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Cariogenic Potential of Starchy Foods

전분식품의 치아우식유발력에 관한 연구

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Graduate School

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Abstract

Cariogenic Potential of Starchy Foods

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Starch is a common source of fermentable carbohydrates. It's slow absorption and degradation make it superior among other low molecular carbohydrates. Yet, current studies reflect incoherent claims about the cariogenic potentiality of starch.

An evaluation analyzing the correlation of microbial and various physiochemical factors and demineralized quantity (radioisotope ^{32}P) which was recently developed and introduced using polyacrylamide hydroxyapatite disc (PAHA) was performed through 11 starchy foods to determine the cariogenic potentiality *in vitro* affecting dental caries.

Test subjects (11 starchy foods) were treated and prepared specified by a modified method from the Association Official Analytical Chemists (AOAC). Subjects included total 5 group of 11 starchy foods, measured both physiochemical & microbial factors; moisture content, total starch, hydrolyzed starch, pH, titratable acidity, total sugar, reducing sugar, texture,

and total viable cell count after inoculation of *S.mutans* and demineralized quantification and degree of radioisotope ^{32}P using PAHA; liquid scintillation count, scanning electronic microscopy and confocal laser scanning microscopy.

Pearson correlation and stepwise regression were performed as a statistical evaluation to analyze the caries-associated variable by SPSS software for Windows (version 23.0, SPSS Inc., Chicago, IL, USA) and IBM Watson Analytics (cognitive analytics).

The total average of moisture content, starch, hydrolyzed starch, pH, titratable acidity, total sugar, reducing sugar, TPA (hardness, springiness, cohesiveness, chewiness and adhesiveness) and total viable cells after inoculation of *S. mutans* in 11 test foods were 32.3%, 67.4%, 9.3%, 5.8, 0.38%, 245.1 mg/g, 17.5 mg/g, 2409.0, 0.57, 0.43, 621.5, -38.8 and 2.22×10^6/ml.

With Pearson correlation coefficients (r) of caries-associated variables, including total starch, hydrolyzed starch, titratable acidity, reducing sugar and TPA results, including hardness, cohesiveness, chewiness, adhesiveness were significant at $p < 0.001$ and pH, total sugar and springiness (TPA) presented $p < 0.05$ significance. Hydrolyzed starch, adhesiveness, total viable cells of *S. mutans*, moisture content and titratable acidity affected cariogenic potentiality significant at $p < 0.001$, reducing sugar presented $p < 0.01$ significance, springiness significant at $p < 0.05$ and an adjusted R^2 at 0.904 showed $p < 0.05$ significance, analyzing caries-associated variable factors by stepwise regression analysis.

Caries associated multivariate analysis was authorized to assess cariogenic potential of starchy foods. Through statistical validation,

quantifying demineralization *in vitro* by measuring radioisotope ^{32}P released from PAHA disc model showed a significance with other physiochemical factors.

With PAHA, this study managed to quantify the starch-induced demineralization *in vitro*. While the method holds limited information than *in situ* or *in vivo* model in terms of providing oral physiological process, it standardized the various types, proportion and characteristics of both individual or bovine models and the PAHA disc was the best candidate. As a standardized matter, which is commonly totaled as enamel compound due to chemical resemblance, it clearly presented a reliability and reproducibility throughout the research. Finally, this data was contributed to the national oral health to mark the cariogenic potential index in starchy foods.

Keywords: Cariogenicity, Confocal laser scanning microscopy (CLSM), Polyacrylamide hydroxyapatite (PAHA), Reducing sugar, Starch, Texture Profile Analysis (TPA)

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List of Abbreviations

ANOVA: one-way analysis of variance

AOAC: association of official analytical chemists

CFU: colony forming per unit

CLSM: confocal laser scanning microscopy

CPI: cariogenic potential index

CPM: counts per minute

DW: distilled water

GI: glycemic index

HI: hydrolytic index

II: insulin index

KRIBB : Korea research institute bioscience & biotechnology

Na-CMC: sodium-carboxymethylcellulose

OD: optical density

PAHA: polyacrylamide hydroxyapatite

QDA: quantitative descriptive analysis

Ra: roughness arithmetic mean height

Rc: mean height of the roughness profile elements

Rku: roughness kurtosis

Rmax: roughness maximum depth

Rp: roughness maximum peak height

Rq: roughness root means square height

Rsk: roughness skewness

Rt: roughness total height

Rv: roughness maximum valley depth

Rz: roughness maximum height of the surface

Sa: surface arithmetic mean height

Sc: mean height of the roughness profile elements

SEM: scanning electronic microscopy

Sku: surface kurtosis

Smax: surface maximum roughness depth

S. mutans: *Streptococcus mutans*

Sp: surface maximum peak height

Sq: surface root mean square height

Ssk: surface skewness

St: surface total height

Sv: surface maximum valley depth

Sz: surface maximum height of the surface

TEMED: N, N, N´, N´- tetramethylethylenediamine

TPA: texture profile analysis

VHI: Vickers hardness index

W/V: weight/volume

Literature Review

Starch is one of the largely dispersed dietary carbohydrates with different types, textures, the degree of hydrolysis. This polymeric molecule is considered to be nutritionally superior to other low-molecular-weight carbohydrates (mono- and disaccharides) in comparison, due to relatively slow degradation and absorption (Holm *et al*., 1988). Generally, dietary starch refers to an extensive range of processed and manufactured foods with varied composition and components. Thus, further research assessing cariogenic potential of starch should be based on single starch food to avoid such disparities. The potential of caries development based on starch has been subjected for past decades (Grenby, 1996; Lingström, 2000; Moynihan, 1998 & 2002; Rugg-Gunn, 1993). Synergistic cariogenic influence of starch and sucrose has shown coherent elaboration, while the impartial role of the starch has been controversial (Firestone, 1982; Ribeiro *et al.*, 2005; Shaw & Murray, 1980).

In etiological perspective, there are 5 factors that are considered to be required for starch-caries development: the amount and frequency of exposure, its bioavailability, co-cariogen effect, microbial flora, plaque-pH degrading capacity, the effect of hyposalivation and their impact on root caries. Throughout the evaluation of frequency and amount factor, human intervention studies have shown a limited and unequivocal records associated to cariogenicity, therefore, required an exemplification of cases. In animal studies, it was noted that starch has comparatively lower cariogenic than

sucrose (Moynihan, 1998; Rugg-Gunn, 1993). Both the presence of an acidogenic plaque and salivary amylase induced digestion were reported as a major catalyst of starch-induced cariogenicity throughout the *in vitro* studies (Brudevold *et al.*, 1988). Several *in vitro* experiments shared the view focused on the role of degradable starch regarding sucrose-induced *S. mutans* glucan synthesis and microbial adherence (Balekjian *et al.*, 1980; Gibbons & Newbrun, 1977; Nygaard, 1970; Vacca-Smith *et al.*, 1996a & 1996b).

The starch-caries issue has been considered to be rather stochastic till this day, yet several hypotheses have refuted its safety to oral health. The necessity of such assessment is unlikely to be changed in foreseeable future, therefore, a comprehensive method to estimate the possibility of starch-inducive caries development is required.

I. Starch

The total consumption of carbohydrates has increased during the past years and in 1991 reached 356 g/person/day in Sweden, of which starch made up 55%. Other common dietary carbohydrates are sucrose, lactose, glucose, and fructose. The consumption of starchy products, for example, potato chips and cheese doodles, has increased in 1991. In western, starch exists as a wheat form used in diverse food with substantial proportion within dietary carbohydrates. This phenomenon was observed both in Michigan, USA and UK making up almost 50% of total carbohydrate (Burt & Szpunar, 1994; Rugg-Gunn *et al.*, 1986).

Occurrence and Structure

Starch is synthesized by plants and its slow absorption and degradation characteristics were renowned as nutritionally superior to other low molecular weight carbohydrates including monosaccharides and disaccharides (Holm *et al.*, 1988). Depending on the species, the starch may be observed as a varied form of granules which is water-insoluble, range from 1 to 100 μm in diameter (Würsch, 1989). It is polymer structure (α-D-glucose) mainly constituted with amylose and amylopectin which is packed with a high molecular order with compact hydrogen bonding. In raw plants, when exposed to either heat, pressure or mechanical stress, the gelatinization initiates. Through this stage, an irreversible destruction of the crystalline structure within granules become a presence. It may even rupture, fractures and ultimately become soluble (Colonna *et al.*, 1992). Wheat starch

gelatinizes in the temperature range 58-70˚C. Under low water conditions, elevated temperatures and pressure are necessary to obtain partial or complete gelatinization. Thus, the melting temperature increases as the water content is reduced (Eliasson *et al.*, 1980).

