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Confocal laser scanning microscopy of dextran–rice starch mixtures

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ABSTRACT

The microstructure of rice starch and dextran–rice starch mixtures was studied using confocal laser scanning microscopy (CSLM) and scanning electron microscopy (SEM). Surface pores and channels of rice starch were looked for. Channels could not be found in rice starch granules after reaction with 3-(4-carboxybenzoyl)-quinoline-2-carboxaldehyde (CBQCA). Fluorescein-labeled dextran was mixed with rice starch in order to locate hydrocolloid molecules in hydrocolloid–rice starch mixtures. The results showed that FITC-dextran (ave. Mw 4000; FD4) penetrated into raw rice starch granules, with the degree of penetration varying from granule to granule. FD4 could penetrate throughout cooked rice starch granules. FITC-dextran with an average Mw of more than 10,000 could not penetrate either raw or cooked rice starch granules.

1. Introduction

Rice starch is one of the important starches that can be produced and used in Thailand. The major concerns of the use of starch in the food industry are gelatinization, retrogradation and syneresis, each of which can be affected by the addition of hydrocolloids (Alloncle, Lefebvre, Llamas, & Doublier, 1989; Bahnassey & Breene, 1994; Christianson, Hodge, Osborne, & Detroy, 1981; Funami et al., 2005; Sae-kang & Suphantharika, 2006; Sudhakar, Singhal, & Kulkarni, 1996). When xanthan is used in starch-based frozen food products, the undesirable effects of low temperature on textural quality can be avoided (Sae-kang & Suphantharika, 2006).

Dextran is a water-soluble glucan produced from sucrose by enzymes on the cell surface of lactic acid bacteria or and produced by *Leuconostoc mesenteroides*. Dextran is composed of D-anhydroglucosyl units joined by α-(1 → 6)-linkages in the main chains with α-(1 → 3)-linkages at branch points. Dextran of various molecular weights is produced by employing *L. mesenteroides* dextransucrase and sucrose at various concentrations (Falconer, Mukerjea, & Robyt, 2011).

The study of starch–hydrocolloid paste microstructures helps to understand their rheology, texture, and sensory characteristics. The use of fluorescein-labeled hydrocolloids allows visualization of their location in starch dispersions (van de Velde, Weinbreck, Edelman, van der Linden, & Tromp, 2003). Confocal laser scanning microscopy (CSLM) was used to investigate starch microstructure (Choi, Woo, Ko, & Moon, 2008; Huber & BeMiller, 2000; Kim & Huber, 2008) and the behavior of starch–hydrocolloid mixtures (Tromp, van de Velde, van Riel, & Paques, 2001; van de Velde et al., 2003). Many researchers have studied the microstructure of corn, sorghum, wheat, and triticale starch granules (Fannon, Shull, & BeMiller, 1993; Huber & BeMiller, 2000; Kim & Huber, 2008; Naguleswaran, Li, Vasanthan, & Bressler, 2011; Zhao, Madson, & Whistler, 1996). Noisuwan, Hemar, Wilkinson, & Bronlund, (2011) studied the adsorption of Alexa Fluor™-labeled sodium caseinate (NaCAS) and Alexa Fluor™ labeled whey protein isolate (WPI) on normal and waxy rice starch granules. They concluded that fluorescence-labeled NaCAS and WPI adsorbed on the granule surface of normal and waxy rice starch. However, they did not study the effect of molecular weight (Mw) and the effect of starch gelatinization on the penetration of hydrocolloids into rice starch. Moreover, they did not study microstructure of normal and waxy rice starch such as surface pores and starch channels. In this research, the microstructure of rice starch and rice starch–dextran mixtures was studied before and after cooking the starch. Dextran of various molecular weights labeled with fluorescein was used to determine the location of the hydrocolloid in dextran–rice starch mixtures.

2. Materials and methods

2.1. Materials

Normal rice starch and waxy rice starch were supplied by Cho Heng Rice Vermicelli Factory Co., Ltd. (Nakhon Pathom, Thailand). Tapioca starch was purchased in a local market from Thai Wah

