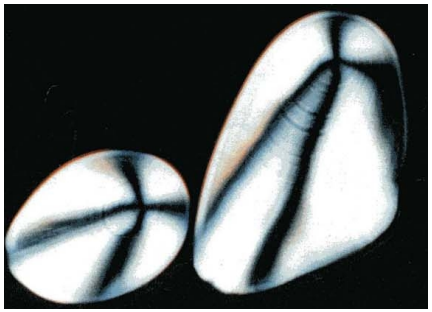
National Agriculture
and Food Research Organization

The enzymatic digestibility and phosphate content in potato starches



Takahiro Noda

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Hokkaido Region (NARCH), JAPAN

Workshop Japan-New Zealand (JST),
11 October 2010, Tokyo.

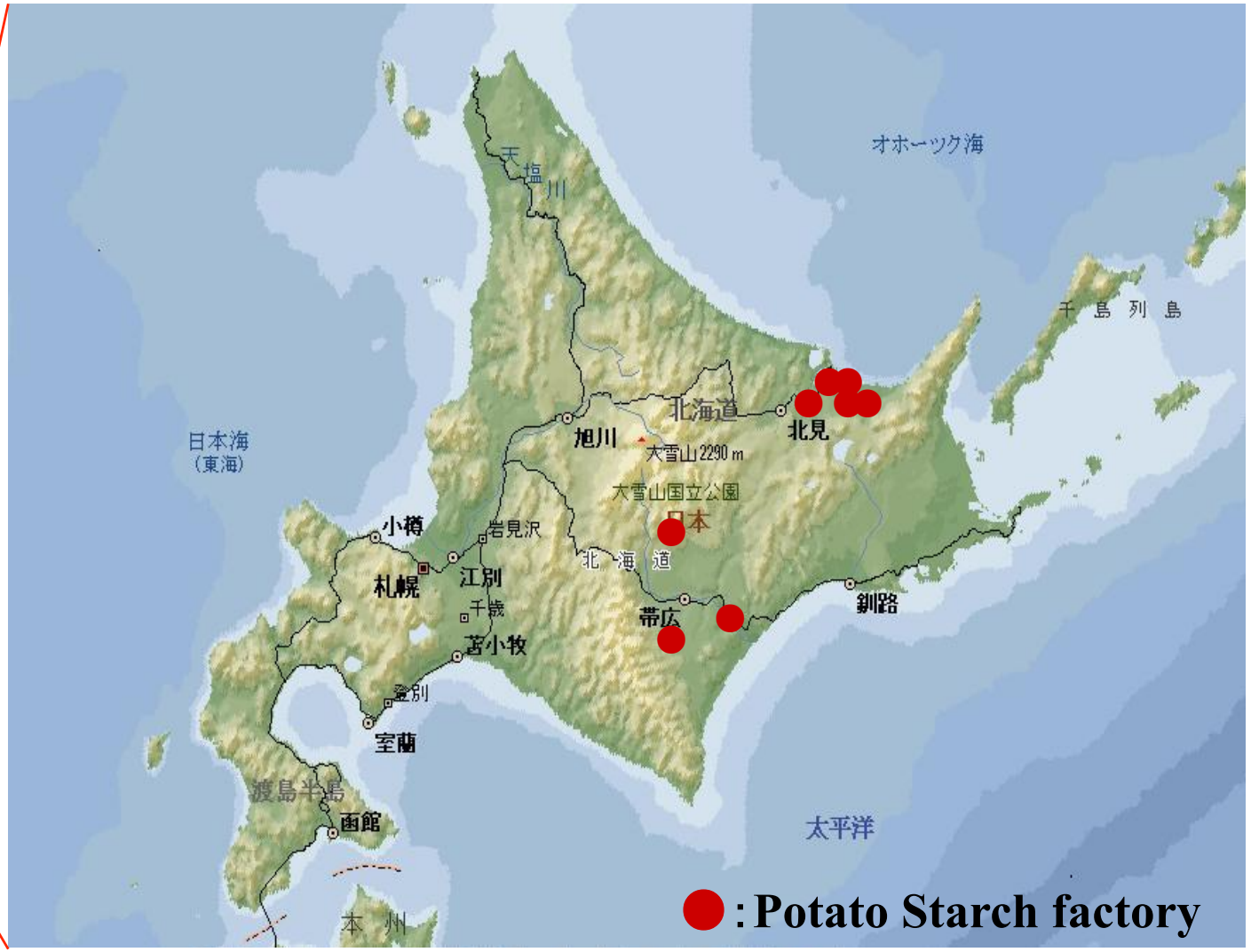
Starch

- Commonest storage carbohydrate in plants
- Largest source of carbohydrate in human food
- A semi-crystalline composite substance
- Consist of two alpha-glucan polymers; amylose and amylopectin
- Amylose: amylopectin controls most of functional properties

