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Enzymatic hydrolysis of potato starches containing different amounts of phosphorus

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ABSTRACT

The rapid hydrolysis of potato starches differing in phosphorus content, as well as sweet potato, cassava and yam starches, was accomplished by treatment of gelatinised starches with bacterial liquefying α-amylase at 50 °C for 1 h, followed by *Bacillus licheniformis* α-amylase at 55 °C up to 24 h, and then by glucoamylase at 40 °C for a further 24 h. Among the potato starches, the high-phosphorus starches showed higher starch resistant capacity than the medium-phosphorus starches, as well as other tuber and root starches. The hydrolysis rate of tuber and root starches was not greatly influenced by their amylose content and median granule size. Only glucose was detected in the almost completely hydrolysed tuber and root starch samples, indicating that the concomitant enzymes treatment could hydrolyse both the α-1,4 and α-1,6 linkages of the starches examined.

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1. Introduction

Starchy substances constitute the major part of the human diet for most of the people in the world, as well as many animals. Starch granules are quite resistant to penetration by both water and hydrolytic enzymes, due to the formation of hydrogen bonds within the same molecule and with other neighbouring molecules. Starch may be hydrolysed by amylolytic enzymes such as α-amylase. For example, when potato starch is the substrate, its hydrolysis yields mixtures of different saccharides, i.e. maltodextrins, and their precise compositions are of considerable commercial interest. The hydrolysed products are widely used in the food, paper and textile industries (Crabb & Mitchinson, 1997; Marchal, Jonkers, Franke, deGooijer, & Tramper, 1999; Nigam & Singh, 1995; Pandey et al., 2000). Amylases have many applications in the food, textile, paper and pharmaceutical industries (Gupta, Gigras, Mohapatra, Goswami, & Chauhan, 2003). Generally, fungal glucoamylase, as well as bacterial or fungal α-amylase, may be used together to convert starch to simpler sugars. The practical applications of this type of enzyme mixture include the production of corn syrup and the conversion of cereal mashes to sugars in brewing.

The potato, an important upland crop in Japan, is used most widely as a raw material in the food industry and, in 2005, 39% of the total net production was utilised for starch production. Potato starch is known to contain a small amount of covalently-bound phosphate groups in its components, 1 in 200–500 glucose residues on average being phosphorylated. The phosphate groups are attached to C-6 and C-3 (Hizukuri, Tabata, & Nikuni, 1970; Posternak, 1951; Tabata & Hizukuri, 1971) of the glucosyl residues and are located mostly in the B-chain (the chain with one or more side-chains (Peat, Whelan, & Thomas, 1952)) of amylopectin (Takeda & Hizukuri, 1982). As amylolytic enzymes are incapable of bypassing the phosphorylated glucosyl residue, phosphoryl-oligosaccharides are released from the digestion of potato starch with amylase (Abe, Takeda, & Hizukuri, 1982; Kamasaka et al., 1995; Takeda, Hizukuri, Ozono, & Suetake, 1983). Marshall and Whelan (1970) recommended that trace amount of α-amylase should be present in a glucoamylase preparation, for the quantitative conversion of starch and glycogen into glucose. They showed that *Rhizopus niveus* glucoamylase was supposedly free from α-amylase (slight adulteration was noticed later (Marshall, 1978)) and transglucosidase hydrolysed waxy-maize starch, shellfish glycogen and rabbit liver glycogen nearly completely and potato amylose partly (90.1%). Further, it was reported that purified *Cladosporium resinae* glucoamylase fully hydrolysed waxy-maize starch and potato amylopectin partially (approx. 65%) (McCleary & Anderson, 1980). It was also found that the saccharogenic amylase (glucoamylase) of *Aspergillus awamori* hydrolysed potato starch up to 90% into glucose and left limit dextrin enriched with phosphate groups (Ohta & Ueda, 1967; Ueda, 1956). Furthermore, they also suggested that the starches containing phosphate esterified with some glucose units, such as potato, tapioca, arrowroot and so on, would not be completely saccharified by amylase only, without the action of phosphatase, which is active toward glucose 6-phosphate. Recently, glucose was detected only in the hydrolysate of potato

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starch granules, gelatinised potato starch and gelatinised soluble starch by action of the crude glucoamylase (Li et al., 2007). Gelatinisation of starches increased their susceptibility to enzyme degradation, in comparison to their native form (Konsula & Liakopoulou-Kyriakides, 2004; Noda et al., 2008).

