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Physicochemical characteristics of phosphorylated cross-linked starch produced by reactive supercritical fluid extrusion

Khanitta Manoi[a,b,*], Syed S.H. Rizvi[a]

[a] *Institute of Food Science, Cornell University, Ithaca, NY 14853-7201, USA*
[b] *Agro-Industry Department, Naresuan University, Phitsanulok 65000, Thailand*

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ABSTRACT

Cross-linking of starch blends by phosphorylation using reactive supercritical fluid extrusion was investigated. A starch blend was mixed with 9% (starch basis) sodium trimetaphosphate and extruded at 60–70 °C with 0.1 M or 0.2 M NaOH solution and 0–1.5% (dry feed) supercritical carbon dioxide (SC-CO_2). Physicochemical and morphological characteristics of extruded starch phosphates were evaluated and compared with unextruded and extruded starch controls. Degree of substitution (DS = 0.05) and incorporated phosphorus content (P = 0.92%) was highest in the starch phosphates produced with 0.2 M NaOH, indicating the highest level of cross-linking. Cross-linking of starch at highest reaction pH was also evidenced by its lowest water solubility and highest proportion of distarch monophosphate investigated by ^{31}P NMR. Introduction of phosphate cross-links into the starch restricted the molecular structure mobility, leading to an increase in the gelatinization temperature (by up to 7 °C). SC-CO_2-based expansion provided light weight and non-porous skin starch foams with excellent water resistance which would be desirable properties for their utilization as a biodegradable material.

1. Introduction

Natural polymers, such as starches, have a range of potential applications in markets currently dominated by petroleum-based materials because they have the advantages of being renewable, biodegradable, abundantly available (especially wheat and corn), and low in cost. Most importantly, their properties can be tailored to meet specific demands. Petroleum-based polymers create solid waste disposal problems, and thus attention has been focused on the application of starch as a biodegradable thermoplastic material (Davis & Song, 2006; Tharanatha, 2003). Starch is the major component in cereal grains and is composed of amylopectin (branched polymer, ~70%, 4000 glucose units) and amylose (linear polymer, 1000 glucose unit) (Buléon, Colonna, Planchot, & Ball, 1998). However, starch alone is hardly functional as a material of utility due mainly to its poor mechanical properties (e.g. brittleness) and its hydrophilic nature, which both pose problems in its commercial use. Consequently, native starches are invariably modified mechanically, physically or chemically and/or combined with plasticizer or polymeric additives to overcome such disadvantages.

Cross-linking is a key technique for modifying the properties of starches and can be achieved by adding intra- and inter-molecular bonds at random locations in the starch granules (Singh, Kaur, & McCarthy, 2007). Cross-linking tends to limit the interaction of starch with water and provides a structural integrity of starch-based biodegradable materials during exposure to pressure and moisture (El-Tahlawy, Venditti, & Pawlak, 2007). Cross-linking is generally performed by treating starches (semi-dry or slurry) with reagents capable of forming either ether or ester linkages between hydroxyl (–OH) groups on starch molecules. Polyfunctional chemicals such as phosphorus oxychloride ($POCl_3$), sodium trimetaphosphate (STMP), sodium tripolyphosphate (STPP), epichlorohydrin (EPI), a mixture of adipic and acetic anhydrides, and a mixture of succinic anhydride and vinyl acetate have been commonly used to cross-link starches (Hirsch & Kokini, 2002; Singh et al., 2007). $POCl_3$ is an efficient cross-linking agent in aqueous slurry at pH > 11 in the presence of a neutral salt (Hirsch & Kokini, 2002; Singh et al., 2007). EPI is poorly soluble in water and less uniformly distributed. Additionally, both $POCl_3$ and EPI are toxic and flammable (MSDS, 1996; Woo & Seib, 1997). STMP, on the other hand, is cited as an efficient cross-linking agent and is a solid of low toxicity with no reports about the adverse effects on humans (Woo & Seib, 1997). The phosphorylation reaction produces either monostarch phosphates via substitution or distarch phosphates via cross-linking depending on the reaction conditions including concentration of STMP, amylose content, pH, time and temperature. Singh et al. (2007) stated that a combination

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[*] Corresponding author at: Institute of Food Science, Cornell University, 151 Stocking Hall Food Science Department, Ithaca, NY 14853-7201, USA. Tel.: +1 607 255 7904; fax: +1 607 254 4868.
E-mail address: km275@cornell.edu (K. Manoi).

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of substitution and cross-linking can provide stability against acid, thermal, and mechanical degradation of starch and delay retrogradation during storage. Starch phosphates prepared with STMP have been reported to enhance the stability of rice by minimizing granule rupture, solid loss, and water uptake (Rutledge, Islam, & James, 1972; Rutledge, Islam, & James, 1974).

However, the conventional procedures of starch phosphate preparations are time-consuming and require many steps to achieve the desired degree of chemical bonding. Reactive extrusion, on the other hand, can be advantageously utilized to modify starch as reported by several researchers (Chang & Lii, 1992; Kim, Shih, Champagne, & Daigle, 1999; Landerito & Wang, 2005; Nabeshima & Grossmann, 2001; O'Brien & Wang, 2009; O'Brien, Wang, Vervaet, & Remo, 2009; Seker & Hanna, 2005; Seker, Sadikoglu, & Hanna, 2004; Seker, Sadikoglu, Ozdemir, & Hanna, 2003a; Seker, Sadikoglu, Ozdemir, & Hanna, 2003b). The use of an extruder as a chemical reactor offers a larger operational flexibility as a result of the broad range of processing conditions in pressure, shear and temperature, the possibility of multiple injection, controlled residence time, and degree of mixing (Xie, Yu, Liu, & Chen, 2006). Landerito and Wang (2005) and Chang and Lii (1992) reported that the reactive extrusion process greatly increased the amount of incorporated phosphorus by 3–4 times, compared with the conventional method. The controlled temperature and high-shear pressure used in extrusion contribute to the enhanced phosphorylation.

