The Possible Role of Starch in Oral Calcification: The *In Vitro* Formation of Hydroxyapatite is Regulated by a Combination of Protein and Mineral Content in Dietary Starch Flour

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Abstract: The effects of twelve kinds of dietary starch flour, i.e. rice (non-glutinous and glutinous), wheat (soft, medium, and hard), barley (roasted), buckwheat (inner layer and straight), corn, sweet potato, kudzu, and tapioca on *in vitro* calcium phosphate precipitation were investigated using the pH drop method. The induction time was elongated by the addition of all of the kinds of flour. Although the rate of amorphous calcium phosphate (ACP) formation was not affected, the rate of transformation of ACP to hydroxyapatite (HAP) was either stimulated or inhibited by the different types of flour. The following observations were made: (1) When the lipid content below 0.2% (w/w), any of the types of starch had a stimulatory effect on the transformation of ACP to HAP. (2) When the lipid content around or above 1.0% (w/w), the value of the product of (protein content) × (mineral content) seems to determine the effect of starch.

Keywords: Amorphous calcium phosphate, calcium phosphate precipitation, dietary starch flours, hydroxyapatite, oral calcification.

INTRODUCTION

Health problems such as cardiovascular disease, obesity and related disorders, dental caries, and cancer are believed to be related to diet [1]. Dental caries is definitely related to total sugar intake; in etiological studies, sucrose was identified as the specific cause of this problem [2,3]. While the effects of dietary carbohydrates on oral hygiene have been extensively investigated in relation to dental caries [3,4], the relationship between the dietary components and the promotion of oral calcification has not been well elucidated.

Oral calcification includes both remineralization of tooth enamel and dental calculus formation. The former can be caused by both endo- and exogenous factors such as salivary flow, salivary mineral levels, and salivary components, antibacterials, fluoride from extrinsic sources, and dietary components [5]. Dental calculus is a calcified dental plaque composed primarily of calcium phosphate mineral salts. Its formation and prevalence have been reported to be associated with the periodontal diseases [6]. The level of calculus formation is affected by several factors including age, gender, diet, oral hygiene, and diabetes [6]. Approximately forty years ago, Baer *et al.* [7] and Baer and White [8] reported the effects of various types of starch on experimental calculus formation in rats. Starch is a major component of the human diet throughout the world. In the countries of Asia, Europe and other global regions, starch from sources such as rice and wheat is found in a wide variety of foods and constitutes a high percentage of total dietary carbohydrate [4]. Recently, we reproduced Baer's *in vivo* results with our *in vitro* experiment we found that reagent grade starch from corn, rice, and potato remarkably stimulated the rate of transformation of amorphous calcium phosphate (ACP) to hydroxyapatite (HAP) *in vitro* [9]. However, the types of starch we used in that study were those of reagent grade that were more highly purified as compared to ordinary dietary starch taken as food. In order to critically study the diet-related oral calcification, it is important to use starch products manufactured for daily meal and snacks.

In countries relying on rice as the main staple, up to 60% of daily calories are obtained from cereal products [10]. There are two kinds of rice: Glutinous rice (comprised of 100% amylopectin) is used to make rice cakes because of its sticky nature, while non-glutinous rice (about 80% amylopectin) is taken as daily meal in Asia. Both kinds of rice flour are used for making cakes, snacks, and sweets. Both sweet potato and tapioca (cassava), two major starchy crops used in many tropical countries [11,12], are used for snacks in Japan. Sweet potato starch is traditionally used for the production of an alcoholic beverage [13]. Kudzu is a traditional Chinese herb and its starch is used for making cakes and sweets [14], while its root extract is used to treat alcohol abuse patients [15]. Three kinds of wheat flour are used for different purposes; hard flour is for baking [16], medium flour is for noodles [17], and soft flour is for cake or tempura. Corn flour is used to prepare basic nutritional foods in several Latin American countries [18], and for sweets, snacks, and cakes in Japan. It is also used for feeding cattle [19]. Compared to wheat and rice starch, barley starch is not as commonly used as a food ingredient. It is the prime source of starch (and hence glucose) for the production of alcoholic beverages in most industrial countries [20]. A variety of buckwheat dishes are served throughout the world; noodle

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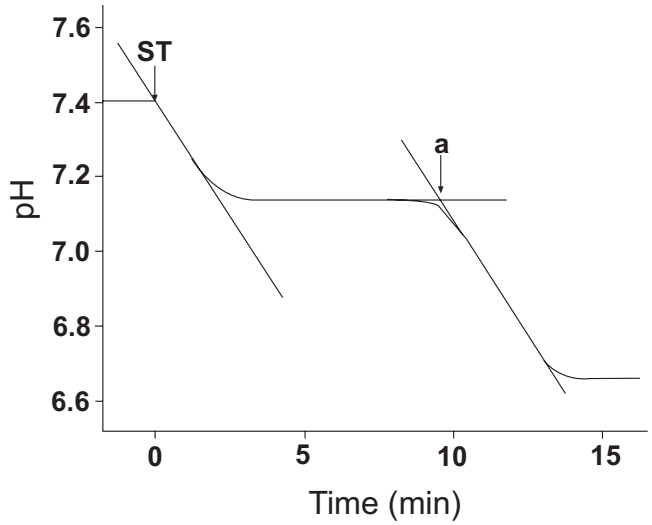

Fig. (1). Representative recording of the rate of pH change induced by the addition of phosphate (ST) to the reaction mixture. Three millimoles calcium and 3 mM phosphate were used. The final volume of the assay solution, which contains 2 mM Hepes buffer (pH 7.4), was 2 ml. The reaction mixture was stirred at 37 ˚C. Changes occur in two distinct steps. The rate of ΔpH/min was determined by drawing a tangent of the first (for the rate of ACP formation) and the second (for the rate of transformation of ACP to HAP) slope, respectively. Induction time was determined between ST and the point (a), which is the intersection (a) between the baseline and the extended line of tangent of second slope.

dishes made from buckwheat flour are popular in Japan, China, and Italy [21].

Understanding the popularity of starch-related dishes, and knowing the need for the investigation on their possible role in promoting oral calcification, we undertook this project to study the *in vitro* effects of different types of starch on calcium phosphate formation. We used the pH drop method to follow the reaction *in vitro* [22]. Fig. (**1**) shows an example of the pH record. When phosphate and calcium ions are mixed at pH 7.4, the formation of amorphous calcium phosphate (ACP) can be monitored from the first pH drop. The transformation of ACP to hydroxyapatite (HAP) *in vitro* can be followed from the second pH drop. The changes occur in two distinct steps as shown in the figure. The effectiveness of any agents that affect the calcium phosphate precipitation can be experimentally studied by measuring (i) the rate of the pH decrease at the first step, (ii) the onset time (as indicated by "a" in the figure) of the second rapid decrease, and (iii) the rate of the pH drop at the second step [22,23]. Since some compounds and ions are stimulatory [23-25], while others are inhibitory or not effective [22,26,27], this pH drop method has come to be used for analyzing the effects of foods and their components. As an example in the case of foods, we have previously applied this method to distinguish bovine bone gelatin from porcine skin gelatin using the difference in their stimulatory patterns of *in vitro* calcium phosphate precipitation [28].

In this project, we used twelve kinds of commercial starch dietary flour, i.e. rice (non-glutinous and glutinous), wheat (soft, medium, and hard), barley (roasted), buckwheat (inner layer and straight), corn, sweet potato, kudzu, and tapioca, and investigated their effects on the *in vitro* formation of calcium phosphate precipitates.

MATERIALS AND METHODS

Chemicals

Corn starch of reagent grade was purchased from Sigma Aldrich Japan Co. (Tokyo, Japan) and used as standard starch. Dietary starch flour: Rice (non-glutinous) (Joshin-ko;

Table 1. Constituents and Contents in Dietary Starch Flour Used in this Study

	Constituents					
	Contents	Water	Proteins	Carbohydrates	Lipids	Ashes
Rice (non-glutinous)	% (w/w)	14.0	6.2	78.5	0.9	0.4
Rice (glutinous)	% (w/w)	12.5	6.3	80.0	1.0	0.2
Wheat (soft)	% (w/w)	14.0	8.0	75.9	1.7	0.4
(medium)	% (w/w)	14.0	9.0	74.8	1.8	0.4
(hard)	% (w/w)	14.5	11.7	71.6	1.8	0.4
Barley (roasted)	% (w/w)	3.5	12.5	77.1	5.0	1.9
Buckwheat (inner layer)	% (w/w)	14.0	6.0	77.6	1.6	0.8
(straight)	% (w/w)	13.5	12.0	69.6	3.1	1.8
Corn	% (w/w)	14.0	6.6	76.1	2.8	0.5
Sweet potato	% (w/w)	17.5	0.1	82.0	0.2	0.2
Kudzu	% (w/w)	13.9	0.2	85.6	0.2	0.1
Tapioca	% (w/w)	14.2	0.1	85.3	0.2	0.2

Values on the Table were cited from the book of Kagawa [29].