At present, experimental data on the enzymatic hydrolysis of gelatinised starches are not available, particularly for different cultivars of potato starches containing higher amounts of phosphorus. As starch is cooked to produce starch syrup, it is important to estimate the enzymatic digestibility of gelatinised starch in the food industry. So the objective of the present investigation is to explore the hydrolytic profiles of potato starches with comparatively high-phosphorus content, as well as sweet potato, cassava and yam starches, by successive actions of two α-amylases and glucoamylase, using a minimum concentration of these enzymes.

2. Materials and methods

2.1. Chemicals

D-Glucose (anhydrous) and calcium chloride dihydrate were purchased from Nacali Tesque Inc., Kyoto, Japan, while maltose monohydrate and sodium chloride were purchased from Wako Pure Chemical Industries, Osaka, Japan. Other reagents used were of analytical grade.

2.2. Experimental starch samples

Information regarding tuber and root starches used in the present study is in Table 1. Of the experimental starches, 28 potato samples consisting of 19 cultivars were cultivated in 2005 and 2006 at the experimental farm of the National Agricultural Research Centre for Hokkaido Region (NARCH), Memuro, Hokkaido, Japan. Starches were isolated from those samples, following the procedure described previously (Noda et al., 2004). Four potato starches, produced by the Jinno Starch Co. (Sarabetsu, Hokkaido, Japan) in 2005 were purchased for experimental purposes. Three different sized potato starch samples, which were produced by air classification at Nakashari Starch Factory, Shari Agricultural Cooperative Association, Shari, Hokkaido, Japan, were collected for the study. In addition, one potato starch sample of unknown cultivar was purchased for the study from Toukouren Starch Factory, Urahoro, Hokkaido, Japan. The sweet potatoes of two different cultivars were cultivated in 2005 at the experimental farm of the National Agricultural Research Centre for Kyushu–Okinawa region (KONARC), Miyakonojo, Japan. Starches were isolated from these samples, as reported previously (Noda, Takahata, Nagata, & Monma, 1992). Two sweet potato starches of unknown cultivar were purchased from Haraigawa Starch Factory, Kimotsuki Agricultural Cooperative Association, Kanoya, Kagoshima, Japan. Cassava starches of three unknown cultivars, isolated from cassava tubers grown in Thailand, in 2005, was obtained from Nippon Starch Chemical Co., Ltd., Osaka, Japan. Yam starch, obtained from Kawanishi Agricultural Cooperative Association, Obihiro, Hokkaido, Japan, was isolated from fresh yam tubers, as reported previously (Zaidul, Norulaini, Omar, Yamauchi, & Noda, 2007).