Potato starch production in Japan

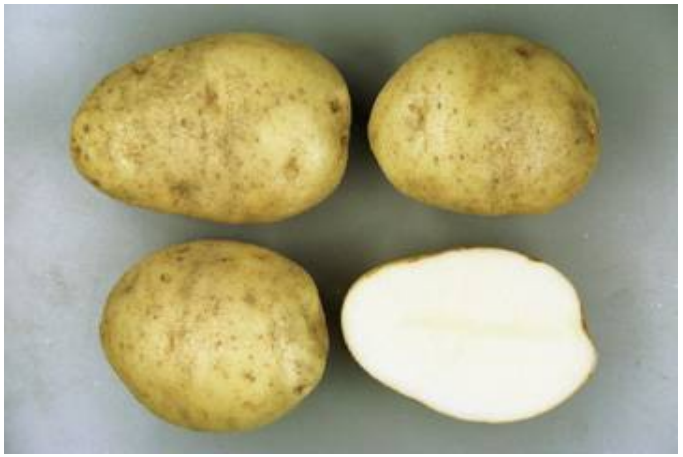
1. In Hokkaido, the northernmost island of Japan, local factories produce potato starch.
2. Benimaru and Konafubuki are typical varieties for starch production.
3. The output of potato starch is 250,000 tons per year.
4. Potato starch has various uses, including a material of “kamaboko” (boiled fish paste), various noodles and glucose-fructose syrup.

Distribution of potato starch factory



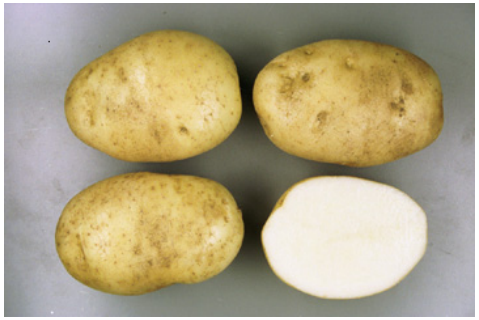
Representative potato cultivars adapted in Japan

Benimaru
(starch production)

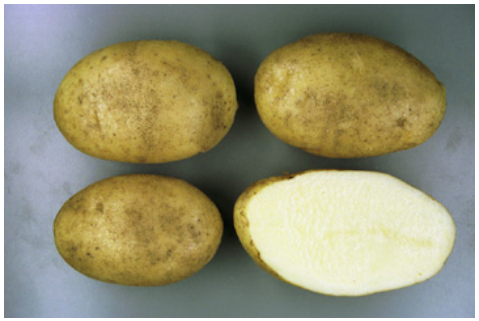


Konafubuki
(starch production)

Irish Cobbler
(market sale)



Toyoshiro
(food processing)



Hokkaikogane
(food processing)

The process for potato starch production

Products made from potato starch in Japan

Various noodles

“Katakuriko”

“Kamaboko”
(boiled fish paste)

Intact use (120000 t/year)

Glucose-fructose syrup
(Isosyrup)

Saccharification products
(120000 t/year)

Potato starch has unique properties

1. The granule size of potato starch is definitely larger than other starches.
2. Potato starch has a wide distribution of granule size, ranging from 5 to 100μm.
3. Compared to other starches, potato starch has a higher phosphorus content.
4. Due to higher phosphorus content, potato starch exhibits extremely high viscosity.

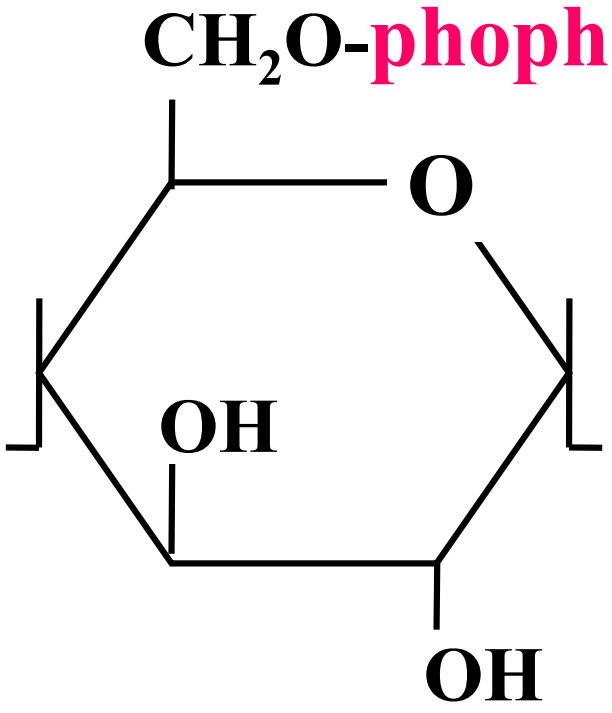
Granule size of root and tuber starches

	Size distribution (μm)
Potato	15-110
Sweet potato	2-42
Taro	3.0-3.5
Cassava	5-40
Kudu	3-23
Lotus	15-40