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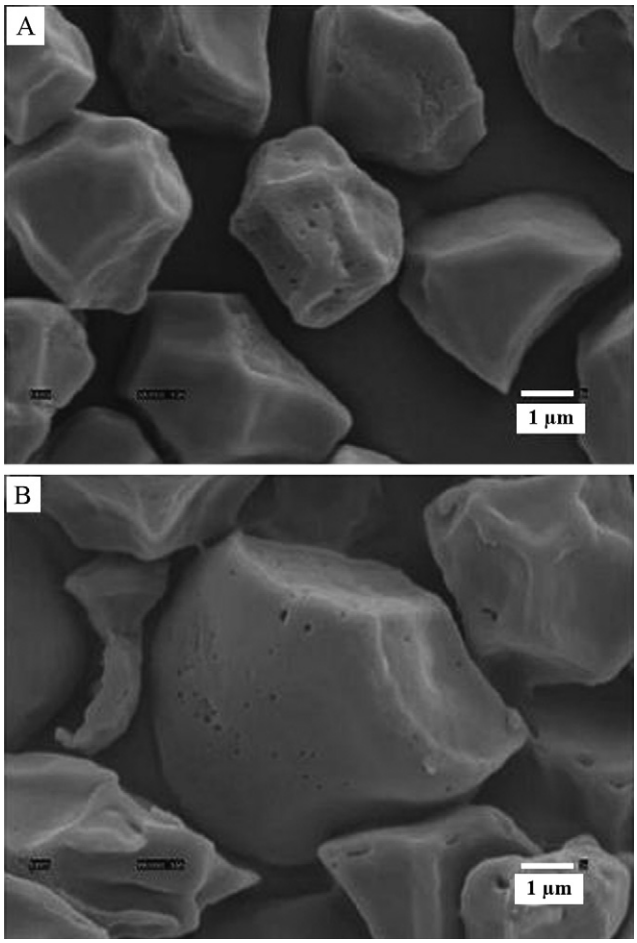

Fig. 1. Scanning electron micrograph of normal (A) and waxy rice starch.

Food Product Public Co., Ltd. (Nakhon Pathom, Thailand). Sorghum starch was kindly supplied by B.R. Hamaker, Whistler Center for Carbohydrate Research, Department of Food Science, Purdue University, USA. Corn starch was obtained from Tate & Lyle N.A. (Decatur, IL, USA). Mercury dibromofluorescein (disodium salt, Merbromin) was purchased from Sigma–Aldrich (St. Louis, MO, USA). CBQCA Protein Quantitation Kit (C-6667) was purchased from Invitrogen (Carlsbad, CA, USA). FD4 (FITC-dextran ave. Mw 4000) and FD10 (FITC-dextran ave. Mw 10,000) were purchased from Sigma–Aldrich. The dextrans were derived from *L. mesenteroides*, strain B512.

2.2. Preparation of cooked dextran–rice starch mixtures

Dispersions of rice starch were prepared at 3% (w/v) concentration. A weighed amount of rice starch was placed in an eppendorf tube. Distilled water was pipetted into the tube, which was then vortex stirred. FITC-dextran was added to the starch dispersion at 1% (w/v) concentration, and the mixture was vortex stirred at room temperature for 10 min. The mixture (70 μL) was heated in a water bath at 95 °C for 15 s, and then cooled rapidly in a water-ice bath.

2.3. Merbromin treatment

Starch samples were treated with merbromin according to the method of Huber and BeMiller (1997, 2000). Starch samples (2.5 mg) were suspended in a merbromin solution, and the suspension was mixed using a multi-wrist shaker (Lab-Line Instruments, Melrose Park, IL, USA) for 90 min at room temperature. The starch was recovered by centrifugation, washed a few times with absolute ethanol, and air-dried in the dark.

2.4. CBQCA treatment

The method used for 3-(4-carboxybenzoylquinoline-2-carboxaldehyde) (CBQCA) staining was that of Han, Benmoussa, Gray, BeMiller, and Hamaker (2005). Starch (7 mg) was mixed with 0.1 M sodium borate buffer (pH 9.3) (135 μL), 20 mM KCN (5 μL) and 5 mM CBQCA (10 μL), respectively. Samples were incubated for 3–5 h and observed using CLSM.

2.5. Amyloglucosidase

Starch (10 mg) was treated with 2 units of amyloglucosidase (Sigma–Aldrich, St. Louis, MO, USA) in 1 mL of 0.2 M sodium acetate buffer, pH 4.5, while being shaken with a multi-wrist shaker at room temperature for 24 h. The reactions were inactived in a boiling water bath for 10 min. Starch samples were recovered by centrifugation, washed with distilled water 5 times, 70% ethanol 1 time and 100% ethanol 1 time, and allowed to air-dry.

2.6. Scanning electron microscopy (SEM)

Samples were mounted on stubs with double-sided sticky tape, coated with AuPd for 3 min, and imaged in a JEOL JSM-840 SEM (JEOL USA Inc., Peabody, MA) using 5 kV accelerating voltage, 10 mm working distance, aperture 3 and probe current 3×10^{-11}. Magnifications were 5000× and 10,000×. Digital images were captured using 1280 × 960 resolution and 160 s dwell time.