Table 2. Mineral Contents in Dietary Starch Flour Used in this Study

	Contents (mg/100 g)								
	Na	**K**	**Ca**	**Mg**	**P**	**Fe**	**Zn**	**Cu**	**Mn**
Rice (non-glutinous)	2	89	5	23	96	0.8	1.0	0.19	0.75
Rice (glutinous)	2	3	5	6	45	1.1	1.2	0.17	0.55
Wheat (soft)	2	120	23	12	70	0.6	0.3	0.09	0.50
(medium)	2	100	20	18	74	0.6	0.5	0.11	0.50
(hard)	2	80	20	23	75	1.0	0.8	0.15	0.38
Barley (roasted)	2	490	43	130	340	3.1	3.8	0.41	1.81
Buckwheat (inner layer)	2	410	17	190	400	2.8	2.4	0.54	1.09
(straight)	1	190	10	83	130	1.7	0.9	0.37	0.49
Corn	1	200	3	31	90	0.6	0.6	0.08	0.13
Sweet potato	1	4	50	4	8	2.8	0.1	0.01	ND
Kudzu	2	2	18	3	12	2.0	Tr	0.02	0.02
Tapioca	1	48	28	5	6	0.3	Tr	0.03	0.09

Values on the Table were cited from the book of Kagawa [29].
ND: Not-detected.
Tr: Trace.

Maruyo Milling, Yamaguchi, Japan), rice (glutinous) (Shiratama-ko; Maehara Co. Ltd, Hyogo, Japan), wheat (soft) (Hakuriki-ko; Nisshin Foods Co. Ltd, Tokyo, Japan), wheat (medium) (Churiki-ko; Nisshin Foods Co. Ltd, Tokyo, Japan), wheat (hard) (Kyoriki-ko; Nisshin Foods Co. Ltd, Tokyo, Japan), barley (roasted) (Hattai-ko; Maehara Co. Ltd, Hyogo, Japan), buckwheat (inner layer) (Naiso-hun; Yamae-Hisano Co. Ltd, Tokyo, Japan), buckwheat (straight) (Zenso-hun; Ituki Foods Co. Ltd, Kumamoto, Japan), corn (Maruyo Milling, Yamaguchi, Japan), sweet potato (Maruyo Milling, Yamaguchi, Japan), kudzu (Maehara Co. Ltd, Hyogo, Japan), and tapioca (Watashino Daidokoro Co. Ltd, Miyazaki, Japan) were used. The constituents and contents of the dietary starch flours used in this study are shown in Table **1**. The mineral contents are shown in Table **2**. α-amylase (1,000 - 1,500 units/mg) from human saliva (type IX-A), gluten (wheat protein), and rutin hydrate (buckwheat flavonoid) were purchased from Sigma-Aldrich Co. (St. Louis, MO, U.S.A.). Other reagents were obtained from ABIOZ Co. Ltd. (Osaka, Japan).

Measurements of pH

A pH meter (F-21, Horiba, Japan) with a combination type pH electrode (6378-10D, Horiba, Japan) and a recorder were used for pH measurements. The final volume of the assay solution was 2 ml [22]. The reaction mixture was stirred continuously during the pH measurement procedure, and the temperature was maintained at 37 ± 0.1˚C.

Assay of Amorphous Calcium Phosphate (ACP) Formation, Its Transformation to Hydroxyapatite (HAP), and the Induction Time by the pH Drop Method

Both stock solutions, 100 mM $Ca(NO_3)_2$ and 100 mM KH_2PO_4, were prepared in 2 mM Hepes Buffer (pH 7.4). To a 1.88 ml buffer solution (2 mM Hepes buffer; pH 7.4), 60 µl of the calcium stock solution was added. Then, 60 µl of the phosphate stock solution was added to start the reaction. The final concentrations of calcium and phosphate were both 3 mM. An example of the recording is shown in Fig. (**1**).

For the study of the effect of dietary starch flour, each of the following types of flour was added to the reaction buffer at a final concentration of 0.1 - 5.0 mg/ml, 5 min before the addition of phosphate: Rice (non-glutinous), rice (glutinous), wheat (soft), wheat (medium), wheat (hard), barley (roasted), buckwheat (inner layer), buckwheat (straight), corn, sweet potato, kudzu, and tapioca. Final concentrations of 0.1 - 5.0 mg/ml of corn starch (reagent grade) were used as the starch standard. Final concentrations of either 12.5 - 125 µg/ml of gluten or 0.25 - 2.0 mg/ml (0.38 - 3.0 mM) of rutin were used. We also tested the starch hydrolysates formed by α-amylase (10 µg/ml). The concentration was 2.0 mg/ml for each of wheat (soft), wheat (medium), wheat (hard), barley (roasted), buckwheat (inner layer), buckwheat (straight), sweet potato, and corn flour or corn starch (standard). In order to investigate the effect of maltose [10.0 mg/ml (27.8 mM); a product of α-amylase hydrolysis] alone or α-amylase (10 µg/ml) alone, each of these was added separately to the reaction chamber in the absence of starch products.

The formation of amorphous calcium phosphate (ACP; ppm Ca^{2+}/min), its transformation to hydroxyapatite (HAP; ppm Ca^{2+}/min), and the induction time (min) were calculated from the recording of the pH changes. The rate of pH decrease was converted to the rate of consumption of calcium (parts/10^6/min) [22,28]. The induction time was determined according to the method of Blumenthal *et al.* [30].

The coefficients of variation calculated from this method were below ~10%.

Hydrolysis of Dietary Starch Flour by α-Amylase

Each of three kinds of wheat flour (soft, medium, and hard), barley flour (roasted), two kinds of buckwheat flour (inner layer and straight), sweet potato flour, and corn flour suspension (2.0 mg/ml) or corn starch solution (2.0 mg/ml) in 2 mM Hepes buffer (pH 6.9) containing 6 mM NaCl was mixed with 10 μg/ml α-amylase (10 - 15 units/mg) from human saliva (type IX-A) and incubated at 37℃ for 30 min.

X-Ray Diffraction

Twenty min after mixing 3 mM calcium and 3 mM phosphate at 37℃ in the absence or presence of 2.0 mg/ml standard corn starch, 2.0 mg/ml tapioca flour, 2.0 mg/ml rice flour (glutinous), 2.0 mg/ml wheat flour (hard), or 2.0 mg/ml buckwheat flour (straight), the solution was centrifuged at 2,200 × g for 5 min. The calcium phosphate precipitates collected were examined by X-ray diffraction analysis (Rint-

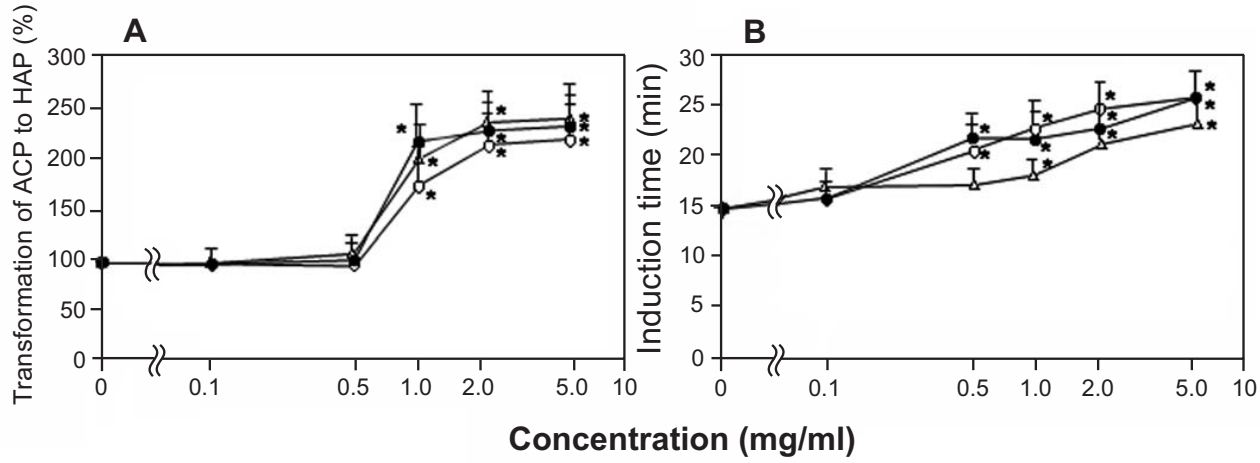

Fig. (2). Transformation of ACP to HAP (% of control) by dietary starch flour. (A) Sweet potato (○), kudzu (●), and tapioca (Δ). (B) Induction time (min) by dietary starch flour. Sweet potato (○), kudzu (●), and tapioca (Δ). The transformation of ACP to HAP and the induction time were measured by the pH drop method. The concentration of calcium and phosphate were 3 mM each. Additives were mixed with the reaction mixture 5 min before the addition of 3 mM phosphate. The final volume of assay solution, which contains 2 mM Hepes buffer (pH 7.4), was 2 ml. The reaction mixture was stirred at 37 ± 0.1°C. Values were converted to the rate of consumption of calcium (parts/10^6/min) [22].