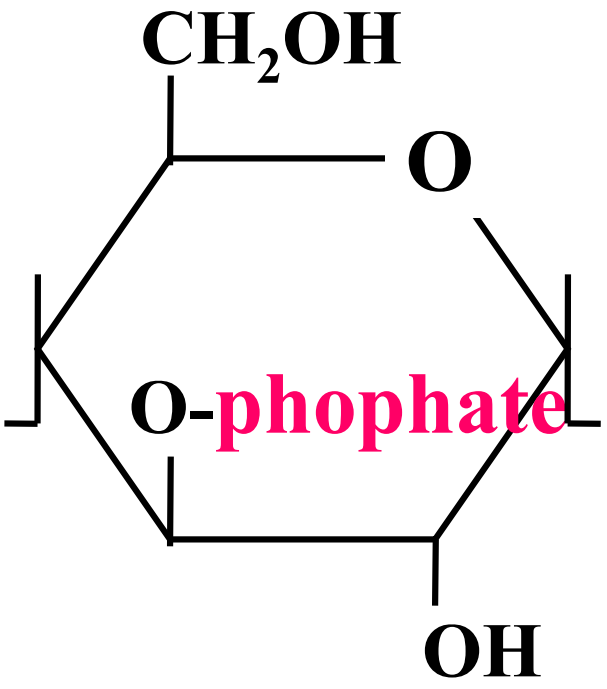
Large-sized

Hoover (2001) Carbohydr. Polym.

Starch bound phosphate(1/1)



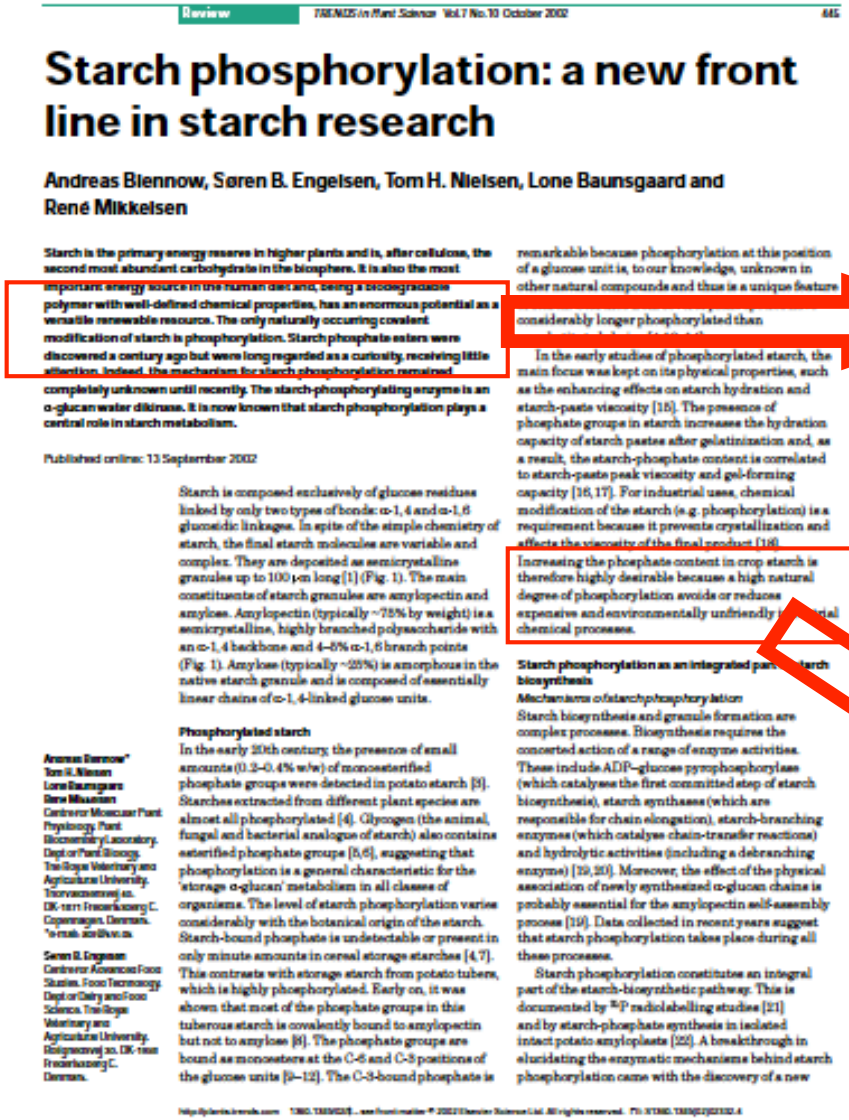
P at C-6
glucosyl residue



P at C-3
glucosyl residue

Starch bound phosphate exists in tuber starches.

Starch bound phosphate (2/2)



Review TRENDS in Plant Science Vol.7 No.10 October 2002 445

Starch phosphorylation: a new front line in starch research

Andreas Blennow, Søren B. Engelsen, Tom H. Nielsen, Lone Baunsgaard and René Mikkelsen

Starch is the primary energy reserve in higher plants and is, after cellulose, the second most abundant carbohydrate in the biosphere. It is also the most important energy source in the human diet and, being a biodegradable polymer with well-defined chemical properties, has an enormous potential as a versatile renewable resource. The only naturally occurring covalent modification of starch is phosphorylation. Starch phosphate esters were discovered a century ago but were long regarded as a curiosity, receiving little attention. Indeed, the mechanism for starch phosphorylation remained completely unknown until recently. The starch-phosphorylating enzyme is an α-glucan water dikinase. It is now known that starch phosphorylation plays a central role in starch metabolism.