2.7. Confocal laser scanning microscopy (CLSM)

Starch powder was sprinkled onto a glass slide. The glass slide and cover slip were glued together with a thin film of paraffin wax. A Bio-Rad Radiance 2100 MP Rainbow System (Bio-Rad Laboratories, Hercules, CA, USA) equipped with a Nikon Eclipse TE2000 (Nikon, Melville, NY, USA) inverted microscope was used to image starch granules at an excitation wavelength of 488 nm. CLSM digital images were acquired using the BioRad Laser Sharp program.

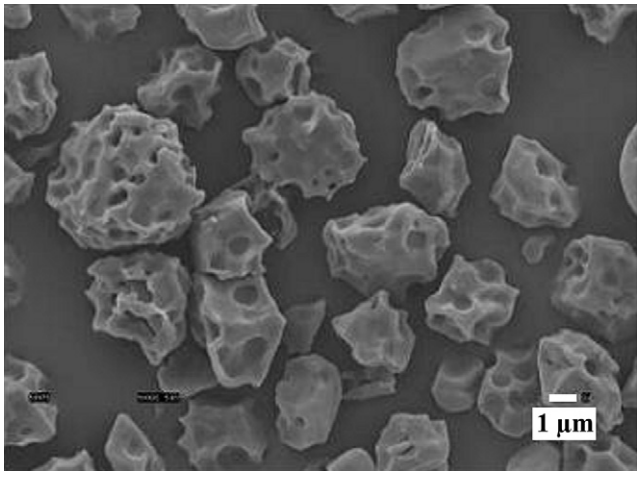

Fig. 2. Scanning electron micrograph of waxy rice starch treated with amyloglucosidase.