Table 3. Various Dietary Flour did not Affect Amorphous Calcium Phosphate (ACP) Formation

Dietary flours	Ca^{2+} consumption (ppm/min)					
	Concentration used (mg/ml)					
	0.0	0.1	0.2	0.5	1.0	5.0
Rice (non-glutinous)	123 ± 1	124 ± 12	123 ± 1	126 ± 1	124 ± 12	124 ± 12
Rice (glutinous)	123 ± 12	123 ± 12	123 ± 12	123 ± 12	123 ± 12	122 ± 12
Wheat (soft)	123 ± 12	124 ± 12	125 ± 12	124 ± 12	124 ± 12	124 ± 12
Wheat (medium)	123 ± 12	124 ± 12	124 ± 12	124 ± 12	124 ± 12	124 ± 12
Wheat (hard)	123 ± 12	124 ± 12	125 ± 12	125 ± 12	124 ± 12	123 ± 12
Barley (roasted)	123 ± 12	123 ± 12	121 ± 12	124 ± 12	124 ± 12	124 ± 12
Buckwheat (inner layer)	123 ± 12	124 ± 12	125 ± 12	121 ± 12	121 ± 12	122 ± 12
Buckwheat (straight)	124 ± 12	124 ± 12	124 ± 12	124 ± 12	124 ± 12	124 ± 12
Corn	123 ± 12	123 ± 12	122 ± 12	122 ± 12	122 ± 12	124 ± 12
Sweet potato	123 ± 12	124 ± 12	123 ± 12	125 ± 12	121 ± 12	122 ± 12
Kudzu	123 ± 12	123 ± 12	23 ± 12	24 ± 12	24 ± 12	124 ± 12
Tapioca	123 ± 12	124 ± 12	124 ± 12	124 ± 12	124 ± 12	124 ± 12
Corn starch	123 ± 12	123 ± 12	121 ± 12	121 ± 12	122 ± 12	122 ± 12

The ACP formation was measured by the pH drop method [22]. The concentration of calcium and phosphate were 3 mM each. Additives were added to the reaction mixture 5 min before the addition of 3 mM phosphate. The final volume of assay solution, which contains 2 mM Hepes buffer (pH 7.4), was 2 ml.
The reaction mixture was stirred at 37 ± 0.1°C. Values were converted to the rate of consumption of calcium (parts/10^6/min) [22].

2100V, Rigaku, Japan: CuKα radiation, 40 kV and 30 mA) [22].

Statistical Analysis

Data were obtained from 3 - 5 measurements and expressed as the means ± standard deviations. Statistical comparisons were made by ANOVA and Scheffe's Test using a statistics software program. The difference was considered significant at $p<0.05$.

RESULTS

ACP formation, its transformation to HAP, and induction time after the addition of starch flour from sweet potato, kudzu, and tapioca with low lipid content [<0.2% (w/w)]

As shown in Fig. (**2A**), sweet potato starch flour stimulated the rate of transformation of amorphous calcium phosphate (ACP) to hydroxyapatite (HAP); the stimulation was 220% of the control at the concentration of 2.0 mg/ml. Both kudzu flour and tapioca flour were also stimulative; the respective stimulation rate was 232% and 238% at the same concentration.

As shown in Fig. (**2B**), the concentration dependence of the induction time was similar for all types of flour from sweet potato, kudzu, and tapioca. It ranged from 1.6 to 1.8 times the control at the concentration of 5.0 mg/ml. Furthermore, these three types of starch flour did not affect the rate of ACP formation at all (see Table **3**).

ACP formation, its transformation to HAP, and induction time after the addition of starch flour from rice, wheat, barley, buckwheat, and corn with high lipid content [≳1.0% (w/w)]

As shown in Fig. (**3A**), both glutinous and non-glutinous rice flour stimulated the rate of the transformation of ACP to HAP. Their stimulation ratios were 243 and 142% of the control (no addition; 13.0 ± 1.3 Ca^{2+} ppm/min) at the concentration of 2.0 mg/ml, respectively. As to the three kinds of wheat flour (which have different protein content), their effects are different. Wheat (soft) flour had the highest stimulatory effects, 230% of the control at the same concentration. The stimulation by medium wheat flour was less (152% of the control). Hard wheat flour had no effect. As shown in Fig. (**3B**), corn flour stimulated the reaction, was 185% of the control at the concentration of 2.0 mg/ml. Standard corn starch (reagent grade) was stimulative; the stimula-

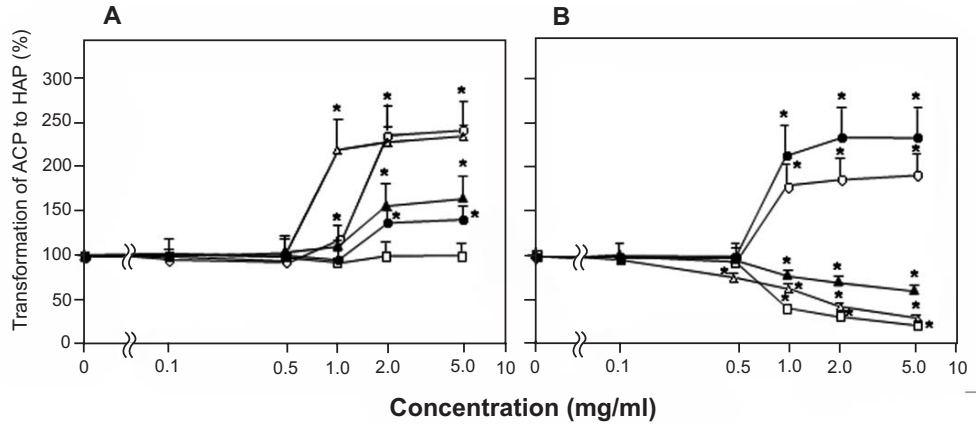

Fig. (3). Transformation of ACP to HAP (% of control) by dietary starch flour. (A) Rice (glutinous) (○), rice (non-glutinous) (●), wheat (soft) (Δ), wheat (medium) (▲), and wheat (hard) (□). (B) Corn (○) corn starch standard (●), barley (Δ), buckwheat (inner layer) (▲), and buckwheat (straight) (□). The transformation of ACP to HAP was measured by the pH drop method. The details are the same as those shown in Fig. (**2**). *Significant difference ($p<0.05$) when compared to the control (no addition).