Supercritical fluid extrusion (SCFX), a novel extrusion technology for production of highly expanded starch foams, was developed by Rizvi and Mulvaney (1992). Instead of steam this process uses supercritical CO_2 (SC-CO_2) as a blowing agent. SC-CO_2 is an environmentally friendly solvent, chemically inert, physiologically safe and readily available. Thermodynamically, SC-CO_2 has a liquid-like density and gas-like diffusivity and viscosity which leads to rapid wetting and allows penetration of complex structure. The supercritical conditions of CO_2 are relatively easy to achieve (critical temperature = 31 °C, critical pressure = 7.38 MPa) (Rizvi, Mulvaney, & Sokhey, 1995). This allows extrusion conditions to be maintained below 100 °C. A major advantage of CO_2 expansion over steam expansion is the combination of low temperature and low energy input with diffusion-controlled expansion, which provides a strategy for control of the number and size of cells and thus the morphology of the extrudate. The potential of using SCFX for producing puffed products, microcellular foams, and cross-linked starch extrudates has been reviewed elsewhere (Alavi, Gogoi, Khan, Bowman, & Rizvi, 1999; Ayoub & Rizvi, 2007; Mulvaney & Rizvi, 1993). The SCFX process dynamics can be divided into two stages: (I) flow of starch melts containing SC-CO_2 through the nozzle and (II) exit of extrudate from the die. The expansion process consists of three steps: (a) dissolving SC-CO_2 in the polymer melt to form a polymer and SC-CO_2 solution, (b) cell nucleation caused by rapid pressure drop and (c) cell growth and extrudate expansion at the die exit as the pressure quenches to atmospheric level (Alavi et al., 1999; Mulvaney & Rizvi, 1993; Rizvi et al., 1995).

The SCFX process is more versatile and controllable. In this process, the pressure drop can be manipulated by adjusting the operating conditions, therefore the cell size, cell density, and product expansion can be varied to produce a wide range of products having desired mechanical properties. Cross-linking of starch blends by phosphorylation via reactive SCFX for producing starch foam has not been previously reported. The aims of this work were to study the cross-linking of native/pre-gelatinized starch blends and to create cross-linked microcellular starch foams by reactive SCFX with STMP. Investigation of the effects of NaOH concentration and SC-CO_2 injection on the physicochemical and morphological characteristics as well as the ^{31}P NMR spectroscopy of extruded starch phosphates was the main focus of this study.

2. Materials and methods

2.1. Materials and feed formulation

Native (Midsol-50) and pre-gelatinized (Pregel-10) wheat starch were obtained from MGP Ingredients Inc. (Atchison, KS). Food-grade sodium trimetaphosphate (STMP) was purchased from Voigt Global Distribution Inc. (Lawrence, KS). Deuterium oxide (D_2O), sodium hydroxide (NaOH), and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma Chemical Company (St. Louis, MO). Pancreatic α-amylase (Pancreatin, 3 Ceralpha Units/mg) and amyloglucosidase (3300 Units/mL) were obtained from Megazyme International Ireland Ltd. (Bray, Ireland). All other chemicals used were reagent-grade. A mixture of native (40%, w/w) and pre-gelatinized wheat starch (60%, w/w) was used as a control-based formulation. Two levels of STMP powder (9 and 15%, total starch weight) were added to the starch mixture and mixed at ambient temperature for 1 h before feeding to the extruder.

2.2. Production of phosphorylated starch extrudates by extrusion process

2.2.1. Operating conditions

Extrusion of starches was performed using a pilot-scale Wenger TX-52 Magnum (Wenger Manufacturing, Sabetha, KS, USA) co-rotating twin-screw extruder. This extruder was specially configured for the process with 4.5 heads, a barrel diameter of 52 mm, and a length to diameter ratio (L/D) of 28.5:1. The reactive SCFX process was performed with a screw speed of 120 rpm, product temperature of 60–70 °C, and feed rate of 35 kg/h. The die was fitted with one circular insert of 4.2 mm diameter × 18.90 mm length. The average specific mechanical energy (SME) was 50 kW h/ton. The schematic of cross-linking of starch by phosphorylation via reactive SCFX process is illustrated in Fig. 1. In-barrel moisture content of the starch melt was maintained at 55% (dry feed basis) by injection of water (control) or NaOH solutions. To study the effect of alkalinity on the physicochemical properties of the phosphorylated starch extrudates, two different concentrations of NaOH solutions (0.1 and 0.2 M) streams were injected to the extruder at the mixing zone.

2.2.2. Supercritical CO_2 injection

A pilot-scale supercritical fluid system was used for injecting SC-CO_2 into the starch polymer melts at L/D of 24 through four injection valves located around the extruder barrel. The die pressure was maintained higher than the pressure inside the barrel for continuous SC-CO_2 flow into the starch melt, at the desired rates (1 and 1.5%, dry feed basis) and pressure (10–15 MPa). Product temperatures were monitored by a thermocouple at the end of the extruder. The cylindrical extrudates emerging from the die were collected on metal trays, dried in the convection oven at 75 °C for 3 h and then placed at room temperature for 24 h or until the final moisture content was approximately 5%. The dried extrudates were stored at room temperature in sealed containers.