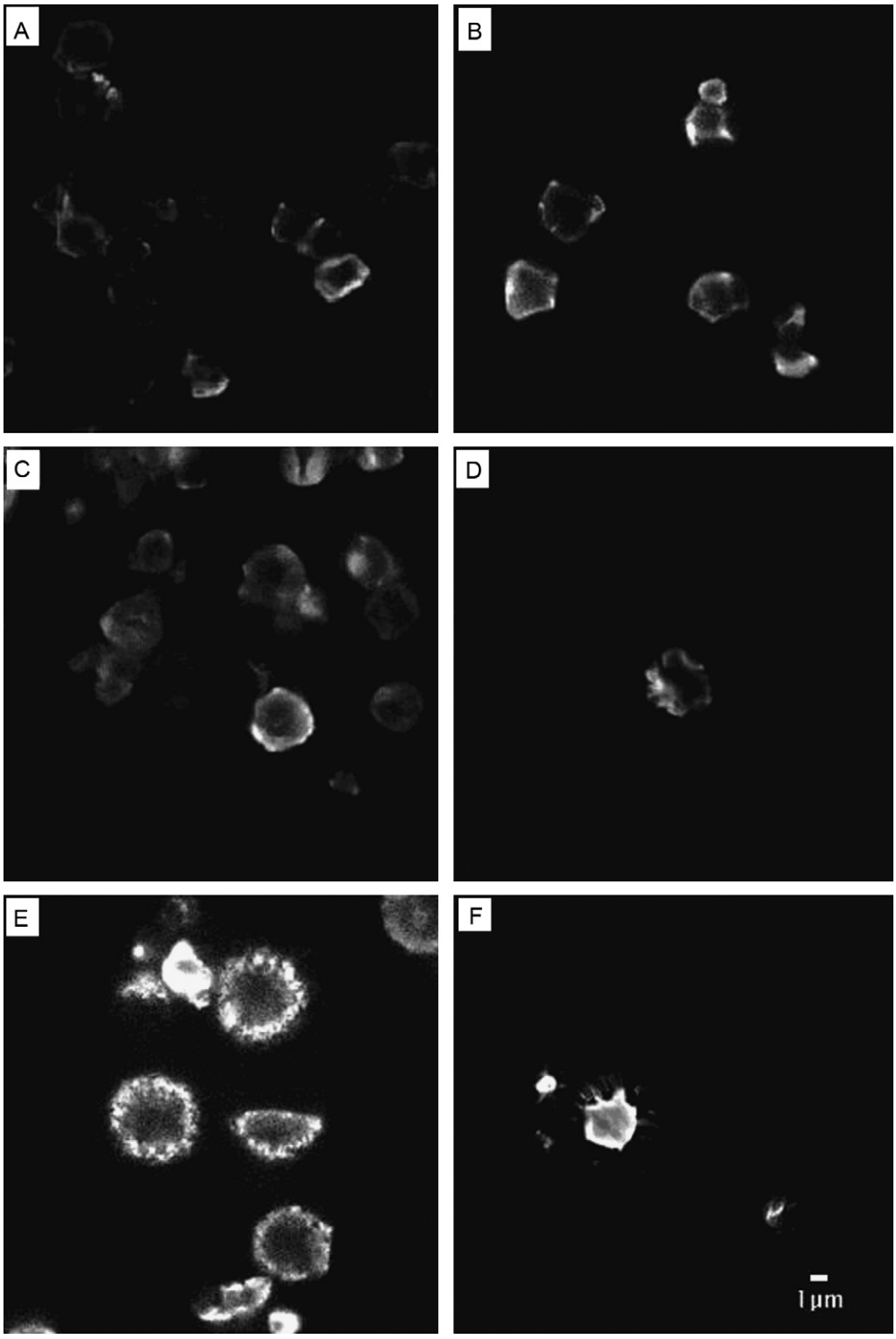

Fig. 3. Confocal laser scanning micrographs of optical sections of normal rice starch (A), waxy rice starch (B) treated with merbromin. Normal rice starch (C), waxy rice starch (D), tapioca starch (E), and corn starch (F) after enzymatic digestion with amyloglucosidase for 24 h and dyed with merbromin.

3. Results and discussion

3.1. Surface pores and channels of rice starch

The surface pores (0.07–0.1 μm in diameter) of starch granules are openings to interior channels. Some starches have surface pores, while the others have none (Fannon, Hauber, & BeMiller, 1992a; Fannon et al., 1993; Huber & BeMiller, 2000). Fannon et al. (1992a) proposed that the presence of channels facilitates enzyme attack of the granule during germination and found that the number of surface pores on corn starch granules varied from granule to granule.

3.1.1. Scanning electron microscopy

It was observed (SEM) that, for the most part, normal and waxy rice starch granules had no pores (Fig. 1). However, a few rice starch

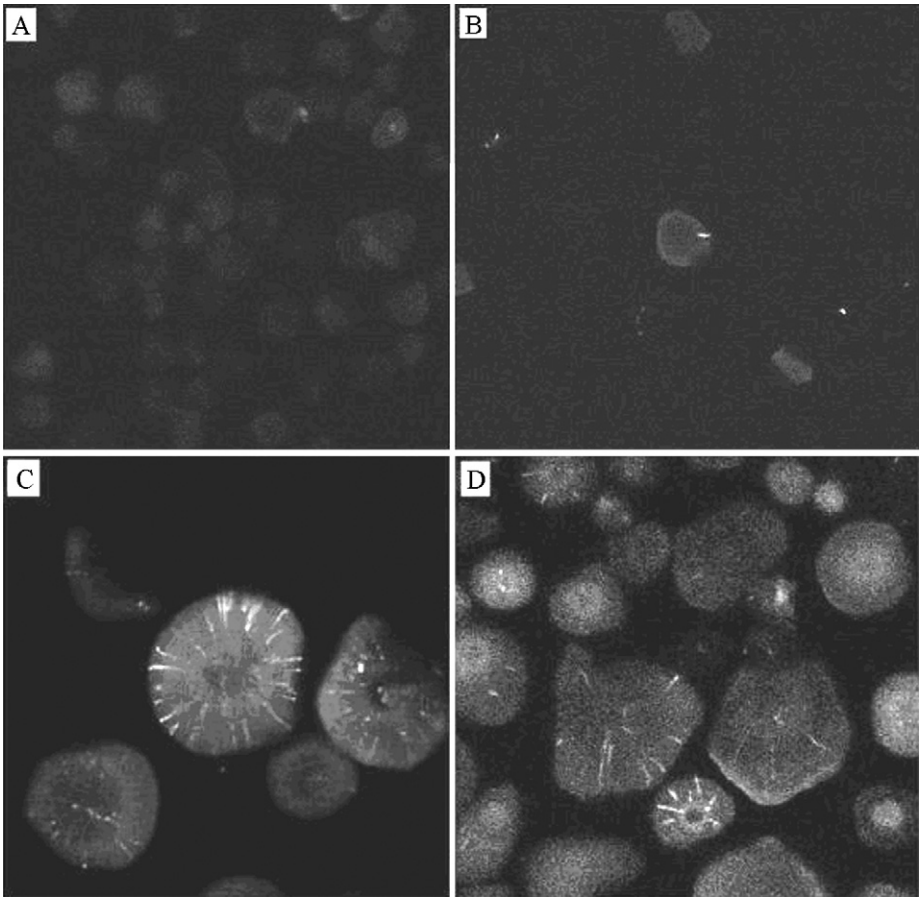

Fig. 4. Confocal laser scanning micrographs of optical sections of normal rice starch (A), waxy rice starch (B), sorghum starch (C), and corn starch (D) dyed with CBQCA (60×, zoom 4).