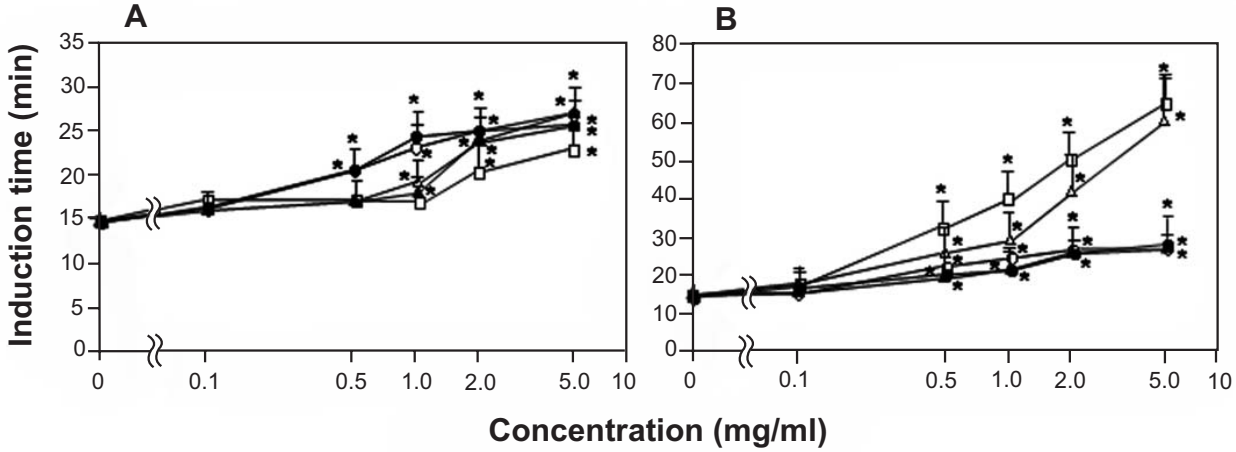

Fig. (4). Changes of the induction time (min) by dietary starch flour. (A) Rice (glutinous) (○), rice (non-glutinous) (●), wheat (soft) (Δ), wheat (medium) (▲), and wheat (hard) (□). (B) corn (○), corn starch standard (●), barley (Δ), buckwheat (inner layer) (▲), and buckwheat (straight) (□). The induction time was measured by the pH drop method. The details are the same as those shown in Fig. (**2**).

tion was 233% of the control at the same concentration. Its stimulation was about 1.25 times higher than that of dietary corn flour and was similar to that of wheat (soft) flour (Fig. (**3A**)). In contrast, barley flour was inhibitory; the inhibition was 35% of the control at the concentration of 2.0 mg/ml. Buckwheat (inner layer) flour inhibited the reaction. The inhibition was 51% of the control at the same concentration. The inhibition by buckwheat (straight) flour was about 2 times of that of inner layer flour (27% of the control at the same concentration).

As shown in (Fig. **4A** and **B**), the concentration dependence of the induction time for flour from glutinous rice, non-glutinous rice, three kinds of wheat (soft, medium, and hard), buckwheat (inner layer), and corn, including standard corn starch, were similar. Their values were 1.6 - 1.9 times higher than that of the control (no addition; 14.8 ± 1.4 min) at the concentration of 5.0 mg/ml. However, at the same concentration, barley and buckwheat (straight) flour had 4.1 and 4.4 times longer induction time as compared to that of the control, respectively (Fig. (**4B**)). These nine types of starch flour did not affect the rate of ACP formation at all (see Table **3**).

Relationship Between the Inhibition of Transformation of ACP to HAP and the Protein Content in Dietary Starch Flour

In an attempt to find a possible cause for the stimulatory/inhibitory effects of the different starch products on the transformation from ACP to HAP, we plotted the relationship between their protein contents (obtained from Table **1**) and their effects on the transformation at 2 mg/ml (Fig. **5**). A low lipid content group [0.2% (w/w)] made up of sweet potato, kudzu, and tapioca and a high lipid content group [≳1.0% (w/w)] of rice (glutinous and non-glutinous), barley, wheat (soft, medium, and hard), corn, buckwheat (inner layer and straight) formed two separate groups. It is obvious that the effects of the latter group on the transformation rate ranged from 0.27 (inhibition) to 2.43 (stimulation) and no rule could be found between the protein content and the transformation rate. We also examined the relationship between the mineral content (obtained from Table **1**) and the transformation rate. However, the data were scattered and no rule could be found for mineral content either (data not shown).

Relationship Between the Inhibition of Transformation of ACP to HAP and the (Protein Content × Mineral Content) in Dietary Starch Flour

Although neither the protein nor mineral content was correlated with the effects on the transformation rate, the product of (protein content × mineral content) seems to be correlated as shown in Fig. (**6**). Sweet potato, kudzu, and tapioca with a low lipid content [0.2% (w/w)] and rice (glutinous and non-glutinous), barley, wheat (soft, medium, and hard), corn, buckwheat (inner layer and straight) with a high lipid content [≳1.0% (w/w)] still formed two different groups. However, the latter group now does evidently follow a rule. Namely, the greater the combined value of the product is, the less the stimulation effect. When the value is 4.68, as in the case of wheat (hard), the stimulation was equal to 1.0 (which means no effect). Flour from rice (glutinous and non-glutinous), wheat (soft and medium), and corn, for which the (protein × mineral) value was <3.60, exerted an effect on the transformation of ACP to HAP which was stimulatory. On the other hand, in the case of flour from buckwheat (inner layer and straight), and barley, for which the (protein × mineral) values was >4.80, the effect was inhibitory.

ACP Formation, its Transformation to HAP, and Induction Time After the Addition of Wheat Gluten and Buckwheat Rutin

As shown in Table **4**, at the concentration range of 12.5 - 125 μg/ml, wheat protein gluten inhibited the rate of trans-

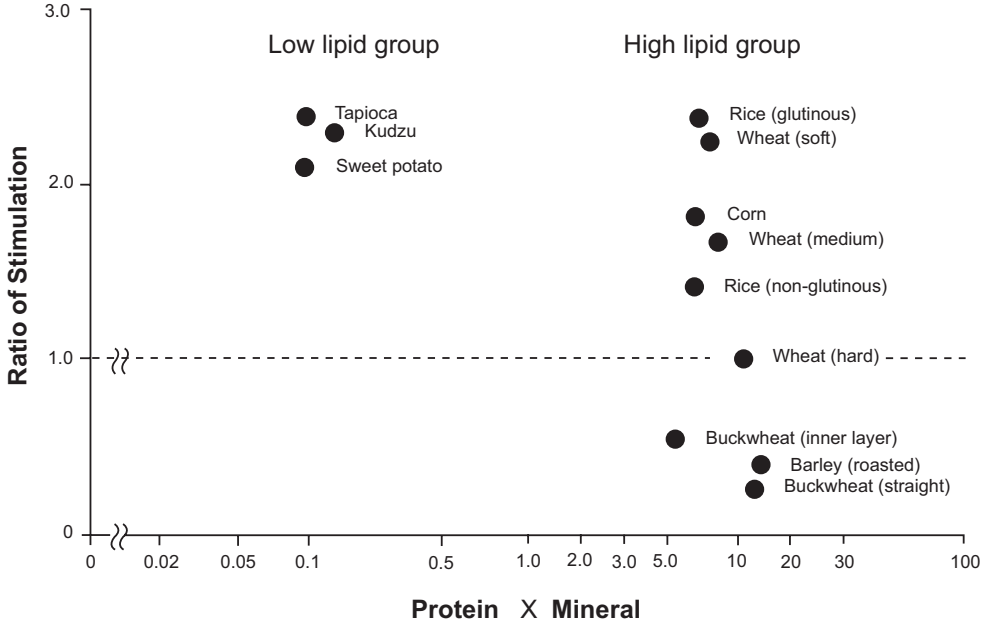

Fig. (5). Relationship between the ratio of stimulation (at the concentration of 2.0 mg/ml) and the protein content of dietary starch flour (See Table 2). First, dietary starch flour was divided into two groups; low and high lipid groups. High lipid group was further classified by the protein content into either stimulatory or inhibitory.

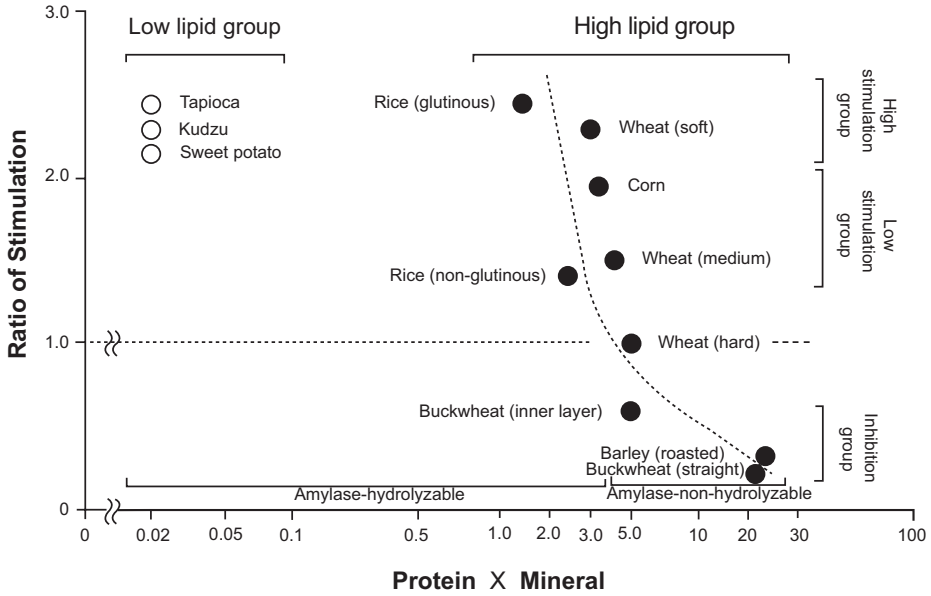

Fig. (6). Relationship between the ratio of stimulation (at the concentration of 2.0 mg/ml) and the value of the product (protein content × mineral content) of dietary starch flour (calculated from values in Table **2**). First, dietary starch flour was divided into two groups; low and high lipid groups. High lipid group was further classified in accordance with the value of (protein content × mineral content) into either stimulatory or inhibitory. At 4.68 on horizontal axis, where the value for wheat (hard) is located, the rate of stimulation was 1.0, namely, no effect. Furthermore, when the value is <3.3, the α-amylase treatment was effective, while when it is >3.6, the treatment was ineffective.