2.3. Starch characteristic analysis

The analyses of the compositional properties of the starches, such as the phosphorus content and median granule size were determined, as reported previously (Noda et al., 2004). The blue values (BVs) of the starches were estimated at 680 nm according to the modified method of Noda et al. (2004), eliminating the step of defatting the starch. The amylose content was calculated from BV according to the equation of Takeda et al. (1983). The BVs of amyloses and amylopectins isolated from potato, sweet potato, cassava and yam starches were determined by Suzuki, Shibanuma, Takeda, Abe, and Hizukuri (1994), Takeda, Tokunaga, Takeda, and Hizukuri (1986), Thitipraphunkul, Uttapap, Piyachomkwan, and Takeda (2003); and Suzuki, Kanayama, Takeda, and Hizukuri (1986), respectively. These BVs were used in the calculation of the amylose content of potato, sweet potato, cassava and yam starches. The enzymatic hydrolysis was performed with slight modification as reported (Kamasaka et al., 1995). Starch sample (0.5%) in 6 mM NaCl, 2 mM $CaCl_2$ solution (49.5 ml) was mixed with 0.5 ml of bacterial liquefying α-amylase (Termamyl 120 L Type L, Novozymes, Krogshosgvej, Denmark). The mixture was gradually heated to 100 °C, to liquefy it. The solution was cooled immediately and then incubated at 50 °C for 1 h in a temperature-controlled water bath Then 0.3 ml of sample solution were withdrawn from the reaction mixture and centrifuged for 2 min at 7000 rpm, 10 °C. After centrifugation, 20 μl clear supernatant was taken in a screw cap test tube, mixed with 480 μl of Milli-Q water and the reaction was stopped by adding an equal volume of Somogyi reagent, 0.5 ml (Abe et al., 1982). The released free sugar was then estimated by Somogyi–Nelson's method (Nelson, 1944; Somogyi, 1952). The hydrolytic

Table 1
Information about the experimental tuber and root starches

Sample no.	Source	Cultivar	Year	Origin
1	Potato	Benimaru	2005	NARCH[a]
2	Potato	Setoyutaka	2005	NARCH
3	Potato	Oojiro	2005	NARCH
4	Potato	Touya	2005	NARCH
5	Potato	Kitamurasaki	2005	NARCH
6	Potato	Shadow Queen	2005	NARCH
7	Potato	Hokkaikogane	2005	NARCH
8	Potato	Unknown	2005	Nakashari Starch Factory
9	Potato	Unknown	2005	Nakashari Starch Factory
10	Potato	Unknown	2005	Nakashari Starch Factory
11	Potato	Unknown	2005	Tokoren Starch Factory
12	Potato	Hokkaikogane	2005	Jinno Starch Co.
13	Potato	Benimaru	2005	Jinno Starch Co.
14	Potato	Norin no. 1	2005	Jinno Starch Co.
15	Potato	Northern Ruby	2005	NARCH
16	Potato	Konafubuki	2005	NARCH
17	Potato	Eniwa	2005	Jinno Starch Co.
18	Potato	Inca Red	2006	NARCH
19	Potato	Touya	2006	NARCH
20	Potato	Hokkaikogane	2006	NARCH
21	Potato	Kitamurasaki	2006	NARCH
22	Potato	Eniwa	2006	NARCH
23	Potato	Benimaru	2006	NARCH
24	Potato	Konafubuki	2006	NARCH
25	Potato	Toyoshiro	2006	NARCH
26	Potato	Oojiro	2006	NARCH
27	Potato	Setoyutaka	2006	NARCH
28	Potato	Irish Cobbler	2006	NARCH
29	Potato	Inca-no-mezame	2006	NARCH
30	Potato	May Queen	2006	NARCH
31	Potato	White Fryer	2006	NARCH
32	Potato	Snowden	2006	NARCH
33	Potato	Shadow Queen	2006	NARCH
34	Potato	Northern Ruby	2006	NARCH
35	Potato	Inca Purple	2006	NARCH
36	Potato	Norin No. 1	2006	NARCH
37	Sweet potato	Akemurasaki	2005	KONARC[b]
38	Sweet potato	Unknown	2005	Haraigawa Starch Factory
39	Sweet potato	Ayamurasaki	2005	KONARC
40	Sweet potato	Unknown	2006	Haraigawa Starch Factory
41	Cassava	Unknown	2005	Nippon Str Chem. Co. Ltd.
42	Cassava	Unknown	2005	Nippon Str. Chem. Co. Ltd.
43	Cassava	Unknown	2005	Nippon Str.Chem. Co. Ltd.
44	Yam	Unknown	2005	Kawanishi Agr. Cooper.