and waxy rice starch granules had small pores on their surface. Fannon et al. (1992a) reported that no pores were found in rice starch. Our assumption was that the pores of rice starch would be too small to observe using SEM, so the presence of surface pores of rice starch was investigated by observing an enzyme-catalyzed digestion pattern. Amyloglucosidase was used to enlarge any pores. The pattern of enzyme digestion on starch which has surface pores was different from starch which has no pores. Enzyme-treated normal corn starch granules had enlarged surface pores, whereas enzyme-treated potato starch showed exocorrosion on the granule surface (Fannon et al., 1992a). It was assumed that waxy rice starch had more potential to find surface pores compared with normal rice starch. When waxy rice starch was treated with amyloglucosidase (Fig. 2), it was more susceptible to enzymatic digestion than normal rice starch.

3.1.2. Confocal laser scanning microscopy

Starch channels and cavities of corn starch and sorghum starch flooded with merbromin showed up as lines or spots when observed using CSLM (Huber & BeMiller, 1997, 2000). Yoshino, Hayashi, and Seguchi (2005) found starch granule surface protein of rice starch by staining starch granules with fluorescamine. However, no channels were found in this study in either native normal or waxy rice starch granules dyed with merbromin (Fig. 3A and B). A very few normal and waxy rice starch granules showed short lines after treatment with merbromin; however, the lines were short compared with those in corn starch granules (Fig. 3F).

The presence of channels affects enzymatic attack (Gallant, Bouchet, & Baldwin, 1997). Corn starch granules treated with microbial glucoamylase for up to 8 h showed enlarged surface pores and channels (approx. 0.1–0.2 μm in diameter) on the granule surface (Imam, Gordon, Mohamed, Harry-O'Kuru, Chiou, Glenn, & Orts, 2006). Gallant et al. (1997) and Hall and Sayre (1969) reported that tapioca starch granules have pores, but we did not find any. Normal rice starch, waxy rice starch and tapioca starch treated with amyloglucosidase showed no channels when dyed with merbromin (Fig. 3C–E). The results were similar to those of Fannon et al. (1992a), who concluded that tapioca starch and rice starch granules had no pores. Corn starch granules treated with amyloglucosidase (Fig. 3F) clearly showed channels when treated with merbromin.

CBQCA was also used to dye proteins in starch granules (Fig. 4), but did not reveal channels in either normal or waxy rice starch granules (Fig. 4A and B). We conclude that normal and waxy rice starch granules have no channels lined with protein. Sorghum and corn starch granules did show channels lined with protein (Fig. 4C and D) as lines arranged radially. The same results were reported by Han et al. (2005). Han et al. (2005) reported that confocal laser scanning micrographs of optical sections of maize starch and sorghum starch showed protein channels by protein staining with CBQCA. They concluded that the channel of maize starch and sorghum starch is filled with protein and connected the central area of starch granule to granule surface. From our results, corn starch dyed with CBQCA (Fig. 4D) showed less channels than sorghum starch (Fig. 4C). Huber and BeMiller (2000) reported the same results with our results that corn starch appeared to have less number of channels than sorghum starch. Sorghum and corn starch (Fig. 4C and D) showed the heterogeneity of channels between granules because of the variations in the number of pores and channels (Huber & BeMiller, 2000).