Table 4. Both gluten and rutin did not affect amorphous calcium phosphate (ACP) formation. Gluten affected both transformation of ACP to hydroxyapatite (HAP) and the induction time, but rutin only influenced transformation of ACP to HAP

Additives	Concentration used	Ca^{2+} consumption (ppm/min)		Induction
		ACP	HAP	time (min)
Gluten	0 μg/ml	123 ± 12	13.0 ± 1.3	14.8 ± 1.4
	12.5 μg/ml	124 ± 12	6.89 ± 0.57	22.7 ± 2.1*
	25 μg/ml	123 ± 13	3.77 ± 0.31*	37.2 ± 3.5*
	50 μg/ml	123 ± 12	2.60 ± 0.20*	56.1 ± 4.7*
	100 μg/ml	125 ± 12	2.34 ± 0.20*	81.0 ± 7.5*
	125 μg/ml	124 ± 12	2.21 ± 0.15*	102 ± 9.5*
Rutin	0.25 mg/ml	123 ± 12	12.7 ± 1.2	14.7 ± 1.4
	0.5 mg/ml	123 ± 12	10.5 ± 1.0*	15.1 ± 1.4
	1.0 mg/ml	125 ± 12	4.16 ± 0.35*	15.4 ± 1.4
	2.0 mg/ml	125 ± 12	3.64 ± 0.30*	14.8 ± 1.4
DMSO[a]	5% (v/v)	123 ± 12	13.5 ± 1.3	15.0 ± 1.4

The ACP formation, its transformation to HAP, and the induction time were measured by the pH drop method. The concentration of calcium and phosphate were 3 mM each. Additives were mixed with the reaction mixture 5 min before the addition of 3 mM phosphate. The final volume of assay solution, which contains 2 mM Hepes buffer (pH 7.4), was 2 ml. The reaction mixture was stirred at 37 ± 0.1°C. Values were converted to the rate of consumption of calcium (parts/10^6/min) [22]. Gluten (1.0 mg/ml) was solubilized with 1.0 ml of 1N NaOH. Then, the pH of the solution was adjusted to pH 7.4 with HCl. Rutin (40 mg/ml) was solubilized in dimethyl sulfoxide (DMSO).

[a]DMSO: Abbreviation of dimethyl sulfoxide.

*Significant difference ($p<0.05$) when compared to the control (no addition).

formation of ACP to HAP and elongated the induction time in a concentration dependent manner. At 125 μg/ml, gluten inhibited the transformation by 84% and elongated the induction time 6.9 times. The concentration required for 50% inhibition of the rate of transformation of ACP to HAP was 17 μg/ml. However, this compound did not affect the rate of ACP formation at all. At the concentration range of 0.25 - 2.0 mg/ml, buckwheat flavonoid rutin inhibited the rate of transformation of ACP to HAP with an increase of the concentration but it did not elongate the induction time. At the concentration of 2.0 mg/ml (3 mM), rutin inhibited the rate by 72%. The concentration needed for 50% inhibition of the rate of transformation of ACP to HAP was 700 μg/ml (1.1 mM). This compound also did not affect the rate of ACP formation at all.

α-amylase Hydrolysis of Sweet Potato, Wheat (Soft), and Corn Flour with Low Values for the Product (Protein × Mineral <3.3)

The values of (protein content × mineral content) for sweet potato, wheat (soft), and corn flour were 0.02, 3.2, and 3.3 (Table **1** and Fig. **6**). As shown in Table **5**, the hydrolysates of sweet potato, wheat (soft), and corn flour with α-amylase (10 μg/ml) inhibited the rate of transformation of ACP to HAP by 51, 37 and 34%, as compared to that before the treatment with α-amylase. However, the induction time of wheat (soft) and corn flour were not significantly changed by treatment with α-amylase. Both the stimulatory effect (on the rate of transformation of ACP to HAP) and the elongating effect (on the induction time) of standard corn starch (reagent grade) were abolished by α-amylase treatment (10 μg/ml) prior to the addition. The degrees of the effects caused by the α-amylase treatment of standard corn starch were similar to those of sweet potato.

α-amylase Hydrolysis of Wheat (Medium and Hard), Barley, and Buckwheat (Inner Layer and Straight) Flour with High Values for the Product (Protein × Mineral Content >3.6)

The values for wheat (medium), wheat (hard), barley, buckwheat (inner layer), and buckwheat (straight) were 3.6, 4.7, 23.8, 4.8, and 21.6, respectively (Table **1** and Fig. **6**). As shown in Table **6**, the α-amylase (10 μg/ml) treatment with either medium or hard wheat flour did not inhibit the rate of transformation of ACP to HAP significantly. In barley (roasted), buckwheat (inner layer), and buckwheat (straight), the treatment also did not inhibit the rate of transformation of ACP to HAP significantly. In all of these cases, the α-amylase treatment did not significantly change the induction time. Maltose (10.0 mg/ml; a product of α-amylase hydrolysis of starch) alone or α-amylase alone did not exhibit any effect.

The X-ray Diffraction Pattern from Tapioca, Rice (Glutinous), Wheat (Hard), and Buckwheat (Hard) Flour

The X-ray diffraction pattern was compared between the conditions of no addition and the addition of dietary starch flour. As the tested starch flours, tapioca flour of the low lipid and high stimulation group, rice (glutinous) flour of the high lipid and high stimulation group, wheat (hard) flour of high lipid and no effect group, and buckwheat (straight) flour of the high lipid and inhibition group or corn starch (standard) were used. The X-ray diffraction pattern from the precipitates in the control experiment had two major peaks (25.9°and 31.8°of the diffraction angle) typical to hydroxyapatite (A in Fig. **7**). The X-ray diffraction pattern of the addition of 2.0 mg/ml corn starch standard is shown in panel B of

Table 5. Flour from sweet potato, wheat (soft) and corn or corn starch after hydrolysis by α-amylase did not affect amorphous calcium phosphate (ACP) formation, but affected both transformation of ACP to hydroxyapatite (HAP) and the induction time

Flour or starch	Concentration used (mg/ml)	Ca^{2+} consumption (ppm/min)		Induction time (min)
		ACP	HAP	
None	0	123 ± 12	13.0 ± 1.3	14.8 ± 1.4
Sweet potato	2.0	123 ± 12	28.6 ± 2.5*	26.0 ± 2.5*
Sweet potato	2.0	121 ± 11	14.1 ± 1.3[#]	14.5 ± 1.4[#]
treated with α-amylase				
Wheat (soft)	2.0	124 ± 12	29.9 ± 2.5*	23.5 ± 2.1*
Wheat (soft)	2.0	123 ± 12	18.9 ± 1.7*[,#]	23.0 ± 2.1*
treated with α-amylase				
Wheat (soft)	2.0	124 ± 12	29.9 ± 2.5*	23.5 ± 2.1*
Wheat (soft)	2.0	123 ± 12	18.9 ± 1.7*[,#]	23.0 ± 2.1*
treated with α-amylase				
Corn	2.0	122 ± 11	24.1 ± 2.3*	27.0 ± 2.5*
Corn	2.0	122 ± 11	15.8 ± 1.3*[,#]	27.0 ± 2.5*
treated with α-amylase				
Corn starch standard	2.0	124 ± 12	29.4 ± 2.5*	26.6 ± 2.5*
Corn starch	2.0	124 ± 12	13.5 ± 1.3[#]	14.7 ± 1.4[#]
standard treated with α-amylase				

Details are the same as those shown in Table **4**.
*Significant difference ($p<0.05$) when compared to the control (no addition).
[#]Significant difference ($p<0.05$) between treatment and not-treatment with α-amylase.