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Starch is composed exclusively of glucose residues linked by only two types of bonds: α-1, 4 and α-1, 6 glucosidic linkages. In spite of the simple chemistry of starch, the final starch molecules are variable and complex. They are deposited as semicrystalline granules up to 100 μm long [1] (Fig. 1). The main constituents of starch granules are amylopectin and amylose. Amylopectin (typically ~75% by weight) is a semicrystalline, highly branched polysaccharide with an α-1, 4 backbone and 4–5% α-1, 6 branch points (Fig. 1). Amylose (typically ~25%) is amorphous in the native starch granule and is composed of essentially linear chains of α-1, 4-linked glucose units.

Phosphorylated starch

In the early 20th century, the presence of small amounts (0.2–0.4% w/w) of monoesterified phosphate groups were detected in potato starch [3]. Starches extracted from different plant species are almost all phosphorylated [4]. Glycogen (the animal, fungal and bacterial analogue of starch) also contains esterified phosphate groups [5,6], suggesting that phosphorylation is a general characteristic for the 'storage α-glucan' metabolism in all classes of organisms. The level of starch phosphorylation varies considerably with the botanical origin of the starch. Starch-bound phosphate is undetectable or present in only minute amounts in cereal storage starches [4,7]. This contrasts with storage starch from potato tubers, which is highly phosphorylated. Early on, it was shown that most of the phosphate groups in this tuberous starch is covalently bound to amylopectin but not to amylose [8]. The phosphate groups are bound as monoesters at the C-6 and C-3 positions of the glucose units [9–12]. The C-3-bound phosphate is remarkable because phosphorylation at this position of a glucose unit is, to our knowledge, unknown in other natural compounds and thus is a unique feature

considerably longer phosphorylated than

In the early studies of phosphorylated starch, the main focus was kept on its physical properties, such as the enhancing effects on starch hydration and starch-paste viscosity [13]. The presence of phosphate groups in starch increases the hydration capacity of starch pastes after gelatinization and, as a result, the starch-phosphate content is correlated to starch-paste peak viscosity and gel-forming capacity [16,17]. For industrial uses, chemical modification of the starch (e.g. phosphorylation) is a requirement because it prevents crystallization and affects the viscosity of the final product [18].

Increasing the phosphate content in crop starch is therefore highly desirable because a high natural degree of phosphorylation avoids or reduces expensive and environmentally unfriendly [illegible] chemical processes.

Starch phosphorylation as an integrated part of starch biosynthesis

Mechanism of starch phosphorylation

Starch biosynthesis and granule formation are complex processes. Biosynthesis requires the concerted action of a range of enzyme activities. These include ADP–glucose pyrophosphorylase (which catalyses the first committed step of starch biosynthesis), starch synthases (which are responsible for chain elongation), starch-branching enzymes (which catalyse chain-transfer reactions) and hydrolytic activities (including a debranching enzyme) [19,20]. Moreover, the effect of the physical association of newly synthesized α-glucan chains is probably essential for the amylopectin self-assembly process [19]. Data collected in recent years suggest that starch phosphorylation takes place during all these processes.

Starch phosphorylation constitutes an integral part of the starch-biosynthetic pathway. This is documented by ^{33}P radiolabelling studies [21] and by starch-phosphate synthesis in isolated intact potato amyloplasts [22]. A breakthrough in elucidating the enzymatic mechanisms behind starch phosphorylation came with the discovery of a new