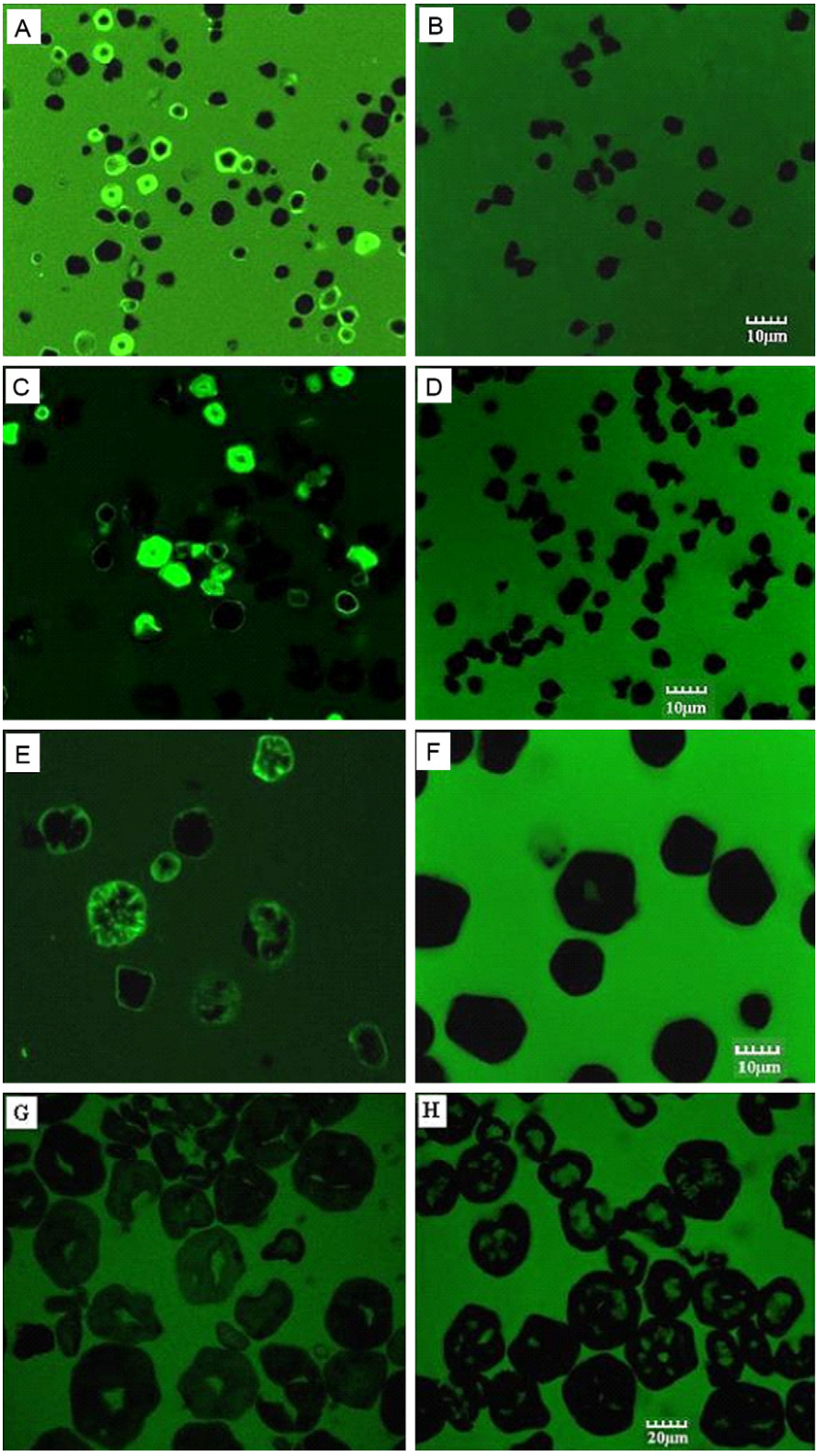

Fig. 5. Confocal laser scanning micrographs of optical sections of normal rice starch (A–D) and sorghum starch (E–H). Raw rice starch mixed with FD4 (A) and FD10 (B). Cooked rice starch mixed with FD4 (C) and FD10 (D). Raw sorghum starch mixed with FD4 (E) and FD10 (F). Cooked sorghum starch mixed with FD4 (G) and FD10 (H).

3.2. Penetration of hydrocolloids into starch granules

The penetration of hydrocolloids into starch granules has been studied by several researchers. Gonera and Cornillon (2002) indicated that xanthan absorbed only onto the surface of raw corn starch and stabilized granules. Fannon et al. (1992a, 1992b) concluded that surface pores of corn starch (approx. 1000 Å in diameter) allowed access of enzyme molecules to the granule

interior. We found that penetration of hydrocolloid molecules into raw starch granules and granule ghosts was a function of the molecular weight of the hydrocolloid. The results in Fig. 5 showed that FITC-dextran (ave. Mw 4000; FD4) penetrated into some raw and cooked rice starch granules, whereas, FITC-dextran (ave. Mw 10,000) did not penetrate through either raw or cooked rice starch granules.