The processed mostly used, when partial or complete gelatinization may take place, are boiling and baking. Examples of commonly consumed products which have undergone this treatment are bread, potato, and rice. In the food industry, several thermal processes are used for pre-cooking of cereal grains and flours. Pre-cooked cereal products for human consumption include, for example, instant gruel and porridge, breakfast cereals, chips, bread, and snacks. Examples of different industrial heat treatment methods resulting in starch solubilization are popping, extrusion cooking, steam-flaking and drum-drying. Products that are extrusion cooked are, for instance, breakfast cereals and cheese doodles and examples of drum-dried products are baby foods like porridge and gruel. Popping is the traditional way of making popped cereals. The steam-flaking process is used for breakfast cereals like cornflakes. All processing modifies the starch to a different extent. After the heat processing, total sugar and adhesiveness have increased compared to raw food, in general.

Enzymatic Availability

α-amylase hydrolyzes the α-1,4-linkages between the glucose molecules, which results in branched and linear malto-saccharides of various degrees of polymerization. The major end-products after complete hydrolysis are maltose, maltotriose, and dextrins (Colonna *et al.*, 1992; Würsch, 1989). Only

small amounts of glucose are formed.

The enzymatic availability of starch is interest of the oral cavity to the colon. Starch is absorbed in the intestines either slowly or rapidly, depending on the structure and treatment of the food product. The molecular weight is minor importance, while other factors affecting the enzyme availability of starch are the physicochemical properties of the food (Holm *et al.*, 1988; O'Dea *et al.*, 1981).

Starch hydrolysis in decomposition stage is initiated in saliva by α-amylase, and can be continued in the plaque after diffusion of different products, where the process is catalyzed by salivary α-amylases and different bacterial enzymes, such as α-glucosidases, glycolytic enzymes, and any bacterial amylases (Birkhed & Skude, 1978; Ruby & Gerencser, 1974; Tatevossian & Newbrun, 1979). After hydrolysis into maltose, maltotriose and glucose, the starch can be used for fermentation by oral bacteria. Certain plaque bacteria produce extracellular amylases, but these are probably of minor importance (Ruby & Gerencser, 1974).

II. Cariogenic Potential of Starch

In terms of bioavailability, only the gelatinized starches are inclusive since they are susceptible to enzymatic breakdown. Starch hydrolyzes into maltose, low-molecular weight dextrins and maltotriose by bacterial amylases and salivary (Mörmann & Mühlemann, 1981). With rapid initiation, entrapped food particles within dentin can produce substrates for acid

production (Imfeld, 1983; Neff, 1967). Under certain condition fermentation of maltose requires bacterial adaptation (Kashket *et al.*, 1996). The effects of food processing were obtained in systemic studies including *in vivo* pH response of plaque (Björck *et al.*, 1984a & 1984b). Severe suspended wheat flour processing conditions such as steam-flaked, dry-autoclaved, extrusion-cooking, drum-drying, popping induced plaque acidogenicity as mentioned order. Boiling also induced a consistent degree of gelatinization with the pH drop. *In vitro* starch hydrolysis was correlated with these pH drop carried by α-amylase (Björck *et al.*, 1984a & 1984b).

The bioavailability of starch has been significantly demonstrated in systemic response research. A range of diverse starchy foods differed in blood glucose and insulin response. The breakdown of consumed starch transformed into maltose and glucose affecting the blood sugar level, glucose in specific. The well-established method for ranking starchy foods by systemic response is the serum insulin level, and the glycemic index in particular (Jenkins *et al*., 1981). Granfeldt *et al*. (1995) suggested that certain foods with partially gelatinized can elicit a high GI. In other terms, indices for an incomplete gelatinization of starch also need to be concerned. Factors, including starch-protein, food texture, starch-lipid interactions, and fiber content have also been reported as moderator of bioavailability of starch, in further contributed to systemic response indexes (Björck *et al.*, 1984a & 1984b). While the variation of starch regarding bioavailability have concrete evidence in medical field including the systemic responses, the subjects has been unrecognized by the dental research even with substantial evidence

concluded that whether the processing method, modification and composition it clearly represented a difference in bioavailability, and presumably the cariogenicity (Foster-Powell & Miller, 1995; Granfeldt *et al.*, 1991 & 1992; Jenkins *et al*., 1981; O'Dea *et al.*, 1981).

The presence of acid production that induced by fermentable sugars are not limited to bioavailability but also to the frequency and the retentiveness of starch. Such developed and westernized demographics exhibited a frequent consumption of starch in addition to regular meals. In this case, such diets are composited with a mixture of sugar and starch rather than in raw formation (Holm *et al.*, 1975; Ismail, 1986; Martinsson, 1972). Throughout the recent decennial between-meal intake of starch has grown exponentially throughout the diet (Bibby & Grimble, 1990). Increased adhesiveness of starch is also discussed as a major contributor of cariogenic potential of starch, though past studies were based on the retention in the whole mouth rather than dentin itself (Bibby *et al.*, 1951; Bibby & Ludwig, 1957; Caldwell, 1970; Gustafsson, 1954). Recent findings suggested that the retention was more adaptive in high starch content foods than the foods with relatively low content of starch. For instance, potato chips indicated a substantial amount of maltose and maltotriose after the consumption. The significant difference between the retention through starch breakdown and the caries development was detected by Lingström *et al.* (1993).

III. Methods for Assessment of Cariogenicity

There have been many attempts to devise a feasible method for assessing the cariogenic potential of foods. The methods used to test the cariogenicity or the caries-promoting properties of different food products, apart from the methods decided at the San Antonio conference (Curzon *et al.*, 1986).

Animal Tests

Animal studies have been widely used during the years. In pathogenesis viewpoint, there are some evident studies which cover that dental caries is essentially the same, both in humans and animals (i.e. rat). Other commonly used animals are hamsters, mice, and donkeys. Substances that promote stances that prevent caries in humans also prevent caries in rats.

Demineralization Models

The ability of organic acids in plaque to cause enamel and dentin demineralization and to produce caries-like lesions *in vivo* has been used by many researchers (Brudevold *et al.*, 1985; Koulourides *et al.*, 1976). Different radiographic methods, including longitudinal, transversal and wavelength-independent x-ray microradiography, represent the only methods for direct analysis of de- and remineralization. Methods like polarized light photo-microscopy, microhardness, and iodide permeability provide indirect measures of mineral flux in enamel and dentin (Arends & ten Bosch, 1992).

Human Plaque-pH Methods

Different methods for studying the process of bacterial fermentation *in situ* have been used since Stephan (1940) made the first attempt to measure acid production in dental plaque. The basic technique for studying the pH response has since then undergone major development and today widely used by researchers all over the world (Rugg-Gunn *et al.*, 1981). The main feature of the plaque-pH model is its benefit carrying evidence of cariogenic potential for different food products in human subjects under normal conditions. Many host factors are involved in this process. Therefore, a plaque-pH curve is the result of both different host factors and factors within the food (Edgar, 1976; Kleinberg *et al.*, 1982).

***In vitro* Studies**

The number of tests has been suggested. The *in vitro* methods address three main factors: i) the inherent properties of food, ii) the ability of food ingredients to support bacterial and plaque growth and to reduce pH, and iii) de- or remineralization (Clarkson *et al.*, 1986). A prolonged incubation of test substrates with saliva and measurement of acid production were performed as a part of *in vitro* studies (Beck and Bibby, 1961; Bibby *et al.*, 1951). Also Bibby. showed the wide range acidogenesis of different types of starches and starchy foods through the exposure of harvested human plaque to a solution of the test substrate at 37°C for 30 minutes (Bibby & Krobicka, 1984). Raw starch (i.e. wheat, corn, potato starch) showed the minimum plaque pH values.

Human Clinical Studies

These can be divided into observational studies and clinical trials. In observational studies, the relationship between the occurrence of the disease and possible causative or preventive factors is recorded. Time factor and the total diet control in ethical aspects are main difficulties within clinical trials. An intra-oral method showed that raw wheat starch-induced negligible amount of demineralization, while cooked wheat starch gels showed significant pH drop and demineralization. (Brudevold *et al.*, 1985). Lingström *et al*. (1994) and Pollard (1995) measured the demineralization by variation in microhardness or microradiography using human enamel slabs of human enamel or dentin covered by plaque formation. This research demonstrated that starchy foods (i.e. bread, corn flakes, bran flakes, boiled rice, and spaghetti) significantly induced enamel demineralization followed by the immersion of enamel slabs.

1. Introduction

The process of enamel breakdown, resulting in a caries defect, can be described as a series of physicochemical events occurring at or in the immediate vicinity of the enamel/oral fluid interface. Whether the substance interaction works through direct or indirect manner, the types of food, texture, composition, solubility, retentiveness and method of usage have been considered as an effective interference regarding caries-process mechanisms.

The effect of food type, composition, texture, retentiveness, solubility and method of usage have been reported as an agent which interferes caries-process mechanisms in different ways, including direct and indirect approaches of the substances. It is the reason that current research regarding the cariogenic potential of food, view as a stochastic problem rather than the direct correlation between triadic (fermentable dietary carbohydrate, microbial acid production and demineralization of enamel) correlation. Among these major contributors, dietary carbohydrate is closely related to environmental aspect, a diet in specific.