2.3. Characterization of starch extrudates

2.3.1. Expansion ratio and piece density

Expansion ratio of the extrudates was calculated by dividing the cross-sectional area of the extrudates by the cross-sectional area of the die (13.85 mm^2 for 4.2 mm die). Piece density of the extrudates was calculated by dividing the sample mass by its volume. The volume of the extrudates was measured using the seed displacement method. An average of six samples was used for these measurements.

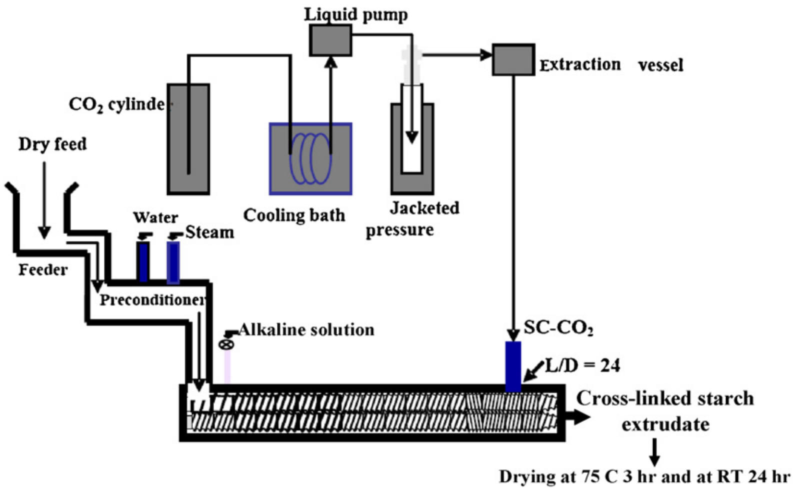

Fig. 1. Schematic of cross-linking of starch by phosphorylation via reactive SCFX process.

2.3.2. *Scanning electron microscopy (SEM)*

Morphological characterization of phosphorylated starch extrudates was performed on images captured by a scanning electron microscope (Leica 440 SEM). The extrudates were cut into 5 mm thick slices perpendicular to the longitudinal axis and mounted on aluminum stubs with double sided conductive carbon tape. A thin strip of conductive carbon paint was brushed on one side of each sample. The sample was then sputter-coated with gold to make the sample conductive at 100 mTorr vacuum. The digital images were obtained from the SEM. All samples were examined using an accelerating voltage of 5 kV.

2.3.3. *^{31}P nuclear magnetic resonance (^{31}P NMR) spectroscopy*

2.3.3.1. Sample preparation for ^{31}P NMR analysis. The excess phosphate in phosphorylated starch samples was removed by dispersing 10 g of starch powder in a 50 mL solution of ethanol and deionized water (1:1). After 5 min of mixing, the mixture was filtered and washed with deionized water (50 mL). The above procedure was repeated four times and the filtrate was discarded. The purified phosphorylated starch was dried at 45 °C for 24 h.

The purified phosphorylated starch was converted to phosphodextrins by enzymatic digestion for the ^{31}P NMR analysis. One gram of starch was dispersed in 20 mL of 100 mM sodium acetate buffer (pH 6.9, containing 2 mM calcium chloride and 0.002% sodium azide) by heating and stirring in a boiling-water bath for 20 min. After cooling, pancreatic α-amylase (500 U) was added to the starch solution and incubated at 25 °C for 20 h. The mixture was then adjusted to pH 4.5 and incubated with amyloglucosidase (200 μL) for 90 min at 60 °C. The mixture was then adjusted to pH 7.0.

2.3.3.2. ^{31}P NMR spectra of the phosphodextrins. ^{31}P spectra were acquired on a Varian UnityINOVA spectrometer equipped with a 10 mm broadband probe-head operating at 243 MHz for ^{31}P observation. For each spectrum 700–1200 transients were collected using 90° (7.25 μs) excitation pulse, 20 kHz spectral width, 16 k points, 6 s relaxation delay and broadband ^1H decoupling. The FIDs were truncated where possible to improve sensitivity. 2–4 Hz Gaussian broadening was applied and the data were zero-filled to 32 k complex points prior to Fourier transformation.

The phosphodextrin solution (5%, 2 mL) was mixed with 2 mL D_2O containing 20 mM EDTA, adjusted to pH 8.0 with 1 M NaOD solution, and then transferred to the 10 mm (diameter) NMR tube. The spectra were processed and analyzed with MestReNova 5.3.2 (MesterLab Research S. L., 2009). Chemical shifts were reported in δ (ppm) from the reference signal of external 85% phosphoric acid (0.0 ppm).

2.3.4. *Phosphorus content*

The phosphorus content of purified phosphorylated starch samples was evaluated using the molybdate blue method of Smith and Caruso (1964). Phosphorus content determination was established on the standard curve of KH_2PO_4 solutions having concentrations of 0.01–2% (w/v). The degree of substitution (DS) was calculated using the equation of Smith and Caruso (1964):

$$\mathrm{DS} = \frac{162\mathrm{P}}{3100 - 102\mathrm{P}}$$

where P = % incorporated phosphorus (w/w, dry basis) of the starch samples.

2.3.5. *Water solubility*

Dried starch extrudates were pulverized using a mill machine (Thomas-Wiley Mill model ED-5, Arthur H. Thomas Co., PA, USA) and sieved through a 250 μm screen (mesh #60). One gram of starch powder was dispersed in 30 mL of deionized water, stirred at 25 °C for 1 h and then transferred to centrifuge tubes. The centrifuge tubes were placed in a water bath at 90 °C for 30 min and centrifuged at 3000 × g for 20 min. The supernatant was decanted into the pre-weighed aluminum pan, dried at 103 °C for 12 h or until a constant weight was obtained. The solubility (S) of starch samples was determined as; S (%) = (weight of dissolved solids in supernatant/weight of sample) × 100.