Our results showed that FD4 can penetrate into raw rice starch granules to different degrees (Fig. 5A). FD4 only penetrated into the matrix of a few raw rice starch granules, whereas, it was absorbed onto the surface of some other granules, showing granule heterogeneity with regard to this property. Penetration of dextran into starch granules needs further study, even though channels were not found in rice starch granules.

The results in Fig. 5 also show the effects of gelatinization on penetration of hydrocolloids into starch granules. Fannon et al. (1992b) showed that granule ghosts are not continuous structures, but rather are quite porous; it can be assumed that the outer surface of granules is the same. Gallant et al. (1997) suggested that granules contain putative channels filled with amorphous material that could be attacked by amylases to produce bore holes. Oates (1997) proposed that the supermolecular helix of amylopectin molecules produced a channel that could be filled with an amylose–lipid complex. Neither of these proposed channels are the very large channels that are openings to surface pores and that can be seen by light microscopy studied by BeMiller et al. (Fannon, Gray, Gunawan, Huber, & BeMiller, 2003), but they may be related to the hypothesis of Atkin, Abeysekera, Cheng, and Robards (1998) that the small size of surface pores would not allow leaching of large molecules of amylopectin from starch granules at ambient temperature, but when the temperature of an aqueous suspension is increased, surface pore size may increase and the release of 400-nm amylopectin molecules from starch ghosts may be allowed. If the enlarged surface pores of cooked starch granules allow the release of amylopectin from granules, hydrocolloid molecules should be able to penetrate into the granules through the same path. Our results showed that only FD4 could penetrate throughout cooked rice and sorghum starch granules (Fig. 5C and G), while the large molecules of FITC-dextran could not enter the matrix of even cooked rice starch granules (Fig. 5D).

4. Conclusions

The presence of surface pores and channels in rice starch granules was investigated using SEM and CSLM and compared with those of sorghum starch, corn starch, and tapioca starch granules. From SEM results, the most of normal and waxy rice starch had no pores. A few rice starch and waxy rice starch granules had small surface pores. However, there was no evidence of channels in normal and waxy rice starch granules studied by using CSLM. The presence of starch channels in corn starch, sorghum starch and tapioca starch was investigated using CSLM. Our results showed no evidence of starch channels in tapioca starch. Corn starch treated with amyloglucosidase clearly showed channels when treated with merbromin. Starches were dyed with CBQCA in order to investigate the presence of channel proteins in starch granules. Channel proteins did not find in either normal or waxy rice starch. Sorghum and corn starch granules did show channels lined with protein. The study of penetration of hydrocolloids into starches showed the evidence of penetration of low-molecular-weight FITC-dextran (ave. Mw 4000) into raw and cooked normal rice starch and sorghum starch. In contrast, high-molecular-weight FITC-dextran (ave. Mw > 10,000) could not penetrate into these starch granules.

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References

Alloncle, M., Lefebvre, J., Llamas, G., & Doublier, J. L. (1989). A rheological characterization of cereal starch–galactomannan mixtures. *Cereal Chemistry*, *66*, 90–93.

Atkin, N. J., Abeysekera, R. M., Cheng, S. L., & Robards, A. W. (1998). An experimentally-based predictive model for the separation of amylopectin subunits during starch gelatinization. *Carbohydrate Polymers*, *36*, 173–192.

Bahnassey, Y. A., & Breene, W. M. (1994). Rapid Visco-Analyzer (RVA) pasting profiles of wheat, corn, waxy corn, tapioca and amaranth starches (*A. hypochondriacus* and *A. cruentus*) in the presence of konjac flour, gellan, guar, xanthan and locust bean gums. *Starch/Stärke*, *46*, 134–141.

Choi, S. J., Woo, H. D., Ko, S. H., & Moon, T. W. (2008). Confocal laser scanning microscopy to investigate the effect of cooking and sodium bisulfite on in vitro digestibility of waxy sorghum flour. *Cereal Chemistry*, *85*, 65–69.

Christianson, D. D., Hodge, J. E., Osborne, D., & Detroy, R. W. (1981). Gelatinization of wheat starch as modified by xanthan gum, guar gum, and cellulose gum. *Cereal Chemistry*, *58*, 513–517.

Falconer, D. J., Mukerjea, R., & Robyt, J. F. (2011). Biosynthesis of dextrans with different molecular weights by selecting the concentration of *Leuconostoc mesenteroides* B-512FMC dextransucrase, the sucrose concentration, and the temperature. *Carbohydrate Research*, *346*, 280–284.

Fannon, J. E., Gray, J. A., Gunawan, N., Huber, K. C., & BeMiller, J. N. (2003). The channels of starch granules. *Food Science and Biotechnology*, *12*, 700–704.