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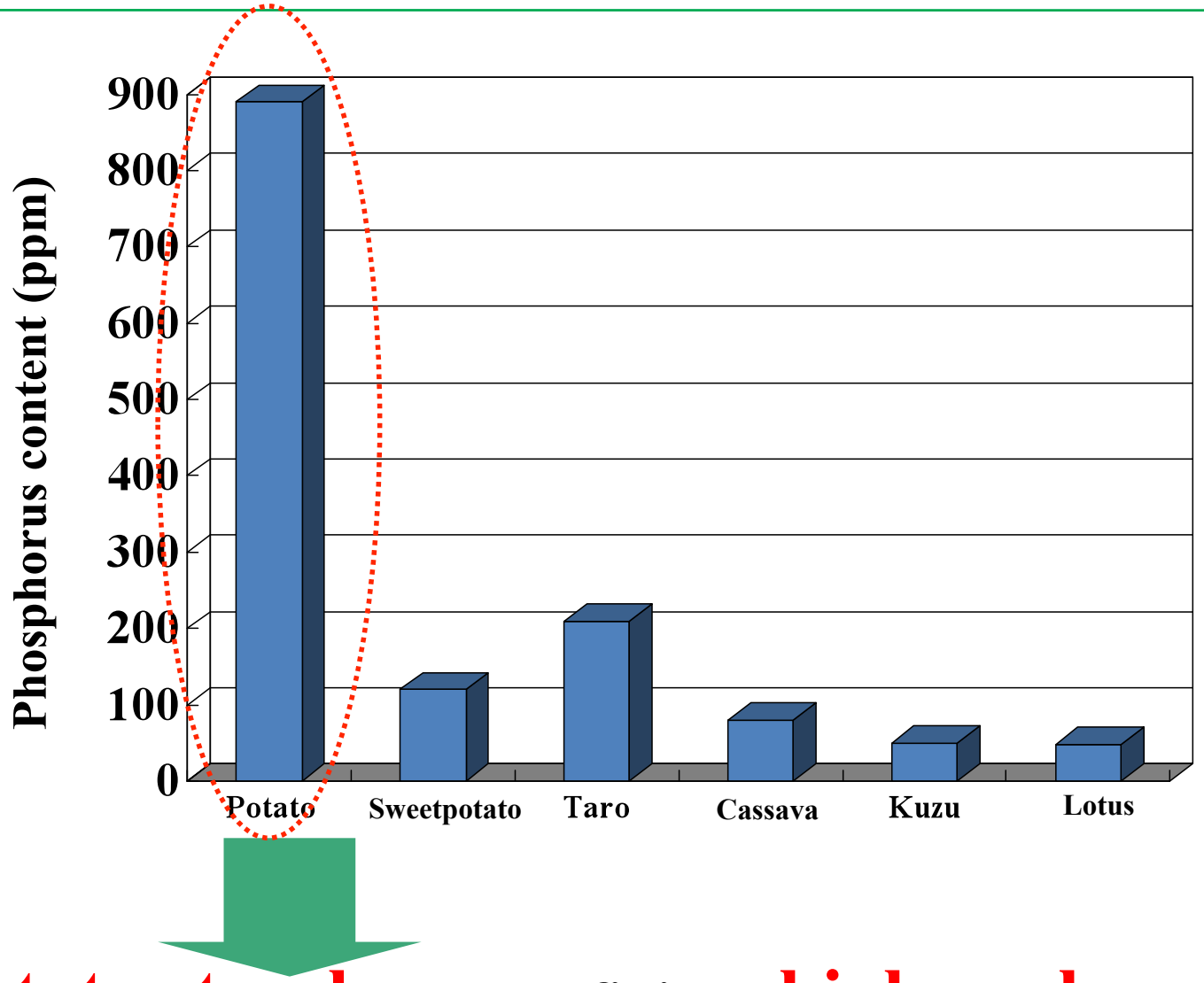
The only naturally occurring covalent modification of starch is phosphorylation. Starch phosphate esters were discovered a century ago but were long regarded as a curiosity, receiving little attention.

Increasing the phosphate content in crop starch is therefore highly desirable because a high natural degree of phosphorylation avoids or reduces expensive and environmentally unfriendly industrial chemical processes.

Blennow et al., *Trends in Plant Science*, 7, 445-450 (2002)

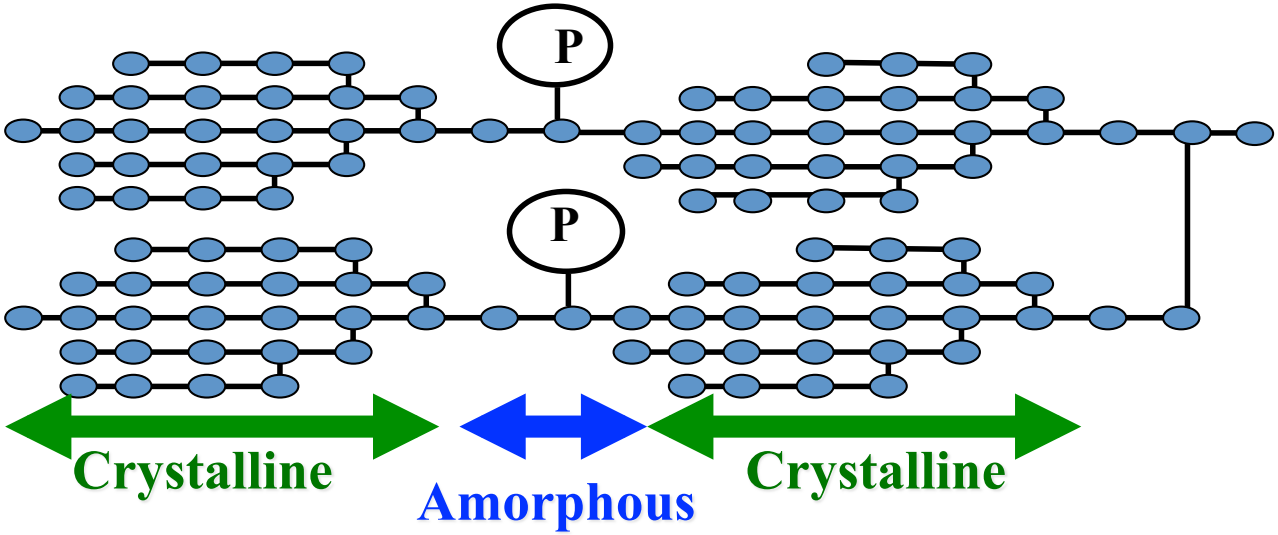
Phosphorus content of root and tuber starches



Potato starch has a definitely higher phosphorus content than other starches.