2.3.6. *Differential scanning calorimetry (DSC)*

The thermal properties of starches were evaluated using a differential scanning calorimeter (DSC, TA Instrument, Model Q 10, New Castle, USA). Two milligrams (2 mg) of starch powder was weighed on the hermetic aluminum pan and 10 mg of deionized water was added. The pan was sealed and equilibrated at 25 °C overnight before carrying out the analysis. An empty pan was used as a reference and the DSC was calibrated using Indium. A sample was heated at 5 °C/min from 20 to 140 °C. The onset temperature (T_o), gelatinization temperature (T_p), and enthalpy (ΔH) were calculated from thermogram using the TA Instruments universal analysis 2000.

Table 1
Piece density and expansion ratio (ER) of starch control and extruded starch phosphate extrudates produced by SCFX.

Samples	% $SC\text{-}CO_2$	Density (g/cm^3)	ER
Extruded starch control 1 (0% STMP, 0.0 M NaOH)	0	0.72 ± 0.01	2.30 ± 0.88
Extruded starch control 2 (0% STMP, 0.1 M NaOH)	0	0.78 ± 0.03	1.78 ± 0.44
Extruded starch control 3 (0% STMP, 0.2 M NaOH)	0	0.81 ± 0.06	1.84 ± 0.52
Extruded starch phosphate 1 (9% STMP, 0.1 M NaOH)	0	0.89 ± 0.02	1.67 ± 0.66
	1	0.50 ± 0.03	2.01 ± 0.62
	1.5	0.32 ± 0.02	4.54 ± 0.70
Extruded starch phosphate 2 (9% STMP, 0.2 M NaOH)	0	0.92 ± 0.02	1.36 ± 0.17
	1	0.40 ± 0.01	4.07 ± 0.28
	1.5	0.29 ± 0.01	5.04 ± 0.71

3. Results and discussion

3.1. Density, expansion ratio and morphology of starch extrudates

The main purpose of the first part of the study was to investigate the effect of cross-linking and $SC\text{-}CO_2$ injection on the density, expansion and microstructure of starch extrudates. The average piece density and expansion ratio of starch extrudates are presented in Table 1. It was observed that alkaline conditions caused yellowish coloration in the starch extrudates. This is due to a secondary starch-browning reaction as reported by Kervinen, Suorti, Olkku, and Linko (1985) and this effect appears to be unrelated to phosphorylation. The cross-linking suppressed expansion of the extrudates. The result showed that without $SC\text{-}CO_2$ injection both starch phosphate extrudates produced with 0.1 and 0.2 M NaOH had slightly higher density and lower expansion than the extruded starch controls. The SEM micrographs of starch phosphate extrudates produced with 0.1 and 0.2 M NaOH and 0% $SC\text{-}CO_2$ are illustrated in Fig. 2. The reduction of expansion in cross-linked starch extrudates was possibly due to the starch degradation and the reduction of melt elasticity induced by cross-linking reaction (Launay, Lisch, & Jowitt, 1984). Ayoub and Rizvi (2007) stated that cross-linking of starch during extrusion tended to increase the viscosity of the melt and created higher density starch extrudates.

The presence of $SC\text{-}CO_2$ during extrusion created the cellular structure of starch extrudates by causing an increase in the pore number concentration of the materials, as shown by the SEM micrographs in Fig. 2. The extrudates produced with $SC\text{-}CO_2$ injection had increased brightness and whiteness in comparison with unexpanded controls. It is important to note that the starch extrudates further expanded during oven drying (75 °C) and the structure was set due to the raising glass transition temperature (T_g) caused by drying (Alavi et al., 1999). With injection of 1.5% (dry feed basis) $SC\text{-}CO_2$, the density and expansion of starch phosphate extrudates