Table 6. Flour from wheat (medium and hard), barley (roasted), buckwheat (inner layer and straight), and corn after hydrolysis by α-amylase did not affect amorphous calcium phosphate (ACP) formation, its transformation to hydroxyapatite (HAP), and the induction time

Flour	Concentration used (mg/ml)	Ca^{2+} consumption (ppm/min)		Induction time (min)
		ACP	HAP	
None	0	123 ± 12	13.0 ± 1.3	14.8 ± 1.4
Wheat				
Medium	2.0	124 ± 12	19.9 ± 1.7*	22.3 ± 2.0*
Medium	2.0	121 ± 12	18.6 ± 1.7*	21.1 ± 2.0*
treated with α-amylase				
Hard	2.0	124 ± 12	12.9 ± 1.0	21.5 ± 2.0*
Hard	2.0	125 ± 12	11.3 ± 1.0	21.2 ± 2.0*
treated with α-amylase				
Barley				
Roasted	2.0	124 ± 12	4.10 ± 0.35*	45.6 ± 4.3*
Roasted	2.0	124 ± 12	3.81 ± 0.35*	43.1 ± 4.0*
treated with α-amylase				
Buckwheat				
Inner layer	2.0	124 ± 12	8.19 ± 0.75*	26.6 ± 2.3*
Inner layer	2.0	123 ± 12	7.06 ± 0.70*	26.9 ± 2.3*
treated with α-amylase				
Straight	2.0	127 ± 12	3.38 ± 0.30*	49.7 ± 4.4*
Straight	2.0	124 ± 12	3.07 ± 0.30*	47.6 ± 4.4*
treated with α-amylase				
Maltose	10.0	124 ± 12	14.1 ± 1.4	13.9 ± 1.3
α-Amylase	0.01	125 ± 12	13.1 ± 1.3	14.0 ± 1.4

Details are the same as those shown in Table **5**.
*Significant difference ($p<0.05$) when compared to the control (no addition).

Fig. **7**. Two major peaks were still evident with a broad peak between 17 - 23°of the diffraction angle. Similar diffraction patterns were obtained for the addition of dietary starch flour of 2.0 mg/ml tapioca (C in Fig. **7**), 2.0 mg/ml rice (glutinous) (D in Fig. **7**), 2.0 mg/ml wheat (hard) (E in Fig. **7**), and 2.0 mg/ml buckwheat (straight) (F in Fig. **7**), although only the intensities from the addition of buckwheat were decreased (F in Fig. **7**).

DISCUSSION

Dietary starch flour, which is a powder product extracted from plant seeds or roots, is an important material in the human diet. The dietary flour used in this study contains 69.6 - 85.6% (w/w) of carbohydrate comprised of starch (Table **1**). Starch has long been a major component of the human diet. In Asia, starch components of the diet, particularly from rice, can be found in a variety of foods and constitutes a high percentage of total dietary carbohydrate. For example, in Japan, rice starch occupied 69% of total carbohydrate in 2002 (Ministry of Health, Labour and Welfare of Japan) [31]. Thus, starch may play an essential role for diet-related oral health.

Using twelve kinds of dietary starch flour, we performed the *in vitro* pH drop measurements and determined the effects on (i) the rate of ACP formation, (ii) the rate of transformation of ACP to HAP, and (iii) the induction time which is the onset time of transformation to HAP. This method was previously shown to be successful in distinguishing bovine bone gelatin from porcine skin gelatin [28]. The extent of the effect on the rate of transformation of ACP to HAP varied in a wide range, namely, from potent stimulation to potent inhibition. All types of flour from glutinous rice, tapioca, kudzu, sweet potato, and wheat (soft) formed one major group, with the highest stimulation by glutinous rice (Figs. (**2A**), (**3A**), and (**6**)). All types of flour from corn, wheat (medium), and non-glutinous rice formed the second group (low stimulatory group) (Figs. (**3A**), (**3B**), and (**6**)). Wheat (hard) flour had no effect. Barley and buckwheat flour (inner layer and straight) comprised an inhibitory group, called "inhibitory" because they inhibited the rate of transformation of ACP to HAP Figs. (**3B**) and (**6**)). From these results, it was found that not all of the types of dietary starch flour stimulates the rate of transformation of ACP to HAP. These results for dietary

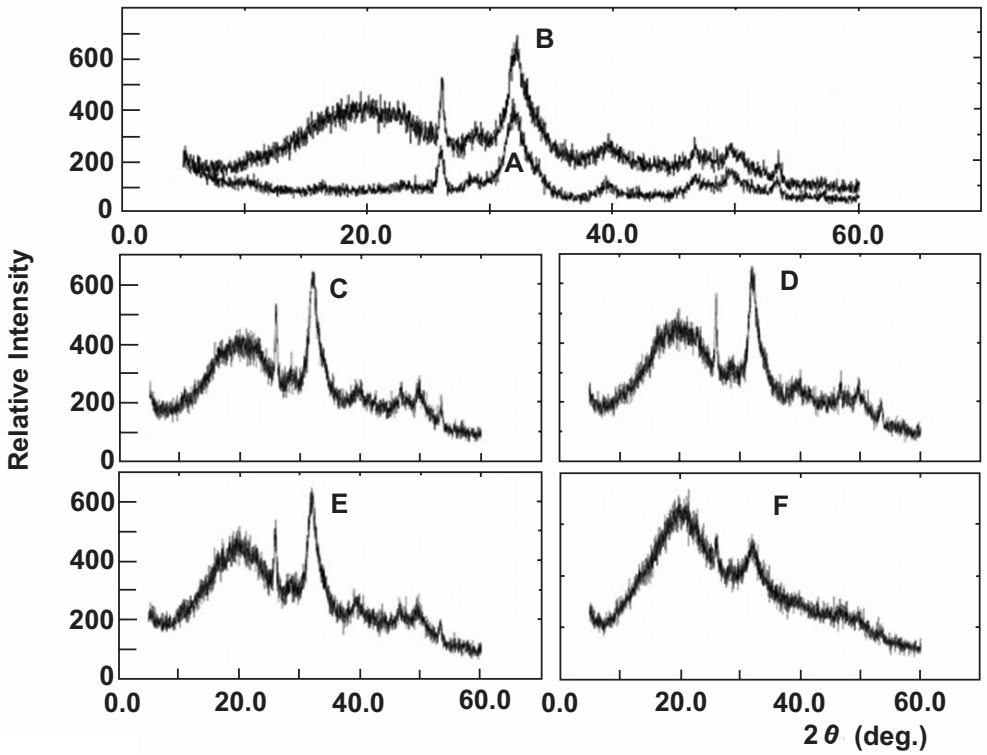

Fig. (7). X-ray diffraction patterns of calcium phosphate precipitates produced with (A) no addition, (B) 2.0 mg/ml corn starch (standard), (C) 2.0 mg/ml tapioca starch flour, (D) 2.0 mg/ml rice (glutinous) starch flour, (E) 2.0 mg/ml wheat (hard) starch flour, and (F) 2.0 mg/ml buckwheat (straight) starch flour. Three mM and 3 mM phosphate were used. Experimental conditions are the same as those in Table **4**.

starch products are much different from those for reagent grade starch [9].

The X-ray diffraction patterns between the conditions of no addition and the addition of either corn starch standard or the dietary starch flour of tapioca, rice (glutinous), wheat (hard), and buckwheat (straight) were similar (Fig. **7**). This finding seems to show that hydroxyapatite was produced as the result of the addition of each of the dietary starch flours. A broad peak with a diffraction angle of between 17 - 23°indicated an organic substance. The decreased intensities of the two major peaks in buckwheat flour (F in Fig. **7**) may be caused by this flour's inhibitory effect.

Since some of the dietary starch flours tested exhibited no stimulatory effect or even an inhibitory effect, we concluded factors other than carbohydrates must possess significant inhibitory effects. In other words, the lipids, proteins and/or ash (minerals), contained in dietary starch flour decreased the rate of transformation from ACP to HAP. These factors were not found in reagent grade starch. As shown in Table **1**, all types of flour contain a certain amount of protein. Starch flour from cereals contains more protein [6.0 - 12.5% (w/w)] as compared to tuberous and non-tuberous roots, both of which contain only 0.1 - 0.2% (w/w) of protein. The ash content, which reflects the mineral content, varied from 0.1 to 1.9% (w/w). As shown in Figs. (**5** and **6**), twelve kinds of dietary starch flour could be categorized by their lipid content, i.e. either below 0.2% (w/w) for sweet potato, kudzu, and tapioca, or approximately 1.0% (w/w) for the other nine flours. When the lipid content below 0.2% (w/w), starch had a 2.2 - 2.4 fold stimulatory effect on the transformation of ACP to HAP at the concentration of 2.0 mg/ml. Since standard corn starch (reagent grade) stimulation was 2.3 fold, it is apparent that the stimulation by these three types of flour is essentially equal to that of the reagent grade starch.