As a major source of dietary carbohydrate, starch was considered as non- or slightly cariogenic (Lingström *et al.*, 2000). In general, starch has been suggested as less cariogenic compared to sugar; however there has been a controversial since its inception (Burt, 1994; Edmondson, 1990; Kalsbeek & Verrips, 1994; Newbrun, 1967; Rugg-Gunn, 1990; Sreebny, 1983; van Houte, 1980; van Palenstein Helderman *et al.*, 1996). This is quite the contrary to sucrose's position, which holds concrete evidence of being major fermentable

carbohydrates which directly affect acid production. Through the incubation of a microorganism (capable of demineralizing tooth enamel) inoculated bread-saliva mixture, an acid production was observed by Miller in 1890. As a result, he suggested the cariogenic potential of starch, more capable than sugar (Miller, 1890). However, since several types of research took starch as cariogenic matter, led general conclusion of starch as less cariogenic substrates than sucrose; experiments on dental biofilm acidogenicity (Imfeld & Mühlemann, 1977; Lingström *et al.,* 1989; Stephan, 1940), animal studies (Bowen *et al.*, 1980 & 1990; Green *et al.*, 1967; Hefti & Schmid, 1979; König *et al.*, 1965), controlled studies in human (Gustafsson, 1954), epidemiological data (Fisher, 1968; Marthaler, 1967; Newbrun, 1980) and *in situ* experiments (Lingström *et al.,* 1994). This observation compromises several inquiries mentioned above. In general, dietary starch often referred as co-cariogen, blocs with sucrose which lead to greater cariogenicity reside in dental biofilm formation (Lingström *et al.,* 2000). As an agent of altering biofilm matrix, highly concentrated insoluble polysaccharides stimulate the degree of cariogenic potential when sucrose in presence as biofilm form (Dibdin & Shellis, 1988). This would result in the accumulation of strong, cohesive, and adherent biofilms on dental surfaces. Accordingly, *S. mutans* showed an adhesive propensity to hydroxyapatite *in vitro* regarding the presence of polysaccharides which may elucidate the physiognomies of retentiveness (Vacca-Smith *et al.,* 1996a & 1996b). Prior research including mentioned above, mostly covered not more than trilateral factors affecting cariogenicity. An extensive analysis was performed to assess cariogenic potential of starchy

foods as multivariate manner *in vitro*.

Therefore, this study provided *in vitro* method using polyacrylamide hydroxyapatite disc (PAHA) to quantify starchy food induced demineralization. While *in vivo* techniques may benefit from a synchronic performance, it is inevitable to compromise with controlling parameters, inter- and intra-individual variations compared to *in vitro* methods. A deployment of polyacrylamide hydroxyapatite disc (PAHA) was vital to this research as a standardized matter of *in vitro* experiment with high reliability and reproducibility. In turn, a whole process including physicochemical observation and the method itself was to provide the purpose of this research; to revitalize and corroborate the cariogenic potential of modern dietary starch. Finally, this study represents the utilization of effective prevention data in dental caries and contribution to national oral health, as to standardize caries potential index of starch-containing foods.

2. Material & Method

2.1 Materials

2.1.1 Foods

Based upon Food Logistic Almanac, total 11 starchy foods were selected and purchased (Table 1).

Table 1. Classification of Starchy Foods

Category	Items
Potatoes	Baked potato Baked sweet potato
Chips & cookie	Potato chip Baked potato chip Butter cookie
Bread	White bread Sponge cake Doughnuts
Cereal	Cornflakes
Fruits	Apple Banana

2.1.2 Artificial Saliva

For artificial saliva, including Na-CMC (sodium-carboxymethylcellulose), glycerin, methyl 4-hydroxy benzoate was acquired from Sigma-Aldrich (Sigma-Aldrich Chemical Co., St. Louis, MO, USA), Shinyo Pure (Shinyo pure chemical Co., Japan), Junsei (Junsei chemical Co., Japan).

2.1.3 Strain

S. mutans (ATCC 25715) was supplied by the Korea Research Institute Bioscience & Biotechnology, cultured aerobically in BHI broth and BHI agar which is purchased from Becton Dickinson (Difco Laboratories, Detroit, MI, USA) supplemented with 1% sucrose and 0.2 units (U) ml^{-1} of bacitracin at 37% for 24 hours rotations.

2.1.4 Hydroxyapatite Disc

To simulate enamel crystalline with hydroxyapatite disc, an acrylamide gel, bis-acrylamide, ammonium persulfate, N, N, N´, N´- tetramethyl ethylene diamine, and hydroxyapatite suspension (suspension in 0.001M 100g w/w, phosphate buffer, pH 6.8, solid content 27%) were purchased from Sigma-Aldrich (USA).

2.2 Methods

2.2.1 Preparation of the Foods

11 starchy foods were classified into 5 types of subject: potatoes (potatoes and sweet potatoes), chips & cookies (potato chips, baked potato chips, and butter cookies), bread (white bread, sponge cake, and doughnuts), cereal (cornflakes), and fruit (apples and bananas). As a control, 10% sucrose solution was used. A modified method defined by Association of Official Analytical Chemists (AOAC) was utilized for preparation before the evaluation. The procedure involved a titration, a reservation at 110˚C for 18 hours. Excessed fat at the degree of 5% of total content was removed. With 25 ml of ether, 5 g of processed samples were centrifuged twice at 1,800 rpm for 15 minutes. Supernatant was discarded with Whatman® qualitative filter paper No.2 (Sigma-Aldrich, USA).

2.2.2 Physico-chemical Factors

2.2.2.1 Starch

Total Starch Content

The method described by Goñi *et al.* (1997) was employed to determine the total starch content. For 30 minutes a 50 mg sample was shaken vigorously. In addition of 2 moles of KOH was followed. Shaken in a water bath containing 1 ml amyloglucosidase (300 U/ml) at 60˚C for 45 minutes. The enzymatic reaction was suppressed by cooling at 10˚C for 10 minutes in a water bath. By using the ABTS method (2,2'-azino-bis (3-ethylbenzthiazoline

-6-sulfuric acid)), free glucose content measurement was offered. 1 ml of the sample solution was added to a 0.12 M phosphate buffer solution, 250 ml melted glucose oxidase, 6 mg of peroxidase, 50 mg of ABTS and 5 ml of supernatant. A spectrophotometric analysis was then performed (DU-650 spectrophotometer, Beckman, Shimadzu, Japan) at 450 nm followed by 30 minutes of arrangement at room temperature. The standard curve was measured by using glucose solution as a standard. The total starch was calculated as glucose × 0.9.

Starch Hydrolysis

Hydrolyzed starch was also quantified using the Goni *et al.* (1997) method. In addition of α-amylase (40 mg/ml) the sample was shaken at 37°C. In a boiling bath water at 100°C, the solution took 5 minutes stand. Then it was cooled until it became 60°C and shaken for 45 minutes before 80 μl (140 U/ml) of amyloglucosidase was added. 1 ml of the supernatant was transferred to a medium of suppressing reaction in every 30 minutes. The ABTS method was used to measure the glucose content in triplicate. After standing 30 minutes at room temperature, a spectrophotometer (Japan) analysis was implemented at 450 nm. Using glucose solution as standard, the standard curve was measured. The total starch was calculated as glucose × 0.9.

2.2.2.2 pH

The pH of the test foods was measured using the Goni *et al.* (1997) method. A digital portable pH meter (Orion model 230A, Thermo Scientific Inc., Beverly, MA, USA) was used for pH assessment at room temperature. Before the measurement, the pH electrode (2A14-KA Analyzer, São Paolo, SP, Brazil) was calibrated using standard buffers of pH 4.0 and 7.0. Each sample measured 10 times repeatedly.

2.2.2.3 Titratable Acidity

Each 10 ml of samples were diluted as directed by the distilled water (DW) 10 ml. The volume of 0.1 N sodium hydroxide as a requirement of neutralizing test foods was calculated to estimate titratable acidity (titration point: pH ± 0.05). Titration was continued until a pH of 7.0, then attained. Each sample's titratable acidity was then expressed with the mole of hydroxyl ions/liter (required to bring the diluted sample from its original pH to neutrality). This was taken as an indication of the buffering potential of the test foods concerned. Each sample measured repeatedly for 10 times.

$$\text{Organic acid (\%, w/v)} = \frac{V \times A \times F \times D}{S} \times 100$$

S : Sample
V: Titrate volume of 0.1 N-NaOH solution (ml)
A: Organic acid volume equivalent to 1ml of 0.1N-NaOH
(lactic acid: 0.009, citric acid: 0.0064)
F: Factor of 0.1 N-NaOH
D: Dilution mutiple

2.2.2.4 Total Sugar

To a 10 ml test tube, 1 ml test foods were added to 1 ml of water containing carbohydrate 1 ml of 5% phenol in 0.1 M sulphuric acid 5 ml cautiously. Right after, vigorously mix the test tube on a vortex mixer and stand to cool at 25–30°C for 20 minutes. Along with 10% sucrose control, all samples were processed in duplicate. The absorbance values were read by spectrophotometer at 490 nm against a reagent blank. Color responses were at the approximate value of glucose 0.69/40 μg. Every sample was measured 10 times repeatedly.