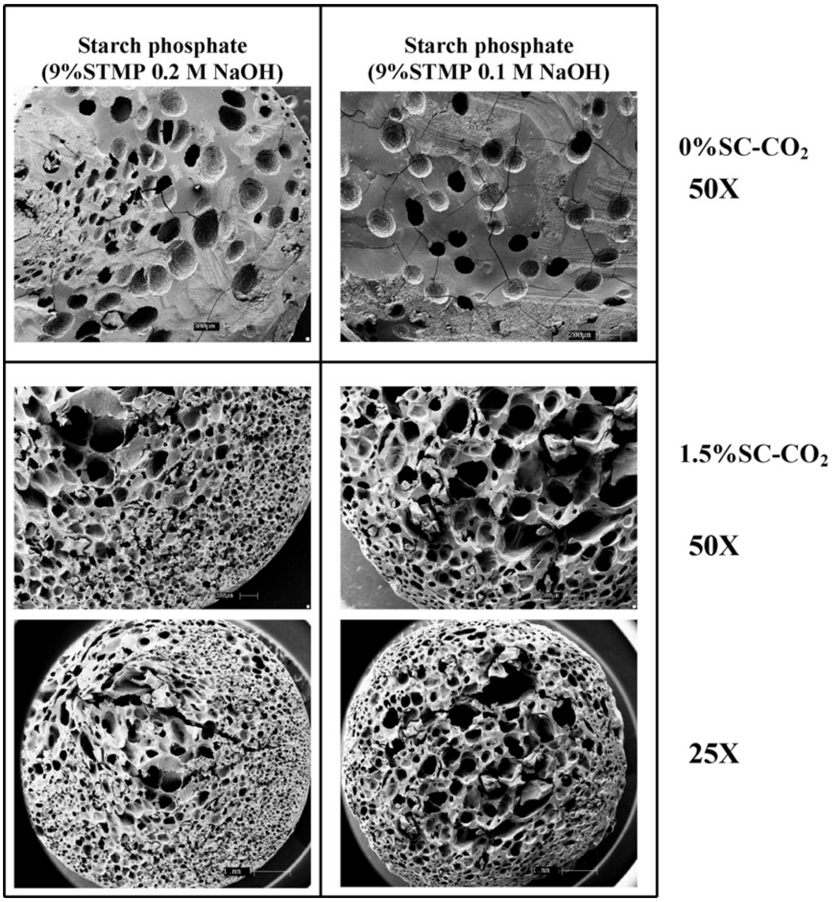

Fig. 2. SEM micrographs of starch phosphate extrudates prepared by SCFX at 0 and 1.5% $SC\text{-}CO_2$.

were significantly enhanced (Table 1). Apparently, the density of both starch phosphate extrudates significantly decreased by ~3 times and the starch phosphates expanded by ~3–4 times compared to the unexpanded product controls. However, SEM images showed that the resulting cellular structure did not show uniform cell size. The large pores were found to be mostly in the center of the extrudates, whereas the smaller pores tended to be toward the edge of the extrudates. This could be related to the rigid and compact structure of starch caused by cross-linking which might have affected the rate of cell nucleation. The structure will have uniform cell size if the nucleation is rapid because the number of nucleation sites and the cell density are increased. On the other hand, the structure will have a wide range in cell sizes if the nucleation is slow because the cells that nucleated first will be much larger the others and lead to a lowering of the cell density (Alavi et al., 1999; Ayoub & Rizvi, 2007). Nonetheless, SC-CO_2-based expansion provided light weight materials having the density in the range that has been previously reported for starch foams (Alavi et al., 1999; Ayoub & Rizvi, 2007). The SCFX process also yielded extrudates with non-porous skin, which could reduce the water penetration. This would be a desirable property for a biodegradable foam material.

3.2. ^{31}P NMR spectra of phosphate compounds in cross-linked starches

Phosphorus nuclear magnetic resonance (^{31}P NMR) has been used to characterize the nature of phosphorus in starches and also has been used to identify the phosphorylation in modified starches (Spyros & Dais, 2009). ^{31}P NMR spectrum of soluble and insoluble fractions of starch phosphates studied by Kasemsuwan and Jane (1994) indicated that cross-linkages (diester bonds), as well as monophosphate derivatives and other types of phosphate esters, were in the insoluble fraction that contained amylose and amylopectin. Therefore, preparation of starch hydrolysate or soluble dextrin is important for the ^{31}P NMR analysis. Digestion of starch samples with α-amylase and amyloglucosidase yielded soluble phosphodextrins and the phosphorus compounds could be detected at a concentration of 5–10% using NMR spectroscopy (Kasemsuwan & Jane, 1996; Sang & Seib, 2007). Kasemsuwan and Jane (1996) and Sang, Seib, Herrera, Prakash, and Shi (2010) stated that the amylolytic enzymes do not hydrolyze the glucosidic bonds near phosphate groups.

^{31}P NMR spectra of starch phosphates obtained by reactive extrusion at different concentration of 0.1 and 0.2 M NaOH solutions compared with that of unextruded control are illustrated in Figs. 3 and 4. The results indicated that both starch phosphates contained five different types of phosphate esters including monostarch monophosphate (MSMP), monostarch diphosphate (MSDP), cyclic-monostarch monophosphate (cyclic-MSMP), distarch monophosphate (DSMP), and grafted starch tripolyphosphate ($STPP_g$). ^{31}P NMR chemical shifts of phosphate derivatives reported in this research are similar with those reported by other studies (Kasemsuwan & Jane, 1994; Lack, Dulong, Picton, Le Cerf, & Condamine, 2007; Sang & Seib, 2007; Sang et al., 2010). The signals at δ 3–5 ppm, δ −5 and −10 ppm, δ 15.9 ppm, δ −1 to 1 ppm corresponded to MSMP, MSDP, cyclic-MSMP, and DSMP, respectively, whereas those at δ −12.5 and −20.5 ppm, δ −7.2, 2.5 ppm corresponded, respectively, to $STPP_g$, PP_i (inorganic pyrophosphate), and P_i (inorganic phosphate). ^{31}P spectroscopy of unextruded starch blend showed a signal mainly at chemical shift −22 ppm which indicates the high level of unreacted STMP (data not shown). It is interesting to note that a minor peak at chemical shift of −1 to 1 ppm which represents diester bonds was also found in the prime (wheat) starch blend used in this study. This finding is consistent with the studies of Kasemsuwan and Jane (1994, 1996) and Lim, Kasemsuwan, and Jane (1994) who reported that normally cereal starches contains endogenous phosphorus in the form of the phospholipids that can be detected as the diester bonds in ^{31}P NMR spectra.

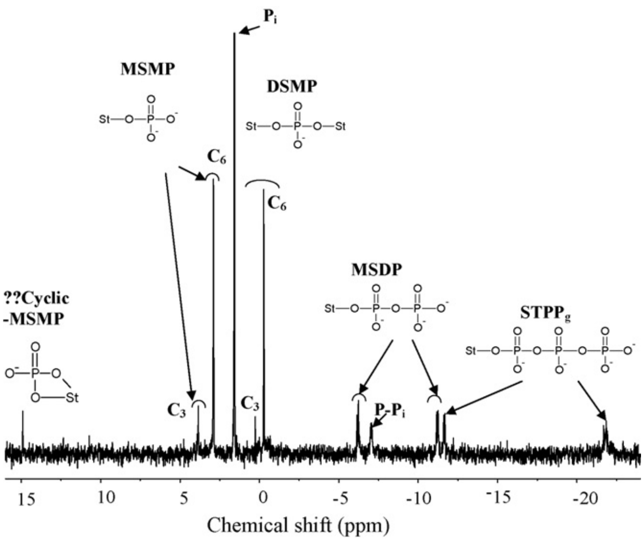

Fig. 3. ^{31}P NMR spectrum of α-amylase/amyloglucosidase digest of the cross-linked starch obtained by extrusion with 9% STMP and 0.1 M NaOH.