We found that gluten (a wheat protein) greatly inhibited the rate of transformation of ACP to HAP, even at a low concentration range (Table **4**). Therefore, it appears that the protein content in the wheat flour is the decisive inhibitory factor in the calcium phosphate precipitation, at least *in vitro*. Particularly, all three types of the wheat (hard, medium, and soft) and two types of buckwheat (straight and inner layer) flour which contain higher protein content (see Table **1**) increased the inhibitory extent on the rate of transformation of ACP to HAP (Figs. (**3A**) and (**B**)). Hard wheat flour exhibit no effect, because the inhibitory effect caused by the protein fraction was compensated by the stimulatory effect of starch. This indicates that the proteins in wheat and buckwheat flour might be a possible inhibitor on the *in vitro* calcium phosphate precipitation. Therefore, in types of starch with a high lipid content [lipid content around or above 1.0% (w/w)], we first attempted to find a relationship between their protein content and the rate of stimulation/inhibition on the transformation of ACP to HAP. However, as shown in Fig. (**5**), we did not find any clear relationship between these two parameters.

Consequently, we tested the hypothesis that the effect on the rate may be correlated with the value of the combined product of (protein content × mineral content). As shown in Fig. (**6**), a good relationship was found between the combination and the rate. When the value was 4.68 (hard wheat), there was no effect on the rate. Flour from rice (glutinous and non-glutinous), wheat (soft and medium), and corn, for which (protein ×mineral) is <4.68, had a stimulatory effect

on the rate of transformation of ACP to HAP, while flour from buckwheat (inner layer and straight), and barley, for which (protein ×mineral) is >4.68, exerted an inhibitory effect. Therefore, the value of the product of (protein content × mineral content) is suggested to be correlated with the effects on the rate of transformation of ACP to HAP.

Almost all of the proteins tested thus far, e.g. bovine serum albumin, casein, proteoglycans, phosphoproteins, and soybean flour, have displayed inhibitory effects on the formation of calcium phosphate precipitates [9,22,32,33]. Blumenthal [33] has reported the mechanism of inhibition of calcification by several different proteins: Proteoglycans exert their delaying action by a steric effect, serum proteins appear to slow the transformation of ACP to HAP by adsorbing on the ACP surface, and phosphoproteins adsorb on the surface of HAP crystals, blocking active growth sites. Certain metal ions are also known to inhibit calcium phosphate precipitation. Mg^{2+} is the most extensively investigated inhibitor. Zn^{2+}, Cu^{2+}, Fe^{2+}, and Mn^{2+} are also reported to have similar effects [23,34]. In terms of the monovalent ions, Na^{1+} has not been shown to cause any effects, while K^{1+} is reported to affect apatite formation [34,35]. Nine mineral elements, i.e. Na, K, Ca, Mg, P, Fe, Zn, Cu, and Mn are present in dietary starch flour (Table **2**). It is conceived that those minerals are able to influence the formation of calcium phosphate precipitates. Considerable amounts of these nine minerals are contained in flour from barley and buckwheat (straight), which were found to be extremely inhibitory of the rate of transformation of ACP to HAP. The content of K calculated as 0.63 and 0.52 mM and that of Mg as 0.27 and 0.39 mM at the concentration of 5.0 mg/ml of flour, respectively. It is reported that at the concentration of 250 μM, Mg^{2+} inhibited the rate of transformation by 30% and the induction time 1.7 times [23]. It has been suggested that K^{1+} and Mg^{2+} enter the structure of the forming HAP nuclei by replacing Ca^{2+} and thus distorts the atomic structure so that the growth of HAP is affected [33,34]. Therefore, the observed correlation between the content of protein × mineral and the rate of stimulation suggests that there may be a synergistic effect of proteins and metal ions.

Rutin has been reported to inhibit ovariectomy-induced osteopenia in rats [36]. However, Notoya *et al*. [37] reported that its aglycon (quercetin which is a flavonoid found in plant foods) reduced the rate of deposition of Ca^{2+} in the osteoblasts in bone tissue culture. Therefore, whether quercetin increases or decreases bone mass *in vivo* is controversial. The inhibition of the *in vitro* calcium phosphate precipitation by rutin (Table **4**) is unique, because there was no effect on the induction time, but the rate of transformation of ACP to HAP was markedly inhibited. Such characteristic effect by rutin may be elicited *in vitro* by an acidic phospholipid, phosphatidylserine (75 μM; Hidaka *et al.* [22]) which is involved in the calcium-phospholipid-phosphate complex in mineralizing tissues [38]. This suggests the possibility that rutin plays an important role in the regulation of tissue mineralization. However, in this study, rutin, which is a buckwheat flavonoid, did not influence the reaction as gluten did, perhaps because its content level is very low (19 - 168 μg/ml; Kreft *et al*. [39]) and because its inhibitory strength was only 1/41 that of gluten (See Results).

The rate of ACP formation was not affected by dietary starch flour (Table **3**). The rate of ACP formation has been shown to be decreased by chelating substances, e.g. pyrophosphate, adenosine triphosphate (ATP), ethyleneglycol-bis-(β-aminoethyether) N,N'-tetraacetic acid (EGTA), and the polyphenols contained in Chinese traditional (Kampo) medicines through the sequestration of calcium from the solutions [22,26]. Furthermore, bovine serum albumin also decreased the rate of ACP formation [22]. As described above, serum proteins bind to the ACP surfaces so potently they cannot be washed off. It appears this powerful adsorption may cause a slowing of the rate of ACP formation. Therefore, all types of dietary starch flour may contain neither chelating agents nor proteins which strongly adsorb onto ACP.

The α-amylase treatment of standard corn starch, sweet potato, corn, and wheat (soft) flour completely abolished their stimulatory effect on the rate of transformation of ACP to HAP (Table **5**). However, the same treatment did not change the inhibitory action of wheat (medium and hard), barley (roasted), or buckwheat (inner layer and straight) flour significantly (Table **6**). The results of these α-amylase effects can also analyzed in terms of our hypothesis, as follows. When the value of the product of (protein × mineral) is above 3.6 (See Fig. **6**), the enzymatic degradation of starch by α-amylase did not further increase the inhibitory effect on the transformation of ACP to HAP. When the value below 3.3, the α-amylase digestion abolished the stimulatory effects of starch on the transformation of ACP to HAP. This may be related to the work of Franken *et al*. [40] who reported a proteinous α-amylase inhibitor in wheat flour.

We previously proposed the mechanisms of action of α-amylase in oral diseases [9] as follows: In the case of low α-amylase activity, the oral reaction proceeds toward the direction resulting in oral calcification (calculus formation or remineralization). In this case, the calculus formation causes a periodontitis and remineralization repair of the decay in dental caries. In contrast, when the hydrolysis by α-amylase is extensive (in the case of high α-amylase activity), the oral reaction results in acid production followed by dental caries.

However, this previous scheme was developed for reagent grade starch. In the case of dietary starch flour, the influence of some factors other than just the starch which is hydrolyzed by α-amylase must be considered in the process of oral reaction. Thus, the previous mechanism needs a modification that should be compatible with actual dietary starch flour which contains both proteins and minerals. When the product of (protein × mineral) is below 3.3, a group in which the seven flours of tapioca, kudzu, sweet potato, rice (glutinous), rice (non-glutinous), wheat (soft), and corn were included, the α-amylase digestion abolished the stimulatory effects of starch on the transformation of ACP to HAP. Therefore, these seven types of flour appear equivalent to the case as seen in the previous scheme. However, for other five types of flour, of which the value of the product of (protein × mineral) is above 3.6, both proteases (that can hydrolyze protein) and chelators (that can bind minerals such as Mg^{2+}), have to be taken into account in the oral reaction. If proteases and chelators from oral bacteria and/or saliva were able to eliminate the influence of proteins and minerals, the oral reaction by the other five flour might

also proceed in accord with the previously proposed scheme that depends solely on α-amylase activity. However, if the effects of the proteases and chelators are inadequate to this task, the oral process would not follow the previous scheme. In such a case, wheat (medium) flour would promote the oral calcification to some extent and wheat (hard) would have neither a positive nor negative effect. However, dietary starch flour from both barley (roasted) and buckwheat (inner layer and straight) would exclusively inhibit oral calcification, because these flours would inhibit the rate of transformation of ACP to HAP.