2.2.2.5 Reducing Sugar

Prepared 4 solutions: A solution (low alkalinity reagent), B solution (30 g $CuSO_4 \cdot 5H_2O$ mixed 200 ml DW and added H_2SO_4 4 drop), C solution (Arsenomolybdate reagent: 25 g ammonium molybdate mixed 3 g sodium bicarbonate), D solution (25 ml A solution mixed 1 ml B solution). Sample 1 ml added D solution 1 ml and heated water bath for 20 minutes. Then cooling for 20 minutes, C solution was added then shook vigorously and stand 20 minutes. After adding 25 ml DW and observed color development (blue) by spectrophotometer (USA) at 520 nm (standard curve: glucose). Each sample measured repeatedly for 10 times.

2.2.2.6 Texture

Rheology property of test foods measured textural analysis (Table 2). A texture analyzer (TA-XT 2, Stable Micro Systems Ltd., Surrey, UK) in texture profile analysis (TPA) mode was utilized. Test samples were positioned in the platform and measured by TPA parameter (circular probe: diameter 2.5 cm × thickness 4.2 cm). At a defined depth (15 mm) and different rates (2, 4, 6, 8, 10 mm s^{-1}) an analytical probe was compressed twice, into each formulation. Carri-Med CSL^2-100 rheometer was used with parallel plate geometry under shearing control at 20.0 ± 0.1°C gauging flow rheograms. Each sample measured for 10 times.

Table 2. Texture Analyzer Set Up Condition Used to Measure the Food Texture

Options	**Texture profile analysis (TPA)**
Pre-test speed	2.5 mm/s
Test speed	5.0 mm/s
Post-test speed	2.5 mm/s
Strain	80%
Trigger type	Auto-100g

2.2.3 Microbial Factor

2.2.3.1 Establishing the Pertinent Incubation Time for *S. mutans*

S. mutans (ATCC 25715) was supplied by KRIBB and cultured in BHI with supplements (1% sucrose and 0.2 units (U) ml^{-1} bacitracin at 37°C for 24-hour rotations) aerobically. The optical density (OD) was measured with a spectrophotometer (Japan) at 30 minutes intervals, after the inoculation (1 μl of *S. mutans* in 10 ml of BHI broth). Incubation was set to 150 minutes. By measuring absorption at 660 nm, which was determined by standard spreading technique based on OD to adjust the number of viable microbes, the bacterial concentration was determined.

2.2.3.2 Count of Viable Cells after Inoculation of *S. mutans* in Test Foods

11 test foods selected total sugar content higher than other items and 10% sucrose solution as the control. Sample 10 g added 3rd DW 100 ml, filtrated Whatman® qualitative filter paper and incubated 37°C for 5 hours. After the inoculation, the incubation time was compared by the cell lag phase at exponential growth. Diluted 1 ml solution in 0.85% saline solution 9 ml and spread 10 fold dilution solution (10^0-10^6) 100 μl in BHI agar plate. Then incubated 37°C for 48 hours and counted colony.

2.2.4 Radioisotope-labeled PAHA Disc

2.2.4.1 Radioisotope Polyacrylamide Hydroxyapatite

Hydroxyapatite (Type I suspension in 0.001 M, phosphate buffer, pH 6.8, approx. 25% solids; Sigma-Aldrich, USA) was added to DW 10ml, acrylamide gel 9 g and bisacrylamide 0.24 g. Then 4ml of prepared solution was assorted with DW 1.7 ml, ammonium persulfate 300 µl, N, N, N', N'-tetramethylethylenediamine (TEMED) 2 µl and 3 g hydroxyapatite.

Polyacrylamide gel 9 ml concentration was set to 20%, 50%, 60%, 70% respectively. The materials were stirred on the magnetic plate, suspended within 300 µl aliquots in a multi-well plate, dried for 2 hours and isolated. Then baked in a furnace for 10 minutes and cooled for 4 hours in desiccators.

The Vickers hardness index of polyacrylamide samples was measured by microhardness tester (HMV-2, Shimadzu, Tokyo, Japan) at a 50 gm load for a 10 seconds dwell time. Polyacrylamide gel at various concentration's microhardness except 60% was undetectable. Therefore, 60% polyacrylamide gel (Vickers hardness index at 36.6 ± 2.8) was selected as pertinent material, then reserved in 100% relative humidity. Although hydroxyapatite discs (PAHA) were produced in controlled situation, equal surface roughness at micro-level wasn't achievable due to brittle characteristics of PAHA.

2.2.4.2 Liquid Scintillation Counter

The standard sugar concentrations were set to 0.5%, 2%, 5%, and 10%. Pre-processed subject solutions were added to 5 ml of artificial saliva (Na-CMC soln., glycerin, KCl, NaH_2PO_4, $CaCl_2$, $MgCl_2$), *S. mutans* and the radioisotope-labeled PAHA. This compound was incubated at 37°C for 150 minutes and then centrifuged at 15,000 rpm for 1 minute. In a radioactive polyethylene bottle, 2 ml of the supernatant was mixed with 7 ml of Aquasol-2 (Packard Bioscience Company, GE Groningen, Netherlands). Using liquid scintillation counter (LS 5000 TA, Beckman Instruments Inc., Fullerton, CA, USA), ^{32}P released from the radioisotope PAHA was measured repeatedly.

2.2.4.3 Confocal Laser Scanning Microscopy

10 ml of pre-processed food samples were added to 5 ml of artificial saliva (Na-CMC solution, glycerin, KCl, NaH_2PO_4, $CaCl_2$, $MgCl_2$), and then incubated for 180 minutes at 37°C after inoculating 1 μl of *S. mutans*. An analysis was performed with confocal laser scanning microscope (HeNe laser, 543 nm excitation wavelength, 2 μl confocal slit, NT 80/20 filter; Carl Zeiss Microscopy GmbH, Munich, Germany). Demineralization effects of subjects induced hydroxyapatite disc (PAHA) were determined based on surface roughness, captured in both 2D and 3D image analysis system (Zeiss LSM Image Examiner, Carl Zeiss Microscopy GmbH, Jena, Germany) in ten times.

2.2.4.4 Scanning Electronic Microscopy

SEM image analysis was performed using S-4700 (Hitachi Ltd., Tokyo, Japan) at a 150 magnification. All assays were repeated 10 times to reduce the disparity in the released amount of ^{32}P radioisotope including the 10% sucrose, as the control.

2.2.5 Statistical Analysis

The statistical analysis used SPSS software for Windows (Version 23.0, SPSS Inc., Chicago, IL, USA) and IBM Watson Analytics. With a significance rate at $p < 0.05$ the data was expressed as the mean ± standard deviation. The statistical evaluation and the amount of ^{32}P released from PAHA in different sugar concentrations were analyzed using one-way analysis of variance (ANOVA) and Scheffé's multiple range test. A statistical evaluation was performed using analysis of Pearson correlation coefficient (r) and stepwise regression to analyze the caries-associated variables. An analysis of Pearson correlation was used for factors between various caries induction and cariogenic potential index based on starchy foods ($p < 0.05$). Stepwise regression analysis used to analyze the caries-associated variables which affect total cariogenic potential index ($p < 0.01$).

3. Results

3.1 Physico-chemical Factors

3.1.1 Starch

Starch Contents

The content of moisture and the total starch in 11 starchy foods were presented in Table 3. Starch content based on the dry weight were as follows: the potato group, 81.5%; the fruit group, 74.6%; the cereal group, 73.7%; the bread group, 70.9% and the chips & cookie group, 70.1%. Throughout the experiment, maltose maintained the high level of proportion which could be explained as constant formation induced by entrapped food particles.

Table 3. Total Starch & Moisture Content in Test Foods

Category	Items	Moisture content (%)	Total starch (%, w/DW)
Potatoes	Baked potato	81.0 ± 0.3	81.2 ± 0.5
	Baked sweet potato	63.4 ± 0.9	81.8 ± 0.4
	Total	72.2 ± 0.6	81.5 ± 0.5
Chips & cookie	Potato chip	1.9 ± 0.1	68.1 ± 0.9
	Baked potato chip	3.0 ± 0.2	68.0 ± 0.6
	Butter cookie	2.7 ± 0.1	74.2 ± 0.8
	Total	2.5 ± 0.1	70.1 ± 0.8
Bread	White bread	32.1 ± 0.3	71.7 ± 0.6
	Sponge cake	28.9 ± 0.2	69.3 ± 0.7
	Doughnuts	13.1 ± 0.4	71.8 ± 0.9
	Total	24.7 ± 0.3	70.9 ± 0.8
Cereal	Cornflakes	3.1 ± 0.1	73.7 ± 0.8
Fruits	Apple	85.1 ± 0.2	73.4 ± 1.1
	Banana	73.4 ± 0.5	75.8 ± 0.7
	Total	79.3 ± 0.4	74.6 ± 0.9

DW, distilled water.