[a] NARCH: National Agricultural Research Centre for Hokkaido Region.
[b] KONARC: National Agricultural Research Centre for Kyushu–Okinawa Region.

reaction was continued further by adding α-amylase from *Bacillus licheniformis* (Sigma–Aldrich Co., St. Louis, MO; 0.5 unit/mg starch) and incubated at 55 °C for up to 24 h. Then crystalline glucoamylase from *Rhizopus* sp. (Oriental Yeast Co., Ltd., Tokyo, Japan; 0.75 unit/mg starch) was added to the reaction mixture and the reaction was continued for another 24 h at 40 °C. The hydrolysis extent was determined by the modified method as reported (Englyst, Kingman, & Cummings, 1992), which was calculated from the released glucose after digestion of Termamyl 120 L, *B. licheniformis* α-amylase, and glucoamylase.

2.4. Thin layer chromatography of hydrolysed sample

The hydrolysis extent was also detected by TLC (Li et al., 2007), using silica gel 60 (Merck, Darmstadt, Germany) and the released free sugar was identified by using glucose and maltose monohydrate as the standard sugars. In this ascending chromatography, the separation was done with the solvent system, 1-butanol: pyridine: water in the proportion of 6:4:3, respectively and the spraying reagent used for detection was: 20 g/l diphenylamine in acetone: 20 g/l aniline in acetone: 850 g/l phosphoric acid (5:5:1, by volume).

2.5. Statistical analysis

The phosphorus content, amylose content, granule size distribution and enzymatic hydrolysis were determined in duplicate and each value is the mean of duplicate measurements. The correlation analysis between the enzymatic hydrolysis extent and other properties (phosphorus content, amylose content, and median granule size) was calculated in potato starches from 36 cultivars as well as in 8 other tuber and root starches.

3. Results and discussion

3.1. Compositional properties

In the present investigation, the compositional characteristics of the tuber and root starches were analysed and presented in Table 2. Based on their phosphorus content, the potato starches were characterised into three classes, as reported previously (Noda et al., 2007): (1) low-phosphorus starches, LPS (308–395 ppm); (2) medium-phosphorus starches, MPS (711–716 ppm), and (3) high-phosphorus starches, HPS (1110–1244 ppm). It was found that the amylose content and median granule size of the tuber and root starches varied significantly. Among the potato starches examined, the amylose content ranged from 15.4% in Inca Red to 25.5% in Setoyutaka, while those of the sweet potato, cassava and yam starches were found to be 16.2–23.4%, 25.3–28.8% and 25.8%, respectively. It should be mentioned that the amylose contents of the same cultivar grown in different years also varied significantly (Zaidul & Yamauchi, et al., 2007). The differences in amylose contents among various starches from potato cultivars may be due to different factors, such as the genotype, environmental conditions, agricultural practice, etc. (Cottrell, Duffus, Paterson, & Mackay, 1995; Kaur, Singh, Ezekiel, & Guraya, 2007; Kim, Wiesenborn, Orr, & Grant, 1995). Remarkably, the potato cultivars differed widely in the phosphorus content of their starch depending on the cultivation time. The phosphorus content varied from 500 to 1132 ppm in 36 potato starches, the average value being 760 ppm, while smaller values (81–231 ppm) were observed in the other tuber and root starches. The range of phosphorus contents of potato starches found in the study showed very good similarity to those reported by Kim et al. (1995) (596–1022 ppm) and Wiesenborn, Orr, Casper, and Tacke (1994) (609–1031 ppm).