Hoover (2001) *Carbohydr. Polym.*

Phosphate in amylopectin molecules

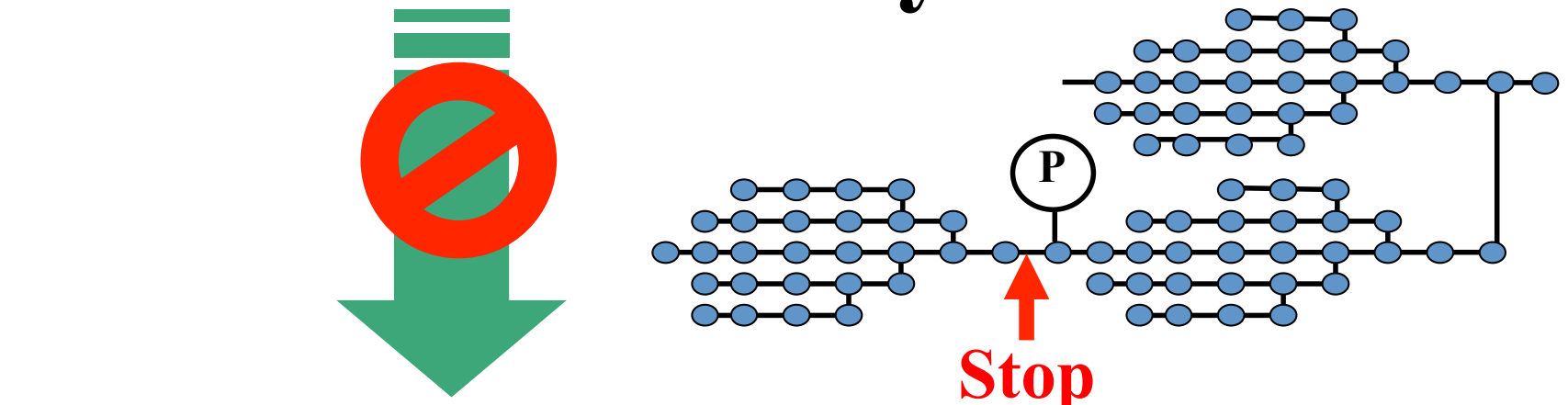


The phosphate group is attached to longer unit-chains with DP>20 of amylopectin (Takeda and Hizukuri (1982) *Carbohydr. Res.*) .

The major part of the total starch phosphate was partition to amorphous part (Blennow et al. (2000) *Int. J. Biol. Macromol.*).

Action of amylases on phosphorylated starch

Action of amylase



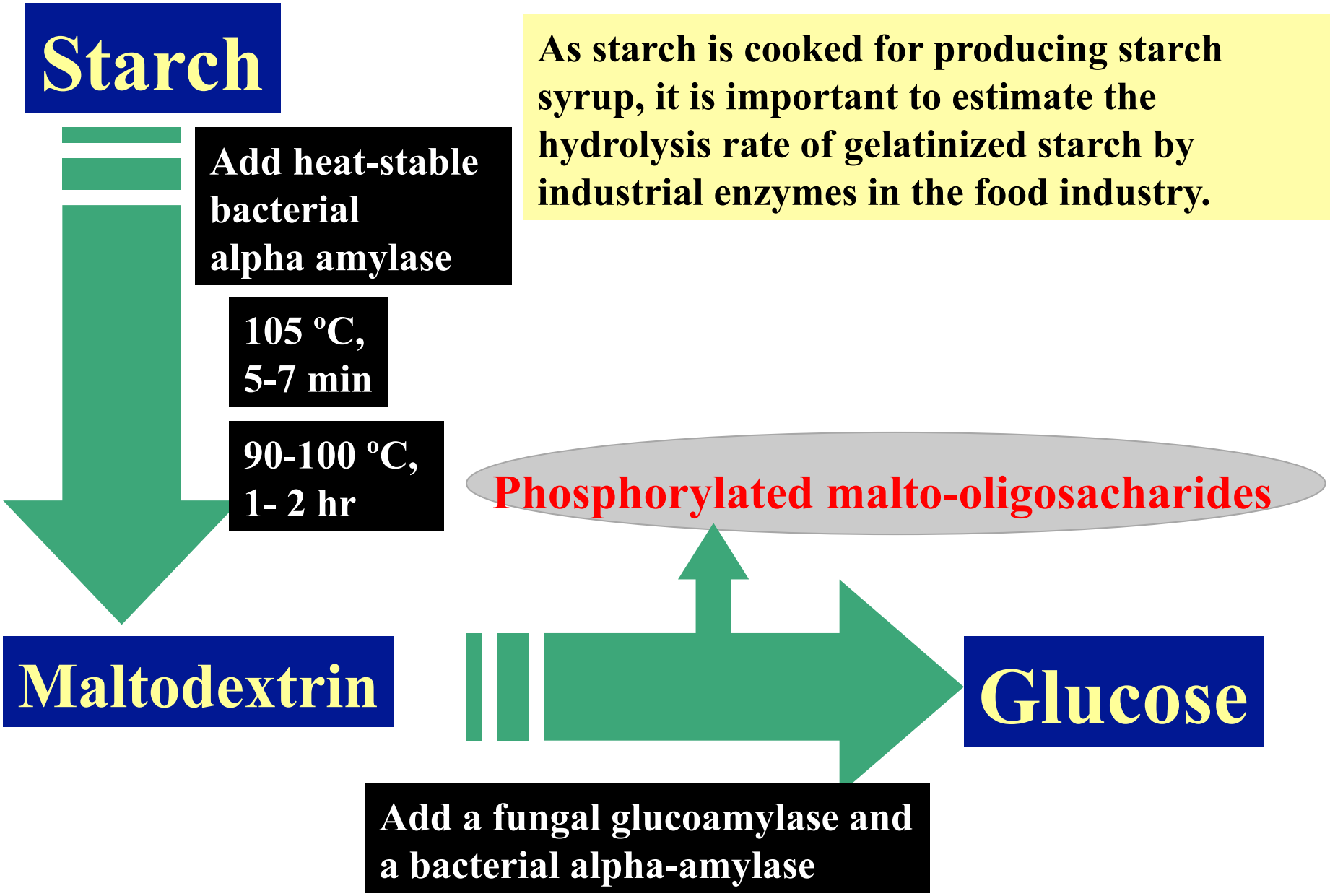
Phosphate groups attached to glucosyl residues of starch