Starch Hydrolysis

Table 4 presented the proportion of starch hydrolysis in 11 starchy foods. Average content among the subjects were as follows: the potato group, 80.8%; the fruit group, 58.7%; the cereal group, 44.3%; the bread group, 35.5% and the chips & cookie group, 22.0%. The average difference (between 0 minute and 180 minutes) was as follows: the potato group, 19.7%; chips & cookie group, 11.2%; the bread group, 5.3%; the cereal group, 12.9% and fruit group, 5.0%. Followed by the result, the overall proportion of starch hydrolysis within test subjects were increased. While baked potato presented a significant increased at 180 minutes, other foods showed the decreasing tendency in starch hydrolysis. Maltose maintained the high level of proportion which could be explained as constant formation induced by entrapped food particles.

Table 4. *In vitro* Starch Digestion of Starchy Foods

(% of total starch hydrolyzed at different times)

Category	Items	Hydrolyzed starch (%)						
		Time (minutes)						
		0	**30**	**60**	**90**	**120**	**150**	**180**
Potatoes	Baked potato	67.1	73.4	74.9	81.0	83.6	84.2	89.1
	Baked sweet potato	55.1	59.8	61.1	62.3	64.2	67.6	72.4
	Total	66.1	66.6	68.0	71.7	73.9	75.9	80.8
Chips & cookie	Potato chip	5.0	8.2	10.1	15.4	17.7	19.3	20.1
	Baked potato chip	11.7	12.0	14.2	15.2	15.5	18.6	20.3
	Butter cookie	15.7	18.1	18.6	18.9	21.5	24.3	25.6
	Total	10.8	12.8	14.3	16.5	18.2	20.7	22.0
Bread	White bread	42.1	43.4	43.5	43.9	44.1	45.0	46.9
	Sponge cake	18.8	19.1	20.3	20.4	21.1	22.0	23.4
	Doughnuts	29.8	30.5	31.0	32.4	34.0	35.6	36.2
	Total	30.2	31.0	31.6	32.2	33.1	34.2	35.5
Cereal	Cornflakes	31.4	37.2	41.7	42.7	42.8	43.5	44.3
Fruits	Apple	48.2	49.1	49.8	50.1	50.5	51.3	51.8
	Banana	59.2	62.7	63.1	63.5	64.1	65.4	65.6
	Total	53.7	55.9	56.5	56.8	57.3	58.4	58.7

Values represent mean % of hydrolyzed starch at different times.

3.1.2 pH and Titratable Acidity

Table 5 showed overall acidity of test subjects, and they divide into titratable acidity and pH. The average pH of test foods was as follows: the potato group, 6.6; the chips & cookie group, 7.3; the bread group, 6.1; the cereal group, 6.2 and the fruit group, 5.1 (control: 7.5). The average titratable acidity of starchy foods was as follows: the potato group, 0.26%; the chips & cookie group, 0.29%; the bread group, 0.54%; the cereal group, 0.26% and the fruit group, 0.67% (control: 0.10%). pH of the chips & cookie group had the highest whereas the fruit group had the lowest. Titratable acidity of the potato and cereal groups had the lowest whereas fruit group had the highest. The total average of pH and titratable acidity in 11 test foods were 5.8 and 0.38%. Compared to control, pH had lower whereas titratable acidity had a higher tendency.

Table 5. pH and Titratable Acidity of Test Foods

Category	**Items**	**pH**	**Titratable Acidity (%, w/v)**
Standard	Sucrose 10%	7.5 ± 0.1	0.10 ± 0.01
Potatoes	Baked potato	6.5 ± 0.2	0.27 ± 0.02
	Baked sweet potato	6.6 ± 0.2	0.24 ± 0.03
	Total	6.6 ± 0.2	0.26 ± 0.03
Chips & cookie	Potato chip	7.1 ± 0.2	0.41 ± 0.02
	Baked potato chip	7.6 ± 0.1	0.30 ± 0.01
	Butter cookie	7.2 ± 0.1	0.15 ± 0.02
	Total	7.3 ± 0.1	0.29 ± 0.02
Bread	White bread	5.2 ± 0.1	0.50 ± 0.01
	Doughnuts	6.3 ± 0.1	0.55 ± 0.02
	Sponge cake	6.9 ± 0.1	0.58 ± 0.01
	Total	6.1 ± 0.1	0.54 ± 0.01
Cereal	Cornflake	6.2 ± 0.1	0.26 ± 0.02
Fruits	Apple	4.6 ± 0.1	0.73 ± 0.04
	Banana	5.5 ± 0.1	0.60 ± 0.02
	Total	5.1 ± 0.1	0.67 ± 0.03

w/v, weight/volume.

3.1.3 Total Sugar and Reducing Sugar

Table 6 indicated the content of total sugar and reducing sugar among the food samples. The average total sugar of samples was as follows: the potato group, 319.0 mg/g; the chips & cookie group, 234.8 mg/g; the bread group, 277.2 mg/g; the cereal group, 140.2 mg/g and the fruit group, 313.1 mg/g (control: 100.9 mg/g). The average reducing sugar of starchy foods was as follows: the potato group, 46.4 mg/g; the chips & cookie group, 0.97 mg/g; the bread group, 15.5 mg/g; the cereal group, 37.1 mg/g and the fruit group, 15.6 mg/g (control: 0.1 mg/g). Total sugar of the potato group had the highest whereas the cereal group had the lowest. Reducing sugar of the potato group had the highest whereas chips & cookie group had the lowest.

The total average of total sugar and reducing sugar in 11 starchy foods were 245.1 mg/g and 17.5 mg/g. Compared to control, total sugar and reducing sugar had a higher tendency.

Table 6. Total Sugar and Reducing Sugar Contents of Test Foods

Category	**Items**	**Total Sugar (mg/g)**	**Reducing Sugar (mg/g)**
Standard	Sucrose 10%	100.9 ± 0.1	0.1 ± 0.0
Potatoes	Baked potato	240.5 ± 0.7	35.3 ± 0.3
	Baked sweet potato	397.5 ± 1.4	57.5 ± 0.5
	Total	319.0 ± 1.1	46.4 ± 0.4
Chips & cookie	Potato chip	255.8 ± 1.3	0.3 ± 0.0
	Baked potato chip	188.7 ± 0.4	0.3 ± 0.1
	Butter cookie	260.0 ± 1.2	2.3 ± 0.2
	Total	234.8 ± 1.0	0.97 ± 0.1
Bread	White bread	260.4 ± 0.7	12.1 ± 0.4
	Doughnuts	275.7 ± 0.8	19.5 ± 0.6
	Sponge cake	295.6 ± 0.5	15.0 ± 0.3
	Total	277.2 ± 0.7	15.5 ± 0.4
Cereal	Cornflake	140.2 ± 1.1	37.1 ± 0.9
Fruits	Apple	385.5 ± 0.7	15.2 ± 0.6
	Banana	240.7 ± 1.0	15.9 ± 0.8
	Total	313.1 ± 0.9	15.6 ± 0.7

3.1.4 Texture

Table 7 represented the textural characteristics of test foods. The average hardness of test foods was as follows: the potato group, 557.9; the chips & cookie group, 3725.3; the bread group, 1798.3; the cereal group, 9051.8 and the fruit group, 1084.9. The average cohesiveness of test foods was as follows: the potato group, 0.35; the chips & cookie group, 0.31; the bread group, 0.51; the cereal group, 0.39 and the fruit group, 0.60. The average chewiness of test foods was as follows: the potato group, 176.0; the chips & cookie group, 989.6; the bread group, 463.5; the cereal group, 1544.6 and the fruit group, 290.4. The average adhesiveness of test foods was as follows: the potato group, -153.5; the chips & cookie group, -39.3; the bread group, -6.0; the cereal group, -0.2 and the fruits group, -11.4 ($p < 0.001$). The average springiness of test foods was as follows: the potato group, 0.71; the chips & cookie group, 0.54; the bread group, 0.49; the cereal group, 0.40 and the fruit group, 0.68 ($p < 0.01$). The total average of 11 test foods was as follows: hardness, 2409.0; springiness, 0.57; cohesiveness, 0.43; chewiness, 621.5 and adhesiveness, -38.8. The stepwise regression of adhesiveness and springiness among rheological characteristics in test foods affected dental caries significantly ($p < 0.05$). It was considered that starchy foods had more chance to cause dental caries due to longer residual time between teeth as adhesiveness was higher and springiness were lower.