Table 2
Compositional properties of the experimental tuber and root starches

Sample no.	Phosphorus content (ppm)	Amylose content (%)	Median granule size (μm)
[a]HPS: 4	986	21.1	29.8
5	956	20.8	38.8
6	850	21.6	33.8
7	918	19.0	39.0
8	992	20.6	14.4
9	900	22.3	22.5
11	847	21.5	35.2
12	825	20.3	36.8
15	812	21.7	31.6
17	878	19.4	37.7
18	1132	15.4	25.8
19	887	21.1	34.0
20	874	20.4	39.7
21	901	20.0	39.2
22	808	18.1	35.6
29	925	23.3	29.3
31	827	22.8	38.2
34	822	22.6	32.4
35	818	21.6	36.3
[b]MPS: 1	501	21.1	39.5
2	533	21.6	33.8
3	538	21.3	33.0
10	673	22.6	41.5
13	599	23.6	44.4
14	541	24.3	34.1
16	753	20.9	39.0
23	528	22.8	42.1
24	772	22.0	39.4
25	613	22.3	30.8
26	572	23.7	34.8
27	573	25.5	30.0
28	678	23.4	31.4
30	572	22.7	41.3
32	709	19.8	32.2
33	756	20.7	35.1
36	500	22.0	32.7
[c]SP: 37	156	16.2	14.5
38	231	23.4	18.3
39	172	17.8	14.5
40	209	20.9	20.6
[d]CS: 41	97	28.8	15.7
42	81	25.3	16.3
43	105	25.4	16.3
Yam: 44	166	25.8	22.8

[a] HPS: high-phosphorus starches.
[b] MPS: medium-phosphorus starches.
[c] SP: sweet potato.
[d] CS: cassava.

On the basis of phosphorus content, we have arranged the experimental potato starches into two classes, as all the experimental potato starches contained 500 ppm phosphorus or higher. There were 19 high-phosphorus starches, HPS, (812–1132 ppm) and two 17 medium-phosphorus starches, MPS (500–756 ppm). As shown in Table 2, the median granule size for HPS varied generally between 29.3 and 39.7 μm, with an average size of 34.7 μm, with the exception of two cultivars, which were found in air-classified potato starches, No. 8 and 9, with the values of 14.4 and 22.5 μm, respectively. The median granule size of the MPS was slightly higher than that of HPS and the values were found to vary between 30.0 and 44.4 μm, with an average size of 36.2 μm; while the values for the sweet potato, cassava and yam starches were 14.4–20.6, 15.7–16.3 and 22.8 μm, respectively. Very similar results were also reported earlier (Noda et al., 2007; Zaidul & Yamauchi, et al., 2007). Lower values of median granule size (16.5–27.5 μm) for potato starches have been reported (Murakami, Asama, Itoh, & Itoh, 1978), as well as higher values (38.7–53.8 μm) (Wiesenborn et al., 1994). This study also indicated that the median granule size

of potato starches varied widely. These variations might be attributed to the differences in cultivars, cultivation times, environmental conditions, soil, growing temperatures, etc.

3.2. Hydrolysis of various starches

The successive action of the enzymes, two α-amylases and glucoamylase, were used to follow the hydrolysis extent of different tuber and root starches. Table 3 shows the hydrolysis rate of HPS, MPS, sweet potato starches, cassava starches and yam starch at three different stages of digestion. Termamyl 120 L (Type L), a bacterial liquefying α-amylase, was found to be active even after heating to boiling for 3–4 min during gelatinisation of starches, and the hydrolysis extent of the HPS, MPS, sweet potato, cassava and yam starches by the enzyme when incubated at 50 °C for 1 h was found to be 28–46%, with a mean value of 35%; 35–48%, with the mean value of 40%; 38–48%, with a mean value of 44%; 35–43%, with a mean value of 40%; and 40%, respectively. The results clearly indicated that the HPS are comparatively more resistant to Termamyl 120 L hydrolysis than the MPS, as well as the other tuber and root starches examined, suggesting that HPS might reduce the digestibility of gelatinised starch to some extent. As the hydrolysis reaction proceeded further with the addition of *B. licheniformis* α-amylase, the degree of hydrolysis of the abovementioned starches by the enzymes were determined to be 60–71%, with a mean value of 65%; 64–74%, with a mean value of 69%; 66–72% with a mean value of 69%; 64–70% with a mean value of 67%; and 71%, respectively. The higher starch resistance capacity of the HPS, as compared to that of the other starches, was also observed in this case. Further continuation of the hydrolysis reaction was performed after addition of crystalline glucoamylase from *Rhizopus* sp. to the mixture. The hydrolysis extents of the experimental tuber and root starches were found to be between 95% and 100%.