Phosphorylated malto-oligosacharides

Takeda et al. (1983) *Biochim. Biophys. Acta*

Kamasaka et al. (1995) *Biosci. Biotechnol. Biochem.*

Starch saccharification process



As starch is cooked for producing starch syrup, it is important to estimate the hydrolysis rate of gelatinized starch by industrial enzymes in the food industry.

Enzymatic digestibility is variable according to:

High amylose, large granule size

Low digestibility of raw starch

High phosphate

Low digestibility of raw and gelatinized starches

?

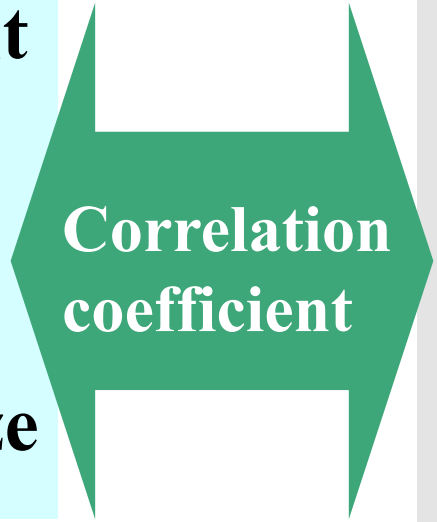
Till to-date, the experimental data on the digestibility of gelatinized starches are not available so much specially using many tuber and root starches.

Objective of this study

Phosphorus content

Amylose content

Meidan granule size



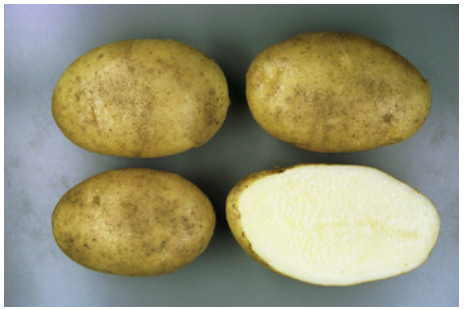
Enzyme digestibility
(after digestion of Termamyl 120L, *Bacillus* alpha-amylase, and glucoamylse)

Potato starches and other tuber and root starches

To describe the effect of phosphorus and amylose contents and median granule size on the rate of gelatinized starch by industrial amylases

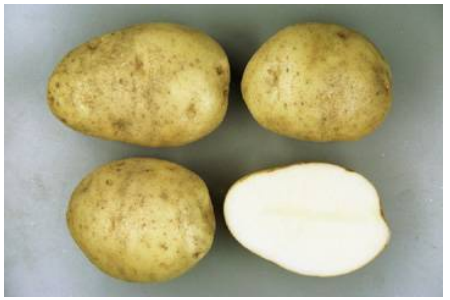
Materials (Starch)

1. Thirty-six potato starches



Hokkaikogane

Benimaru



Konafubuki

etc.