CONCLUSIONS

In order to obtain a better understanding of the relationship between dietary starch and oral calcification, we studied the effects of twelve kinds of dietary starch flour on the *in vitro* formation of calcium phosphate precipitates. The starch flour from rice (non-glutinous and glutinous), tapioca, kudzu, wheat (soft and medium), sweet potato, and corn stimulated the rate of transformation of ACP to HAP. The flour from barley (roasted), buckwheat (inner layer and straight) inhibited it. Wheat (hard) flour had no effect. We attempted to search for the nutritional factors which are responsible for modifying the transformation of ACP to HAP in the different types of dietary starch flour. We developed a hypothesis that the value of (protein content × mineral content), rather than the protein content by itself, modifies the rate of transformation. According to our hypothesis, the greater the value is, the more the inhibition of the transformation of ACP to HAP takes place. Furthermore, this theory is able to account for the results of α-amylase treatment which we had previously reported.

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REFERENCES

[1] Sanders TA. Diet and general health: dietary counselling. Caries Res 2004; 38(Supple 1): 3-8.
[2] Sreebny LM. Sugar and human dental caries. World Rev Nutr Diet 1982; 40: 19-65.
[3] Sheiham A. Dietary effects on dental diseases. Public Health Nutr 2001; 4 (2B): 569-591.
[4] Lingström P, van Houte J, Kashket S. Food starches and dental caries. Crit Rev Oral Biol Med 2000; 11: 366-380.
[5] Featherstone JD. The continuum of dental caries – evidence for a dynamic disease process. J Dent Res 2004; 83 (Spec No C); C39-42.
[6] White DJ. Dental calculus: recent insights into occurrence, formation, prevention, removal and oral health effects of supragingival and subgingival deposits. Eur J Oral Sic 1997; 105: 508-522.
[7] Baer PN, Stephen RM, White CL. Studies on experimental calculus formation in the rats. I. Effect of age, sex, strain, high carbohydrate, high protein diets. J Periodont 1961; 32: 190-196.
[8] Baer PN, White CL. Studies on experimental calculus formation in the rats. X. The effect of various starches. J Periodont 1967; 38: 41-44.
[9] Hidaka S, Oishi A. An *in vitro* study of the effect of some dietary components on calculus formation: Regulation of calcium phosphate precipitation. Oral Dis 2007; 13: 296-302.
[10] Khush G.. Productivity improvements in rice. Nutr Rev 2003; 61: 114-116.
[11] Cock JH. Cassava: a basic energy source in the tropics. Science 1982; 218: 755-762.
[12] Osundahunsi OF, Fagbemi TN, Kesselman E, Shimoni E. Comparison of the physicochemical properties and pasting characteristics of flour and starch from red and white sweet potato cultivars. J Agric Food Chem 2003; 51: 2232-2236.
[13] Endo A, Okada S. Lactobacillus satsumamensis sp. Nov., isolated from mashes of shochu, a traditional Japanese distilled spirit made from fermented rice and other starchy materials. Int Syst Evol Microbiol 2005; 55: 83-85.
[14] Kitada Y, Sasaki M, Yamazoe Y, Nakazawa H. Measurements of the thermal behavior and amylose content of kuzu and sweet potato starches. J Jpn Soc Food Sci Technol 1988; 35: 135-140 (in Japanese).
[15] Keung WM, Vallee BL. Kudzu root: an ancient Chinese source of modern antidipsotropic agents. Phytochem 1998; 47: 499-506.
[16] Hoseney RC, Rogers DE. The formation and properties of wheat flour doughs. Crit Rev Food Sci Nutr 1990; 29: 73-93.
[17] Hou,G. Oriental noodles. Adv Food Nutr Res 2001; 43: 143-193.
[18] Sanchez-Marroquin A, Maya S. Industrial corn flour enrichment with whole amaranth flour and milling reactions in corn-based products. Arch Latinoam Nutr 1985; 35: 518-535.
[19] Dado RG. Nutritional benefits of specialty corn grain hybrids in dairy diets. J Anim Sci 1990; 2: 197-207.
[20] Tester RF. Influence of growth conditions on barley starch properties. Int J Biol Macromol 1997; 21: 37-45.
[21] Ikeda K, Kishida M, Kreft I, Yasumoto K. Endogeneous factors responsible for the textural characteristics of buckwheat products. J Nutr Sci Vitaminol 1997; 43: 101-111.
[22] Hidaka S, Abe K, Liu SY. A new method for the study of the formation and transformation of calcium phosphate precipitates: Effects of several chemical agents and Chinese folk medicines. Archs Oral Biol 1991; 36: 49-54.
[23] Okamoto Y, Hidaka S. Studies on calcium phosphate precipitation: Effects of metal ions used in dental material. J Biomed Mater Res 1994; 28: 1403-1410.
[24] Hidaka S, Abe K. The effects of sodium lauryl sulphate and its oxidative breakdown products on calcium phosphate precipitation and transformation. Archs Oral Biol 1992; 37: 159-165.
[25] Hidaka S, Okamoto Y, Abe K. Possible regulatory roles of silicic acid, silica and clay minerals in the formation of calcium phosphate precipitates. Archs Oral Biol 1993; 38: 405-413.
[26] Hidaka S, Abe K, Takeuchi T, Liu SY. Inhibition of the formation of oral calcium phosphate precipitates: beneficial effects of Chinese traditional (Kampo) medicines. J Periodont Res 1993; 28: 27-34.
[27] Hidaka S, Okamoto Y, Abe K. Elutions of metal ions from dental casting alloys and their effect on calcium phosphate precipitation and transformation. J Biomed Mater Res 1994; 28: 175-180.
[28] Hidaka S, Liu SY. Effects of gelatins on calcium phosphate precipitation: a possible application for distinguishing bovine bone gelatin from porcine skin gelatin. J Food Comp Analy 2003; 16: 477-483.
[29] Kagawa Y. Standard Tables of Food Composition in Japan; Fifth Revised and Enlarged Edition. 1st ed. Tokyo, Japan: Kagawa Education Institution of Nutrition Press 2005.
[30] Blumenthal NC, Betts SF, Posner AS. Nucleotide stabilization of amorphous calcium phosphate. Mater Res Bull 1975; 10: 1055-1060.
[31] Ministry of Health, Labour and Welfare of Japan, The Report of National Nutrition Survey in Japan, 2002, Tokyo: Daiichi Shuppan; 2002, pp. 30-31 (in Japanese).
[32] Termine JD, Peckauskas RA, Posner AS. Calcium phosphate formation *in vitro* II. Effects of environment on amorphous-crystalline transformation. Arch Biochem Biophys 1970; 140: 318-325.
[33] Blumenthal NC. Mechanisms of inhibition of calcification. Clin Orthop Rel Res 1989; 247: 279-289.
[34] LeGeros RZ. Calcium Phosphates in Oral Biology and Medicines. Basel Switzerland: S. Karger 1991.
[35] Wiesmann HP, Plate U, Zierold K, Höhling HJ. Potassium is involved in apatite biomineralization. J Dent Res 1998; 77: 1654-1657.
[36] Horcajada-Molteni MN, Crespy V, Coxam V, Davicco MJ, Remesy C, Barlet JP. Rutin inhibits ovariectomy-induced osteopenia in rats. J Bone Miner Res 2000; 15: 2251-2258.
[37] Notoya M, Tsukamoto Y, Nishimura H, Woo JT, Nagai K, Lee IS, Hagiwara H. Quercetin, a flavonoid, inhibits the proliferation, dif-

ferentiation, and mineralization of osteoblasts *in vitro*. Eur J Pharmacol 2004; 485: 89-96.

[38] Boskey AL. The role of calcium-phospholipid-phosphate complexes in tissue mineralization. Metab Bone Dis Rel Res 1978; 1: 147-142.

[39] Kreft S, Knapp M, Kreft I. Extraction of rutin from buckwheat (Fagopyrum esculentum Moench) seeds and determination by capillary electrophoresis. J Agric Food Chem 1999; 47: 4649-4652.

[40] Franken J, Stephan U, Meyer HE, Konig W. Identification of alpha-amylase inhibitor as a major allergen of wheat flour. Int Arch Allergy Immunol 1994; 104: 171-174.

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