Table 3
Hydrolysis extent (%) of the experimental tuber and root starches by successive action of Termamyl 120 L, *Bacillus licheniformis* α-amylase and glucoamylase

Sample no.	Termamyl 120 L	*Bacillus licheniformis* α-amylase	Glucoamylase
[a]HPS: 4	28	60	95
5	34	66	97
6	36	65	97
7	30	62	97
8	36	68	98
9	36	68	97
11	46	70	100
12	46	71	100
15	36	64	96
17	40	71	99
18	32	66	96
19	30	65	96
20	34	64	96
21	30	61	95
22	32	62	97
29	28	64	95
31	38	65	97
34	33	65	98
35	33	60	96
Mean (n = 19)	35	65	97
[b]MPS: 1	42	69	98
2	43	70	98
3	40	69	97
10	35	69	98
13	44	72	99
14	48	70	99
16	42	66	97
23	42	68	99
24	40	66	99
25	38	66	98
26	35	71	100
27	44	71	99
8	38	74	99
30	38	67	98
32	38	69	98
33	32	64	98
36	39	72	99
Mean (n = 17)	40	69	98
[c]SP: 37	48	70	99
38	44	68	99
39	44	66	99
40	38	72	99
Mean (n = 4)	44	69	99
[d]CS: 41	43	70	100
42	42	68	100
43	35	64	99
Mean (n = 3)	40	67	100
Yam: 44	40	71	99

[a] HPS: high-phosphorus starches.
[b] MPS: medium-phosphorus starches.
[c] SP: sweet potato.
[d] CS: cassava.

Correlation coefficients between compositional properties and hydrolysis extents at three different stages were calculated and the results are shown in Table 4. Phosphorus content was negatively correlated with all three values of hydrolysis extent. In contrast, no significant correlation coefficients were found between median granule size or amylose content, and hydrolysis extents at three different stages, except that amylose content was correlated positively but weakly with hydrolysis extent after the digestion with glucoamylase. It is also established that the amylase action is prevented by the esterified phosphate groups attached to the glucosyl residues of starch (Abe et al., 1982; Takeda et al., 1983).

The present investigation demonstrated that the amylose content and median granular size of the experimental starches after gelatinisation had little or no effect on their hydrolysis, as although the starches contained different percentages of amylose with variation in median granular size, their degrees of hydrolysis were observed to be very similar. It has been established that the starch granule size is an important factor in affecting the digestibility of raw starch by amylase. Several reports have shown that larger starch granules are digested more slowly than smaller granules (Cottrell et al., 1995; Kang, Sugimoto, Kato, Sakamoto, & Fuwa, 1985; MacGregor & Balance, 1980; Noda et al., 2005, 2008). It was indicated that the relative glucoamylase activities towards potato starch granules, raw sweet potato starch and raw corn starch were 74.8%, 8.9% and 7.8%, respectively, at 60 °C (Li et al., 2007). They also reported that even gelatinised corn starch was very poorly digested by crude enzyme. Relative glucoamylase activities (88% and 75%, respectively) towards gelatinised potato starch and sweet potato starch were considerably high compared to

Table 4
Correlation between compositional properties and hydrolysis extent by successive action of Termamyl 120 L, *Bacillus licheniformis* α-amylase and glucoamylase in the experimental tuber and root starches (n = 44)

	Termamyl 120 L	*Bacillus licheniformis* α-amylase	Glucoamylase
Phosphorus content	−0.546**	−0.428**	−0.666**
Amylose content	0.171	0.251	0.385*
Median granule size	−0.170	−0.104	−0.276

*and **: $p < 0.01$ and 0.05, respectively.