^{31}P NMR studies in potato and taro indicated that starch phosphorus was found in the form of covalently bound monophosphate, located either at the C-6 or C-3 position of the glucopyrosyl residues of starch while wheat starch was found to contain mainly C6-monophosphate esters (Lim & Seib, 1993; McIntyre, Ho, & Vogel, 1990). This is in agreement with our ^{31}P NMR results showing that the main phosphorus proportion was bound to the C-6 position and the minor proportion was bound to the C-3 position of the D-glucose residues in modified wheat starch. Although the ^{31}P spectroscopy showed a slight change in the phosphate ester pattern between the extruded starch phosphate prepared with 0.1 M (pH ~ 8) and 0.2 M (pH ~ 11) NaOH, the amount of incorporated phosphorus in starch extruded with 0.2 M NaOH (phosphorus content = 0.92%) was approximately 2.5 times higher than that of starch extruded with 0.1 M NaOH (phosphorus content = 0.39%), while the percentage of phosphorus in the native/pre-gelatinized starch blend was 0.05%. An increase in NaOH concentration from 0.1 to 0.2 M apparently increased the degree of substitution (DS) in the starch from 0.02 to 0.05. The incorporated phosphorus content in starch is

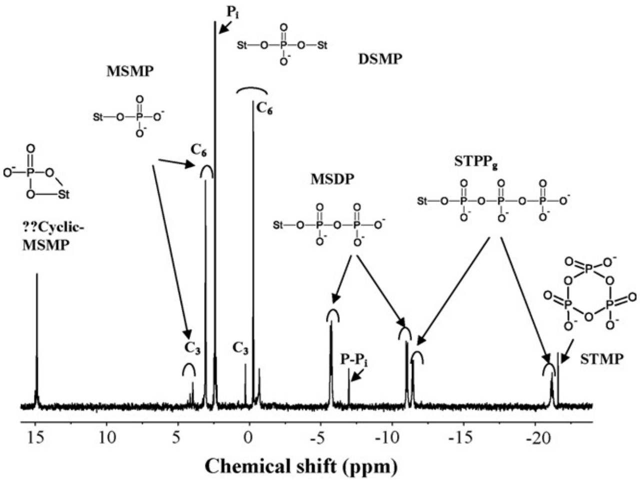

Fig. 4. ^{31}P NMR spectrum of α-amylase/amyloglucosidase digest of the phosphorylated cross-linked starch extruded with 9% STMP and 0.2 M NaOH.

Table 2
Water solubility at 60 °C of starch controls and extruded starch phosphates produced by SCFX.

Samples	SC-CO_2 (% dry feed)		
	0%	1%	1.5%
Unextruded starch control	38.1 ± 1.1	–	–
Extruded starch control 1 (0% STMP, 0.0 M NaOH)	29.3 ± 2.3	–	–
Extruded starch control 2 (0% STMP, 0.1 M NaOH)	27.1 ± 3.7	–	–
Extruded starch control 3 (0% STMP, 0.2 M NaOH)	34.5 ± 2.1	–	–
Extruded starch phosphate 1 (9% STMP, 0.1 M NaOH)	12.5 ± 1.6	27.2 ± 2.2	28.6 ± 1.0
Extruded starch phosphate 2 (9% STMP, 0.2 M NaOH)	6.1 ± 0.2	7.3 ± 0.3	7.1 ± 0.4

directly used to indicate the degree of cross-linking. When a higher amount of phosphorus is found in starch, higher cross-linking is carried out. It is likely that STMP gave more phosphorus-substitution and more cross-linking on starch during a higher pH reaction. This finding is supported by many studies (Kerr & Cleveland, 1959; Lim & Seib, 1993; Sang et al., 2010). According to Lim and Seib (1993), pH plays a dominant role in determining the ratio of starch monoester (monostarch phosphate) to diester (distarch phosphate) bonds. For example, at a reaction pH < 7 the terminal phosphate groups of sodium tripolyphosphate (STPP) are protonated and react rapidly with starch hydroxyl (–OH) groups to produce starch monoesters. Starch diesters, on the other hand, which indicate the cross-linking (St–P–St) can be produced at pH > 8. Sang et al. (2010) prepared the phosphorylated wheat starches at different pH levels and reported that the cross-linking is a major pathway when starch is reacted with STMP at pH 9–12. In addition, it was found that the second most abundant ester form in their modified starch was starch monophosphates. Kerr and Cleveland (1959) also indicated that cross-linking of starch prevailed in alkali-catalyzed reaction at pH > 11 with STMP or STPP (sodium tripolyphosphate). Distarch phosphate linkages are responsible for the stability against heat, acid, shearing, and starch digestion by α-amylase (Sang et al., 2010). However, the intensity of each ^{31}P NMR signal in modified starch samples need to be further quantitatively determined so that the incorporated phosphorus molar ratio bound to each starch phosphate form can be clearly indentified.