Table 7. Textural Characteristics of Test Foods

Category	Items	Hardness	Springiness	Cohesiveness	Chewiness	Adhesiveness
Potatoes	Baked potato	415.7 ± 3.9	0.61 ± 0.03	0.24 ± 0.05	61.0 ± 2.2	-49.1 ± 1.5
	Baked sweet potato	700.1 ± 5.1	0.80 ± 0.07	0.46 ± 0.07	290.4 ± 3.7	-257.9 ± 6.4
	Total	557.9 ± 4.8	0.71 ± 0.05	0.35 ± 0.06	176.0 ± 3.0	-153.5 ± 4.0
Chips & cookie	Potato chip	4301.0 ± 9.7	0.61 ± 0.02	0.28 ± 0.04	1445.6 ± 4.6	-22.4 ± 0.6
	Baked potato chip	4250.5 ± 9.1	0.63 ± 0.03	0.35 ± 0.05	1152.8 ± 2.7	-23.9 ± 0.9
	Butter cookie	2624.3 ± 6.3	0.39 ± 0.04	0.29 ± 0.01	372.0 ± 2.4	-71.7 ± 4.1
	Total	3725.3 ± 8.4	0.54 ± 0.03	0.31 ± 0.03	989.6 ± 3.2	-39.3 ± 1.9
Bread	White bread	1575.7 ± 5.2	0.61 ± 0.05	0.53 ± 0.04	524.8 ± 3.3	-3.9 ± 0.4
	Doughnuts	2470.2 ± 6.8	0.47 ± 0.04	0.39 ± 0.02	441.2 ± 4.5	-6.1 ± 0.2
	Sponge cake	1349.0 ± 5.7	0.38 ± 0.03	0.61 ± 0.05	425.5 ± 2.5	-8.0 ± 0.8
	Total	1798.3 ± 5.9	0.49 ± 0.04	0.51 ± 0.04	463.5 ± 3.4	-6.0 ± 0.5
Cereal	Cornflake	9051.8 ± 10.9	0.40 ± 0.01	0.39 ± 0.03	1543.1 ± 5.0	-0.2 ± 0.1
Fruits	Apple	1968.2 ± 3.1	0.42 ± 0.03	0.55 ± 0.04	535.4 ± 2.3	-8.1 ± 0.4
	Banana	201.5 ± 2.4	0.93 ± 0.02	0.65 ± 0.03	44.5 ± 1.6	-14.6 ± 1.5
	Total	1084.9 ± 2.8	0.68 ± 0.03	0.60 ± 0.04	290.4 ± 2.0	-11.4 ± 1.0

3.2 Microbial Factor

Total Viable Cells after Inoculation of *S. mutans* in Test Foods

After 150 minutes of inoculating *S. mutans* in BHI broth, OD had a tendency to decrease in test samples. The maximum OD of *S. mutans* was observed between 90 minutes and 150 minutes; after 150 minutes, the OD decreased. Therefore, the pertinent incubation time was set to 150 minutes.

Table 8 showed the number of total viable cells after inoculating *S. mutans* and then incubated for 18 hours. The total viable cells of test samples were as follows: the potato group, 0.16×10^6 cfu/ml; the chips & cookie group, 2.53×10^6 cfu/ml; the bread group, 6.05×10^6 cfu/ml; the cereal group, 0.19×10^6 cfu/ml and the fruits group, 0.20×10^6 cfu/ml (control: 7.4×10^6 cfu/ml). Total viable cells of starchy foods were increased as total sugar contents increased. Especially, total viable cells of the bread group had the highest whereas the potatoes group presented the lowest.

Table 8. The Number of Total Viable Cells after Inoculating *S. mutans* in Test Foods and Incubated for 18 hours

Category	**Items**	**Total viable cells ($\times 10^6$ cfu/ml)**
Control	Sucrose 10%	0.74 ± 0.05
Potatoes	Baked potato	0.15 ± 0.03
	Baked sweet potato	0.17 ± 0.02
	Total	0.16 ± 0.03
Chips & cookie	Potato chip	2.31 ± 0.02
	Baked potato chip	2.52 ± 0.03
	Butter cookie	2.75 ± 0.02
	Total	2.53 ± 0.03
Bread	White bread	5.42 ± 1.23
	Sponge cake	6.87 ± 1.59
	Doughnuts	5.87 ± 0.73
	Total	6.05 ± 1.18
Cereal	Cornflake	0.19 ± 0.03
Fruits	Apple	0.20 ± 0.02
	Banana	0.19 ± 0.01
	Total	0.20 ± 0.02

3.3 Radioisotope-labeled PAHA Disc

3.3.1 Comparison of ^{32}P Released from the Radioisotope-labeled PAHA after Inoculating *S. mutans*

Table 9 showed the comparison of released ^{32}P in radioisotope-labeled PAHA after the inoculation with *S. mutans* and the one with 150 minutes of incubation in the sugar solutions. At sucrose concentrations of 0.5%, 2%, 5%, and 10%, The average amount of ^{32}P released from radioisotope PAHA were 7,555 counts per minute (cpm), 8,010 cpm, 8,124 cpm, and 10,027 cpm, at 0.5%, 2%, 5%, and 10% sucrose concentrations. Cpm refers the unit of the released ^{32}P, and the values presented a statistic significance at 95%. Since the standard deviation of 10% sucrose control showed the minimum value, the sample was used to set the standard degree of cariogenic potential.

Table 10 showed the average amount of ^{32}P radioisotope released from the PAHA disc for test foods ($p = 0.032$). The average amount of ^{32}P released for the standard 10% sucrose solution was 10,027.4 cpm. The average amount of ^{32}P released for starch foods was as follows: the potato group, 1,023.1 cpm; the chips & cookie group, 14,709.0 cpm; the bread group, 13,692.6 cpm; the cereal group, 16579.0 cpm and the fruits group, 19340.2 cpm. Fruit groups showed the highest cariogenic potential, while the potato group was the lowest. The average amount of ^{32}P released for the test food groups, such as chips & cookie, bread and cereal were higher than the sucrose control. The average amount of ^{32}P released for starch food group, such as potato was lower than the 10% sucrose control.

Table 9. The Amount of ^{32}P Released from PAHA in Different Sugar Concentration

	Sucrose concentration				
	0.5%	**2%**	**5%**	**10%**	**P-value***
Average amount of ^{32}P ± SD (cpm†)	7,555 ± 1,134	8,010 ± 1,230	8,124‡ ± 1,172	10,027‡ ± 927	< 0.05

*: One-way ANOVA $p < 0.05$, ‡: MCA and Scheffé $p < 0.05$.

†: counts per minute (cpm: unit of ^{32}P as measured by liquid scintillation counting).

Table 10. Demineralized Amount of Phosphorus Radioisotope (^{32}P) Released from the PAHA Disc

Category	Items	Demineralized ^{32}P		P-value
		Quantity (cpm)	Ratio*	
Standard	10% sucrose solution	10,027.4 ± 927.5	1.00	
Potato	Baked potato	978.1 ± 102.0	0.10	
	Baked sweet potato	1,068.1 ± 109.6	0.11	
	Total	1,023.1 ± 105.8	0.11	
Chips &	Potato chip	14,296.0 ± 2,098.0	1.43	
Cookie	Baked potato chip	14,736.8 ± 2,785.1	1.47	
	Butter cookies	15,094.1 ± 2,032.0	1.50	
	Total	14,709.0 ± 2,035.0	1.47	0.032**
Bread	White bread	12,362.0 ± 1,014.2	1.23	
	Sponge cake	14,792.4 ± 1,308.8	1.48	
	Doughnuts	13,923.5 ± 1,247.2	1.39	
	Total	13,692.6 ± 1,190.1	1.37	
Cereal	Cornflake	16,579.0 ± 2,229.1	1.65	
Fruits	Apple	20,003.1 ± 1,857.2	2.00	
	Banana	18,677.3 ± 1,701.5	1.86	
	Total	19,340.2 ± 1,779.4	1.93	

*Demineralized ^{32}P ratio = demineralized ^{32}P amount for the test food / demineralized ^{32}P amount for the standard (10% sucrose).

cpm: counts per minute (unit of ^{32}P as measured by liquid scintillation counting).

**: $p < 0.05$ (DM-ANOVA of quantity of demineralized ^{32}P).

3.3.2 Confocal Laser Scanning Microscopy

The demineralization effect of starchy foods and 10% sucrose as a control were observed through the subsurface lesion of PAHA using CLSM and SEM (Fig. 1), which indicated that 10% sucrose-induced PAHA disc had increased surface roughness with the presence of deep crevices. Food samples, excluding potato groups showed significant increase in the degree of surface roughness when the proportion of hydrolyzed starch to total starch decreased over time ($p = 0.000$). The surface lesion of potato had far fewer crevices and was less irregular than the baseline lesions.

Beyond the microscopic investigation, this study provided the degree of surface roughness data both in 3D and 2D parameters. However, further demineralization quantification and cariogenic potential analysis were based on 3D results, because of 3D surface topography represented both smooth and rough surfaces in optical profiling, while 2D parameters are limited to singular surface profiling.

Fig. 2 showed the variation of surface roughness in sequence (before and after) based on individual parameter values obtained from CLSM. The degree of demineralized quantity in starchy food subjects was illustrated as radar graph and histogram.

Amplitude parameters including Sp, Sv and Sz are evaluated from the absolute lowest to highest points within the surface. The *Sp* parameter represents the maximum peak (highest point of the surface). Through the course of demineralization, cornflake showed the most increased variation ($\Delta = 7.439$ μm) of in maximum peak height, while potato presented the most

negative difference (*Δ* = -1.068 μm). The *Sv* parameter represents the maximum valley depth, in other words, the lowest point of the surface. Contrary to maximum peak height (*Sp*), cornflake showed the most decreased variation (*Δ* = -9.653 μm) in maximum valley depth, while sponge cake presented the most increased (*Δ* = 6.367 μm) value. The *Sz* parameter refers to the maximum height of the surface which showed potato as the most negative variation (*Δ* = -1.68 μm) and banana as a top of the increased difference (*Δ* = 5.637 μm).