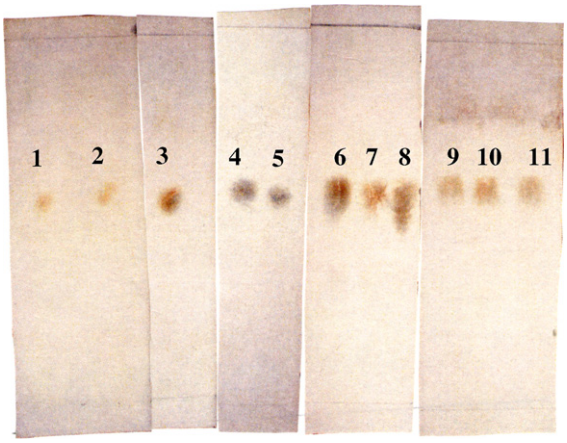

Fig. 1. Thin layer chromatography of the end products of gelatinised tuber and root starches after hydrolysis with the successive action of enzymes; lane 1: glucose; lanes 2–4: almost completely hydrolysed potato, cassava and sweet potato starches; lane 5: maltose monohydrate; lanes 6–8: potato, cassava and sweet potato starches, after hydrolysis with Termamyl 120 L for 1 h, respectively; lanes 9–11: potato, cassava and sweet potato starches, after hydrolysis with Termamyl 120 L plus *Bacillus licheniformis* α-amylase for 24 h, respectively.

non-gelatinised starch (8% and 8.9%, respectively). Very similar hydrolysis rates were observed for the gelatinised starches used in this study by using an enzyme mixture containing bacterial liquefying α-amylase and *B. licheniformis* α-amylase. On the other hand, a high value (74.8%) was obtained by Li et al. (2007) in hydrolyzing non-gelatinised potato starch by glucoamylase only. This result is not understood clearly at this moment but one explanation for this high value may be that potato starch is partially gelatinised at 60 °C. Gelatinised starches show increased susceptibility to enzymatic degradation, in comparison to their native form (Konsula & Liakopoulou-Kyriakides, 2004; Noda et al., 2008). As reported, the presence of phosphate groups in potato amylopectin probably caused essentially 65% hydrolysis with *C. resinae* glucoamylase and because of contamination by a trace amount of α-amylase further hydrolysis occurred, to give conversion of nearly 80% after 20 h incubation (McCleary & Anderson, 1980). Again, it was reported that 81% conversion was attained with *Aspergillus niger* glucoamylase preparation freed from α-amylase and phosphatase when large amounts of the enzymes were used, but after adulteration with α-amylase the hydrolysis approached to an apparent limit of 89% after 24 h (Abe et al., 1982).

It can be observed from Fig. 1 that only glucose was detected in the almost complete hydrolysate of gelatinised potato starch (sample No. 31, lane 2), cassava starch (sample No. 42, lane 3) and sweet potato starch (sample No. 39, lane 4), after digestion with glucoamylase. This implied that the concomitant enzymes treatment could hydrolyse both the α-1,4 and α-1,6 linkages of the starches examined. After hydrolysis of the starches with bacterial liquefying α-amylase for only 1 h and with bacterial liquefying α-amylase plus *B. licheniformis* α-amylase for 24 h, it was observed that the hydrolysate gave in addition to glucose some other spots of limited dextrins (lanes 6–11). From the pattern of spots it may concluded that the hydrolysed product of sweet potato starch contained more different polymers of sugar molecules than the potato and cassava starches. Further study is needed to determine the structure of limited dextrins for obtaining clear information about their sizes. We did not perform the TLC analysis of non-hydrolysed starch samples since they do not give any detectable spot (Li et al., 2007).

In conclusion, the present investigation establishes a method for digestibility using minimum concentration of enzymes, such as two α-amylases and glucoamylase, in potato starches containing higher phosphorus as well as other tuber and root starches. Although higher phosphorus content was associated with the reduced digestibility of gelatinised starches by enzymes, amylose content and median granule size did not affect digestibility greatly. Information regarding the digestibility of gelatinised starches might be important to the food industry, especially, for those companies that make use of potato starch as raw material for producing starch syrup.

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