3.3. *Water solubility*

During reactive extrusion of starch with STMP under alkaline conditions the phosphorus groups reacted with the hydroxyl groups (–OH) of starch, forming distarch (cross-link) phosphate and other starch phosphate derivatives as indicated by ^{31}P NMR results. However, justification of degree of cross-linking by an increase in total incorporated phosphorus content as well as the ^{31}P spectroscopy pattern can be confounded by endogenous phospholipids, and by the formation of phosphomonoesters. For this reason, cross-linking of starch is often evaluated by changes in physical properties such as pasting consistency, thermal properties, swelling, and water solubility (Gunaratne & Corke, 2007; Singh et al., 2007; Woo & Seib, 1997). Singh et al. (2007) stated that a small number of diesters (cross-links) can drastically alter the functional behaviors of starches. The introduction of covalent bonds by phosphate groups causes the structural change in starch granules, reflecting a change in the functional properties of extruded starch such as water solubility and pasting behaviors (Seker & Hanna, 2006). Cross-linking reinforces the structure of starch granules and limits water absorption and solubility of starch, thereby restricting the mobility of the starch chain in the amorphous region (Gunaratne & Corke, 2007). Rutledge et al. (1972, 1974) reported a decrease in both solid loss and water uptake of parboiled rice after cross-linking with STMP. Our preliminary study showed that the extruded starch samples were insoluble in water at room temperature. Therefore, the water solubility of starch samples was determined at 60 and 90 °C and the results are shown in Tables 2 and 3, respectively. The solubility of unextruded and extruded starch controls increased with temperature. For the extruded starch controls, the water solubility increased with an increased NaOH concentration due to the alkaline hydrolysis of starch. On the other hand, extruded starch phosphates produced with 0.1 and 0.2 M NaOH showed much lower water solubility than the unextruded and extruded starch controls. In addition, both extruded starch phosphate samples presented a slight increase in solubility with temperature. The solubility was slightly changed when the temperature increased, perhaps due to the stabilization of starch granules after the cross-linking reaction. With excess cross-linking, phosphorylation could decrease the water solubility of extruded starches. However, the solubility of extruded starch phosphates depends on the reaction pH or NaOH concentration. The lowest water solubility was revealed in the starch phosphate prepared with 0.2 M NaOH, indicating the highest resistance against water penetration. This implies that the degree of cross-linking was more extensive during extrusion at pH 11 (extruded with 0.2 M NaOH) than at pH 8 (extruded with 0.1 M NaOH). This probably occurred because an increase in NaOH concentration increased the number of cross-linked bonds, causing the disruption in intermolecular hydrogen bonding between starch molecules and the reinforcement of granule integrity, thereby reducing solubility (Rutledge et al., 1974). Cross-linked starches exhibit lower solubility than their native equivalents, and solubility further decreases with an increase in cross-link density (Kaur, Singh, & Singh, 2006). Our finding is in agreement with Liu, Ramsden, and Corke (1999) who reported that the formation of distarch phosphates that are favored at high pH levels and act as cross-links can restrict the swelling and hydration of the starch granule. In addition, Seker and Hanna (2006) reported the lowest solubility of cross-linked

Table 3
Water solubility at 90 °C of starch controls and extruded starch phosphates produced by SCFX.

Samples	SC-CO_2 (% dry feed)		
	0%	1%	1.5%
Unextruded starch control	88.1 ± 3.5	–	–
Extruded starch control 1 (0% STMP, 0.0 M NaOH)	39.3 ± 1.9	–	–
Extruded starch control 2 (0% STMP, 0.1 M NaOH)	36.9 ± 3.7	–	–
Extruded starch control 3 (0% STMP, 0.2 M NaOH)	46.3 ± 2.8	–	–
Extruded starch phosphate 1 (9% STMP, 0.1 M NaOH)	13.8 ± 2.6	23.4 ± 2.8	24.4 ± 1.2
Extruded starch phosphate 2 (9% STMP, 0.2 M NaOH)	6.9 ± 0.9	10.8 ± 1.2	10.7 ± 1.1

Table 4
Thermal properties of starch controls and extruded starch phosphates produced by SCFX.

Samples	% SC-CO_2	T_o (°C)	T_p (°C)	ΔH (J/g)
Unextruded starch control	0	55.82 ± 0.38	61.75 ± 0.18	7.99 ± 0.95
Extruded starch control 1 (0% STMP, 0.0 M NaOH)	0	57.28 ± 0.11	61.48 ± 0.18	3.92 ± 0.45
Extruded starch control 2 (0% STMP, 0.1 M NaOH)	0	58.77 ± 0.21	63.63 ± 0.34	3.79 ± 0.23
Extruded starch control 3 (0% STMP, 0.2 M NaOH)	0	58.41 ± 0.28	65.32 ± 0.89	5.86 ± 0.90
Extruded starch phosphate 1 (9% STMP, 0.1 M NaOH)	0	60.49 ± 0.70	65.82 ± 0.22	1.12 ± 0.10
	1	60.99 ± 0.38	65.75 ± 0.51	1.23 ± 0.26
	1.5	61.18 ± 0.44	66.02 ± 0.76	1.81 ± 0.33
Extruded starch phosphate 2 (9% STMP, 0.2 M NaOH)	0	61.80 ± 0.11	68.96 ± 0.25	1.18 ± 0.07
	1	60.88 ± 0.31	67.36 ± 0.25	2.34 ± 0.10
	1.5	60.71 ± 0.21	67.29 ± 0.43	2.24 ± 0.27

starch after extrusion with 0.2 M NaOH and 15% STMP. However, the authors found that increasing NaOH concentration from 0.2 to 1.0 M significantly increased the solubility of extruded starch due to the hydrolysis effect of NaOH on starch granules.