Sa and *Sq* are probably the most commonly used parameters among others, which evaluates over the complete 3D surface. Sa parameter is defined as arithmetic mean height within sample surface. Apple showed the most variation between other subjects (*Δ* = 1.22 μm), while potato presented the modest difference at 0.025 μm. *Sq* states root mean square, which is the standard deviation of surface roughness thus statistically more significant, has more physical grounding than *Sa*. Although, these two parameters are closely correlated (Blunt & Jiang, 2003), *Sq* presents direct relation to surface energy. Likewise, apple presented the most positive variation (*Δ* = 1.697 μm), whereas potato was disclosed as a negative variant (*Δ* = -0.046 μm).

A normal distribution of asymmetry and deviation from a histogram of the surface points of all heights are represented by unitless *Ssk* and *Sku*, which refers to skewness and kurtosis respectively. Basically, skewness indicates the ratio of the mean of heights cubed and the cube of *Sq* within the surface, in other words, it expresses the symmetry of peaks and valleys based on the average central line. Values higher than 0 indicates the prevalence of peaks

while lower than 0 implies valleys. *Ssk* at 0.00 infers Normal distribution (i.e. bell curve). Sponge cake and bread showed the tendency toward the predominance of peaks, while apple, sweet potato, potato, banana, cornflake presented valleys. *Sku* stands as a measurement of the sharpness. It differentiates from *Ssk* by detecting whether the sample spikes are distributed consistently and the degree of spikiness. In other words, the presence of inordinate peaks and valleys are presented in $Sku > 3.00$, while $Sku < 3.00$ refers bumpy surface compared to beforehand. Sponge cake and bread tended to be a thorn, while apple, sweet potato, potato, banana, and cornflake in contrary.

Sc expresses the mean height of the surface of the profile curve elements (an adjacent peak and valley) in a sampling length. Peaks and valleys are identified with minimum heights and lengths. All test subjects showed the decreased value in the variation of *Sc*. Sweet potato was the highest variation among samples at -0.276μm. Although the range of difference between food subjects are minuscule (-0.025 ~ -0.276) compared to other parameters. From the quantitative descriptive analysis, the value of Sc treated as derived attribute since it stabilizes other parameters due to a distinct rate of measurement. The *St* and *Smax* parameter represent total height of the surface and maximum roughness depth respectively. Apple showed the most positive deviation both in *St* (Δ = 9.219 μm) and *Smax* (Δ = 9.578 μm), while cornflake presented the negative (Δ = -2.214 μm, Δ = -4.34 μm).

Fig. 3 illustrated the quantitative descriptive analysis (QDA) profile comparison of CLSM parameter in test foods and an opposite version of Fig. 4 in

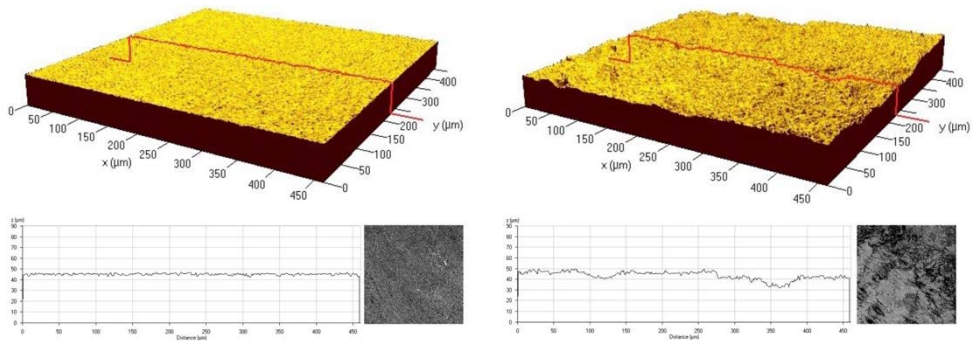
a sequential format. Each radar lines indicates subject foods, x-axis as CLSM parameter and y-axis as a degree of variation.

Fig. 4 represented the Pearson correlation of CLSM parameters and demineralized ^{32}P. Every node except demineralized ^{32}P indicates individual parameters from CLSM. The thickness of the edge and annotated numeric values represent the degree of r-value from Pearson correlation result and only applied to the correlation between Demineralized quantity ^{32}P and CLSM parameters ($p < 0.05$).

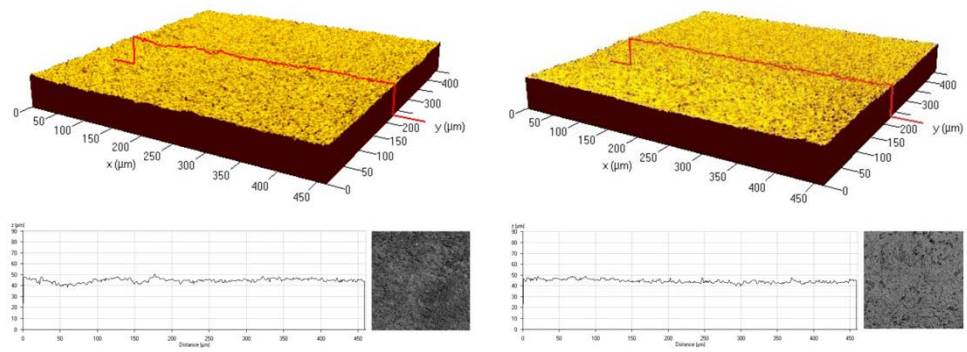
3.3.3 Scanning Electronic Microscopy

Along with CLSM result, Fig. 1 showed the demineralized ^{32}P surface after inoculating *S. mutans* in starchy foods using SEM at 150 magnification. The average amount of ^{32}P released for the 10% sucrose control was 10,027.4 cpm, and the unalloyed PAHA was 9,423.0 cpm. The average amount of ^{32}P released for potato and apple was 978.1 cpm and 20,003.1 cpm, respectively. Fig. 1 displayed an escalated parallel effect between the average amount of ^{32}P released and the demineralization of the radioisotope-labeled PAHA's surface.

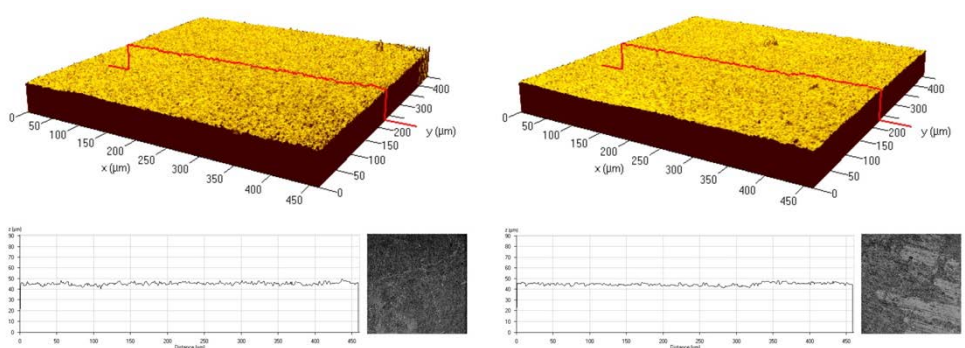
Before *After*



a. Sucrose 10% (Control)



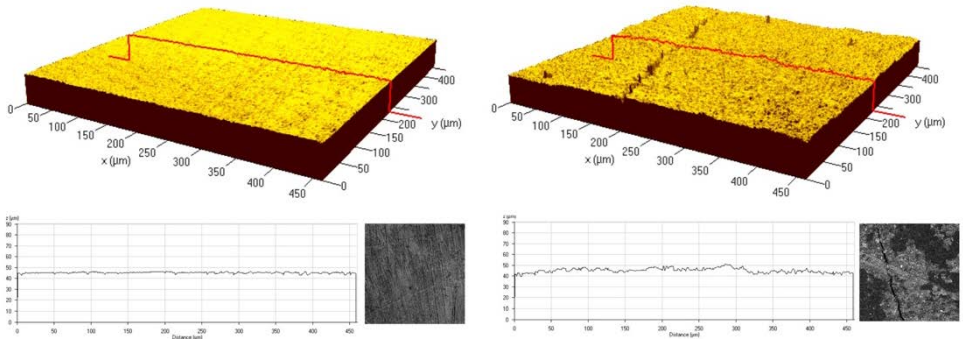
b. Potato



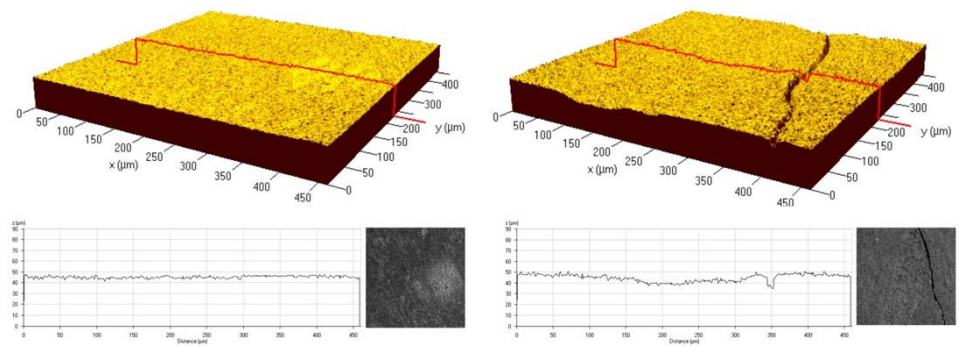
c. Sweet potato

Fig 1. A representative Images of the Demineralization Effect of Starchy Foods and Control (10% sucrose) through Observation of Subsurface Lesion in Polyacrylamide Hydroxyapatite Disc (PAHA) using Confocal Laser Scanning Microscopy (CLSM) and Scanning Electron Microscopy (SEM)

