troots FJ, Venema K, Brummer RJM (2009). Butyrate modulates oxidative stress in the colonic mucosa of healthy humans. J. Clin. Nutr., 28: 88-93.

Han K, Sekikawa M, Shimada K, Sasaki K, Ohba K, Fukushima M (2004). Resistant starch fraction prepared from kintoki bean affects gene expression of genes associated with cholesterol metabolism in rats. Exp. Biol. Med., 229: 787-792.

Hirsch JB, Kokini JL (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. J. Cereal Chem., 79: 102-107.

Hylla S, Gostner A, Dusel G, Anger H, Bartram H, Christl SU (1998). Effects of resistant starch on the colon in healthy volunteers: Possible implications for cancer prevention. Am. J. Clin. Nutr., 6: 136-142.

Larrauri JA, 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.

Li WY, Jin AX, Liu CF, Sun RC, Zhang AP, Kennedy JF (2009). Homogeneous modification of cellulose with succinic anhydride in ionic liquid using 4-dimethylaminopyridine as a catalyst. J. Carbohydr. Polym., 78: 389-395.

Li X, Zhang P, Chen L, Xie F, Li L, Li B (2011). Structure and colon-targeted releasing property of resistant octenyl succinate starch. Food Res. Int., FRIN-03773: 1-7.

Phillips DL, Huijum L, Duohai P, Harold C (1999). General application of raman spectroscopy for the determination of level of acetylation in modified starches. J. Cereal Chem., 76: 439-443.

Thongngam M, Clements DJ (2005). Isothermal titration calorimetry study of the interactions between chitosan and a bile salt (sodium taurocholate). Food Hydrocol., 19: 813-819.

Topping DL, Clifton PM (2001). Short chain fatty acids and human colonic function: Roles of resistant starch and non starch polysaccharides. J. Physiol. Rev., 81(3): 1031-1064.

Vargha V, Truter P (2005). Biodegradable polymers by reactive blending trans-esterification of thermoplastic starch with poly (vinyl acetate) and poly(vinyl acetate-co-butyl acrylate). Eur. Pol. J., 41: 715-726.

Wang X, Li X, Chen L, Xie F, Yu L, Li B (2011). Preparation and characterisation of octenyl succinate starch as a delivery carrier for bioactive food components. J. Food Chem., 126: 1218-1225.

Xu Y, Miladinove V, Hanna MA (2004). Synthesis and characterization

of starch acetates with high substitution. J. Cereal Chem., 81(6): 735-740.
Zhang B, Huanga Q, Luoa F, Fua X, Jiang H, Jane J (2011). Effects of octenylsuccinylation on the structure and properties of high-amylose maize starch. J. Carb. Pol., 84: 1276-1280.