The presence of SC-CO_2 in the extruded starch phosphates produced with 0.1 M NaOH significantly increased the solubility of starch. An increase in solubility of SC-CO_2-treated starches is possibly because during extrusion, injected CO_2 dissolved in water in the polymer melt and formed weak carbonic acid, which decreased the reaction pH. It was observed that in case of starch extrusion with 0.1 M NaOH the pH of the extrudates dropped from pH 8.0–7.0 with 1.5% SC-CO_2 injection which greatly affected cross-linking of starch. On the other hand, the SC-CO_2 injection did not significantly alter the pH of starches extruded with 0.2 M NaOH due to its strong alkalinity and their solubility was unaffected by SC-CO_2.

3.4. Thermal properties by DSC

Gelatinization temperature and enthalpy of starch controls and extruded starch phosphate samples are presented in Table 4. The unextruded control comprising 40% (by weight) native, 60% pre-gelatinized wheat starch, and 9% (starch basis) STMP had a gelatinization temperature of ~62 °C and enthalpy of ~8 J/g. The gelatinization of starch corresponds to the dissociation of the amylose and amylopectin with granules and leaching out of amylose to the continuous phase (Fujita, Lida, & Fujiyama, 1992). Extrusion of starch under alkaline conditions (extruded starch controls) yielded a slight increase in gelatinization temperature of starch compared to its native counterpart. A significant change in onset and peak temperature, and enthalpy was observed in all extruded starch phosphate samples as compared with their parent starch blend. The SC-CO_2 did not significantly change the gelatinization pattern of extruded starch phosphates. Apparently, after phosphorylation all extruded starches gelatinized at higher temperature (66–69 °C) compared to the unextruded control (62 °C). The gelatinization temperature was significantly increased by up to 4 °C in the extruded starch phosphate prepared with 0.1 M NaOH and 7 °C in the extruded starch phosphate prepared with 0.2 M NaOH. Chatakanonda, Varavinit, & Chinachoti (2000) reported an increase in gelatinization temperature (by up to 5 °C) in cross-linked rice starch (using STMP/STTP mixture) with an increase in the degree of cross-linking, while the enthalpy showed no significant change. The authors concluded that the introduction of phosphate cross-links into the starch by STMP tightened the molecular structure, leading to an increase in the gelatinization temperature. The enthalpy (ΔH) of all extruded starch phosphates significantly decreased (Table 4). Enthalpy provides an overall measure (quality and quantity) of crystallinity and indicates the loss of molecular order within the granules (Cooke & Gidley, 1992). The lower enthalpy suggests a disorganized arrangements or lower solubility of the crystals (Chiotelli & Meste, 2002). Our DSC finding indicates the disorganization of starch granules and completed melting of crystalline regions in the cross-linked starch during reactive extrusion and phosphorylation.

The gelatinization combined with the solubility results suggests that the introduction of ester bonds (by phosphate groups) to starch altered the starch functionalities, possibly by tightening the organization in starch molecules and producing low molecular mobility. All of these would cause gelatinization to occur at a higher temperature. Theoretically, the reduction in swelling and solubility of starch resulting from cross-linking delays gelatinization (Carmona-Garcia, Mirna, Guadalupe, Bellanira, & Luis, 2009; Yeh & Li, 1996). Our finding is consistent with the research conducted by Liu et al. (1999) who reported that after phosphorylation the gelatinization enthalpy of all starches greatly decreased. It is believed that phosphorylation reduces stability of the starch structure and, consequently, reduces energy required for the structural transitions in gelatinization. However, some studies reported that cross-linking had little effects on the gelatinization parameters, in contrast to its marked effect on pasting properties (Chang & Lii, 1992; Gunaratne & Corke, 2007; Landerito & Wang, 2005; Sang et al., 2010). Chang and Lii (1992) speculated that the extruded starch phosphates exhibited slightly lower gelatinization temperature, lower enthalpies, and lower paste viscosities than starch phosphates produced by the conventional method. The authors concluded that such behavior could be attributed to the high percentage of damaged starch induced by extrusion process. It suggests that the type and concentration of cross-linking agent, amylose/amylopectin ratio of starch, and modification process significantly affects the extent of change in thermal properties.

The amylose–lipid complex endotherm was apparently shown in unextruded control at 95–100 °C. However, no melting of an amylose–lipid peak was observed in all extruded starch phosphate samples. Possibly the STMP and/or the cross-linking treatment conditions interfered the molecular structure of amylose–lipid complex or the alkali saponified the lipid and somewhat disrupted that complex (Matsunaga & Seib, 1997).

4. Conclusions

Cross-linking of starch blends (40% native, 60% pre-gelatinized wheat starch, w/w) by phosphorylation was achieved by reactive SCFX with 9% (starch basis) STMP. The cross-linking modified the physicochemical properties of extruded starches and differences were exhibited among the starch phosphates prepared under different reaction pH. ^{31}P NMR spectroscopy demonstrated the mixture of distarch monophosphate (cross-link) and monostarch monophospate. Phosphorylation of starch at the highest pH of 11 prepared with 0.2 M NaOH showed the highest effect on water solubility. Degree of substitution (DS = 0.05) and incorporated phosphorus content (P = 0.92%) was highest in this sample, indicating the highest level of cross-linking. The higher temperature and lower enthalpy of gelatinization of starch phosphates is consistent with the lower solubility due to the rigid structure and restricted mobility of starch molecules caused by cross-linking. In general, cross-linking suppressed starch expansion. However, with SC-CO_2 injection, the cross-linked microcellular starch foams were successfully produced. The morphology and the cell distribution of starch foams can be controlled by processing conditions.

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