Fannon, J. E., Hauber, R. J., & BeMiller, J. N. (1992a). Surface pores of starch granules. *Cereal Chemistry*, *69*, 284–288.

Fannon, J. E., Hauber, R. J., & BeMiller, J. N. (1992b). Use of low-temperature scanning electron microscopy to examine starch granule structure and behavior. In R. Chandrasekaran (Ed.), *Frontiers in Carbohydrate Research – 2* (pp. 1–23). Essex, UK: Elsevier Science Publishers.

Fannon, J. E., Shull, J. M., & BeMiller, J. N. (1993). Interior channels of starch granules. *Cereal Chemistry*, *70*, 611–613.

Funami, T., Kataoka, Y., Omoto, T., Goto, Y., Asai, I., & Nishinari, K. (2005). Food hydrocolloids control the gelatinization and retrogradation behavior of starch 2a. Functions of guar gums with different molecular weights on the gelatinization behavior of corn starch. *Food Hydrocolloids*, *19*, 15–24.

Gallant, D. J., Bouchet, B., & Baldwin, P. M. (1997). Microscopy of starch: Evidence of a new level of granule organization. *Carbohydrate Polymers*, *32*, 177–191.

Gonera, A., & Cornillon, P. (2002). Gelatinization of starch/gum/sugar systems studied by using DSC, NMR, and CSLM. *Starch/Stärke*, *54*, 508–516.

Hall, D. M., & Sayre, J. G. (1969). Scanning electron-microscope study of starches. Part I: Root and tuber starches. *Textile Research Journal*, *39*, 1044–1052.

Han, X.-Z., Benmoussa, M., Gray, J. A., BeMiller, J. N., & Hamaker, B. R. (2005). Detection of proteins in starch granules channels. *Cereal Chemistry*, *82*, 351–355.

Huber, K. C., & BeMiller, J. N. (1997). Visualization of channels and cavities of corn and sorghum starch granules. *Cereal Chemistry*, *74*, 537–541.

Huber, K. C., & BeMiller, J. N. (2000). Channels of maize and sorghum starch granules. *Carbohydrate Polymers*, *41*, 269–276.

Imam, S. H., Gordon, S. H., Mohamed, A., Harry-O'Kuru, R., Chiou, B.-S., Glenn, G. M., et al. (2006). Enzyme catalysis of insoluble cornstarch granules: Impact on surface morphology, properties and biodegradability. *Polymer degradation and stability*, *91*, 2894–2900.

Kim, H.-S., & Huber, K. C. (2008). Channels within soft wheat starch A- and B-type granules. *Journal of Cereal Science*, *48*, 159–172.

Naguleswaran, S., Li, J., Vasanthan, T., & Bressler, D. (2011). Distribution of granule channels, protein, and phospholipid in triticale and corn starches as revealed by confocal laser scanning microscopy. *Cereal Chemistry*, *88*, 87–94.

Noisuwan, A., Hemar, Y., Wilkinson, B., & Bronlund, J. E. (2011). Adsorption of milk proteins onto rice starch granules. *Carbohydrate Polymers*, *84*, 247–254.

Oates, C. G. (1997). Towards an understanding of starch granule structure and hydrolysis. *Trends in Food Science and Technology*, *8*, 375–382.

Sae-kang, V., & Suphantharika, M. (2006). Influence of pH and xanthan gum addition on freeze–thaw stability of tapioca starch pastes. *Carbohydrate Polymers*, *65*, 371–380.

Sudhakar, V., Singhal, R. S., & Kulkarni, P. R. (1996). Starch–galactomannan interactions: Functionality and rheological aspects. *Food Chemistry*, *55*, 259–264.

Tromp, R. H., van de Velde, F., van Riel, J., & Paques, M. (2001). Confocal scanning light microscopy (CSLM) on mixtures of gelatine and polysaccharides. *Food Research International*, *34*, 931–938.

van de Velde, F., Weinbreck, F., Edelman, M. W., van der Linden, E., & Tromp, R. H. (2003). Visualisation of biopolymer mixtures using confocal scanning laser microscopy (CSLM) and covalent labelling techniques. *Colloids and Surfaces B: Biointerfaces*, *31*, 159–168.

Yoshino, Y., Hayashi, M., & Seguchi, M. (2005). Presence and amounts of starch granule surface proteins in various starches. *Cereal Chemistry*, *82*, 739–742.

Zhao, J., Madson, M. A., & Whistler, R. L. (1996). Cavities in porous corn starch provide a large storage space. *Cereal Chemistry*, *73*, 379–380.