2. Four sweet potato starches, three cassava starches and one yam starch

Sweet potato

Cassava

Yam

Methods(1)

1. Amylose content

- Blue value (680nm) method according to the equation of Takeda et al. (1983)

2. Mean granule size

- Using Sympatec HELOS Particle Size Analysis

3. Phosphorus content

- Vanado-Molybdate method

Amylose content and median granule size

	Amylose content (%)	Median granule size (µm)
Potato	15.4-25.5	14.0-44.7
Mean (n=36)	21.5	34.6
Sweet potato	16.2-23.4	14.5-20.6
Mean (n=4)	19.6	17.0
Cassava	25.3-28.8	15.7-16.3
Mean (n=3)	26.5	16.1
Yam	25.8	22.8

Phosphorus content (ppm)

Potato Mean (n=36)	500-1132 760
Sweet potato Mean (n=4)	156-231 192
Cassava Mean (n=3)	81-105 94
Yam	166

High-phosphorus, medium-phosphorus and low-phosphorus starches

On the basis of phosphorus content, we have arranged the experimental potato starches into two classes. Namely, there were 19 high-phosphorus starches (HPS) (812-1132 ppm) and 17 medium-phosphorus starches (MPS) (500-756 ppm).

We have defined sweet potato, cassava and yam starches as low-phosphorus starches (LPS) as their phosphorus content ranged from 81 to 231 ppm.

Methods(2)

Starch was suspended in 49.5 ml of 6mM, 2mM $CaCl_2$ solution and 0.5% of **Termamyl 120L Type L.**

Heated to 100 ºC to liquefy

Cool and incubate at 50 ºC for 1 hr

Estimate the reducing sugar to calculate **hydrolysis rate (1)**

And then add ***Bacillus*** **alpha-amylase**

Incubate at 55 ºC for 24 hrs

Estimate the reducing sugar to calculate **hydrolysis rate (2)**

And then add ***Rhizopus*** **sp. glucoamylase**

Incubate at 40 ºC for 24 hrs

Estimate the reducing sugar to calculate **hydrolysis rate (3)**

Hydrolysis rate (%) after digestion of three amylases

	Termamyl 120L	Alpha-amylase	Glucoamylase
HPS (potato)	28-46	60-71	95-100
Mean (n=19)	35	65	97
MPS (potato)	35-48	64-74	97-100
Mean (n=17)	40	69	98
LPS (sweet potato, cassava and yam)	35-48	64-72	99-100
Mean (n=8)	42	69	99

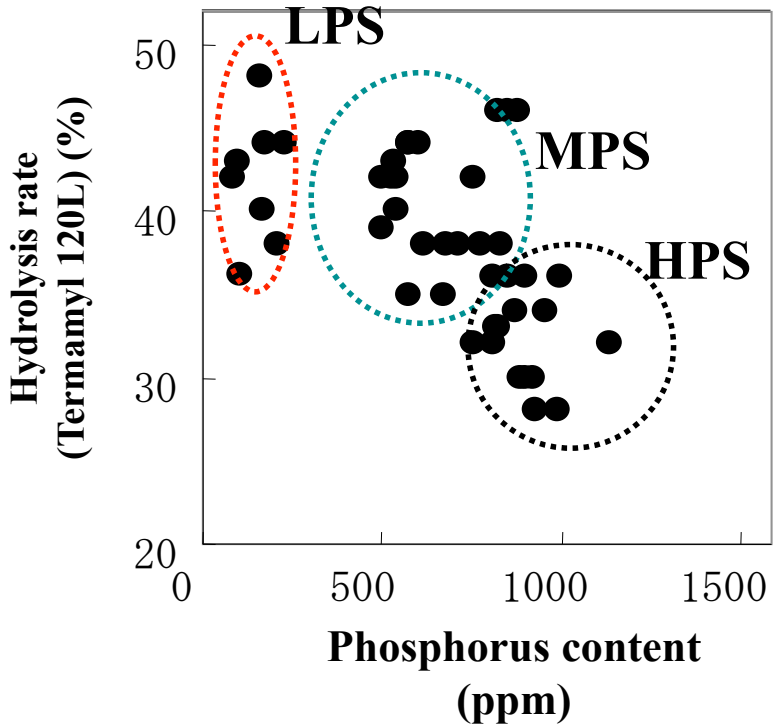
Low

High-phosphorus starches were more resistant to enzyme hydrolysis than middle- and low-phosphorus starches.

Correlation coefficients

Starch digestibility	Phosphorus content	Amylose content	Median granule size
Termamyl 120L	**-0.546****	0.171^{NS}	-0.170^{NS}
***Bacillus* alpha-amylase**	**-0.428****	0.251^{NS}	-0.104^{NS}
Glucoamylase	**-0.666****	0.385*	-0.276^{NS}

*** and ** Significant at the P≤0.05 and P≤0.01, respectively. NS :not significant.**



Higher phosphorus content is associated with lower hydrolysis rate by industrial amylases !!

Conclusion

1. High-phosphorus potato starches were more resistant to enzyme hydrolysis than middle-phosphorus potato starches as well as sweet potato, cassava and yam starches.

2. The hydrolysis rate of tuber and root starches was not largely influenced by their amylose content and median granule size.

3. Information concerning the enzymatic digestion of gelatinized tuber and root starches might be important to the food industry.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Thank you for your attention !!