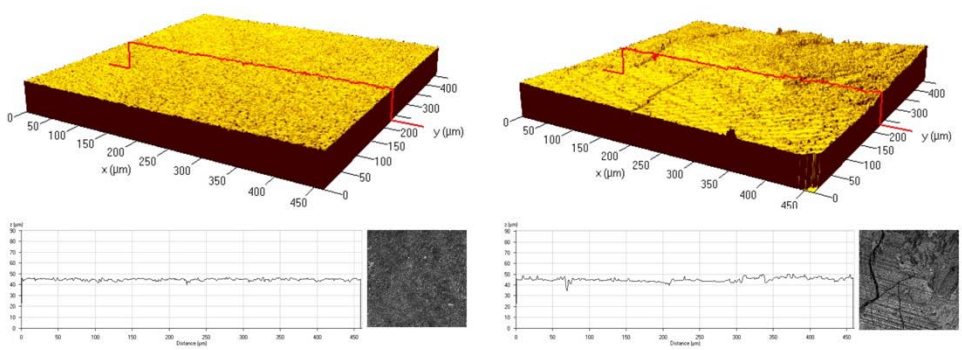
Before *After*



d. Butter cookie



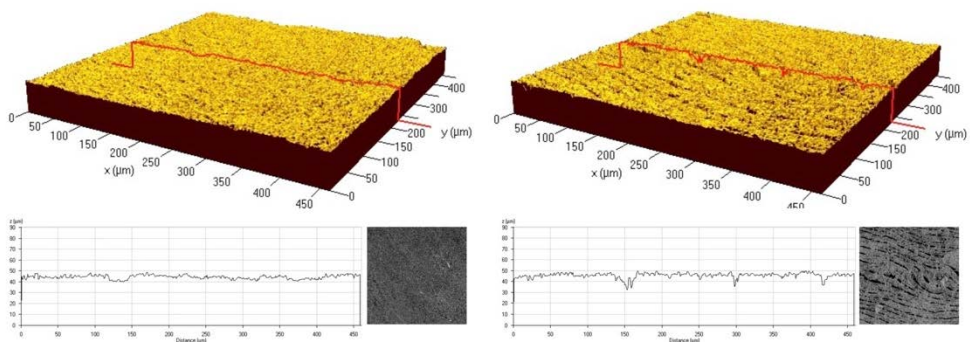
e. Baked potato chip



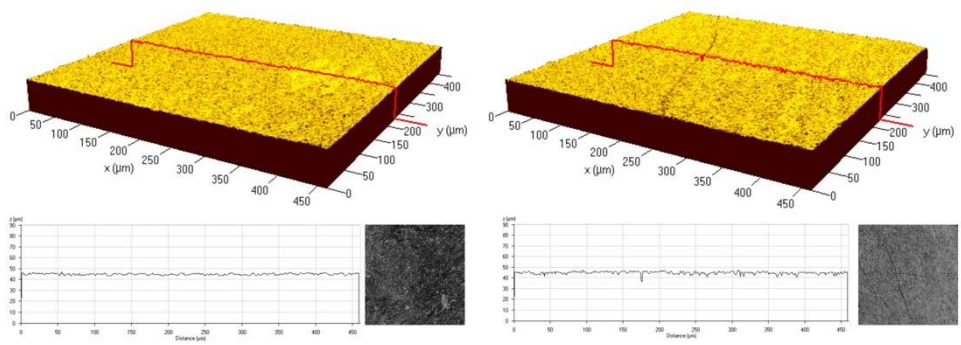
f. Potato chip

Fig 1. Continued

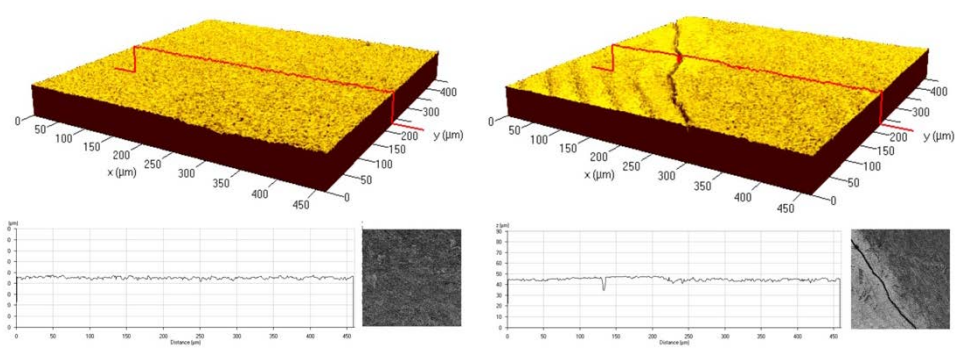
Before *After*



g. Sponge cake



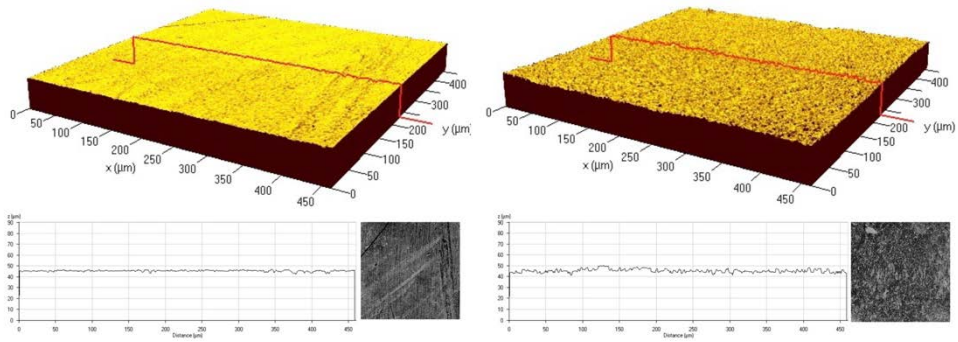
h. White bread



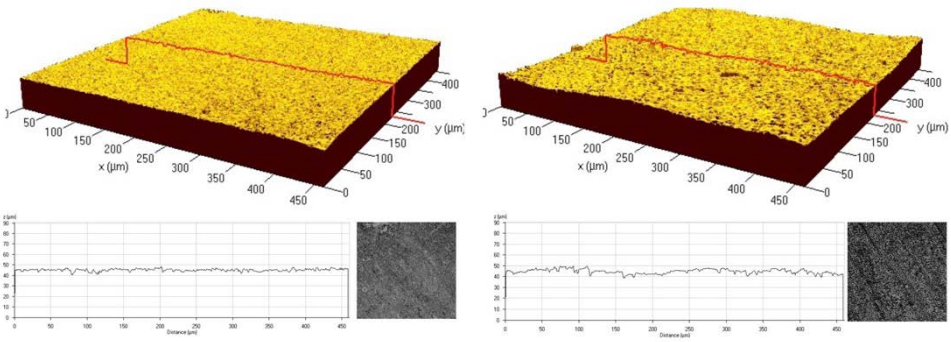
i. Doughnuts

Fig 1. Continued

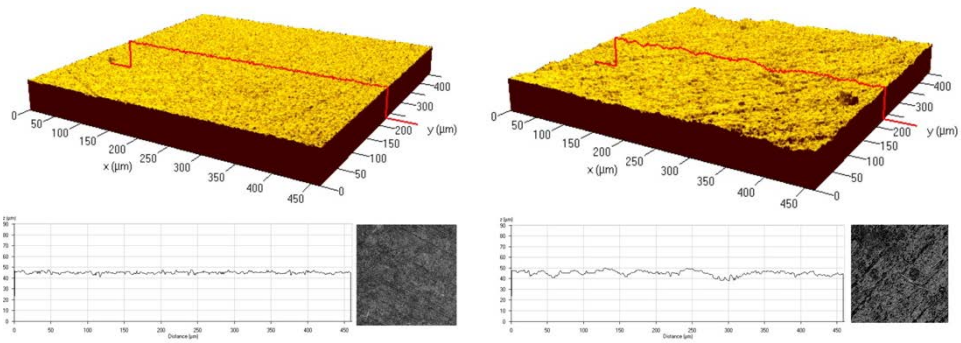
Before *After*



j. Cornflake



k. Banana



l. Apple

Fig 1. Continued

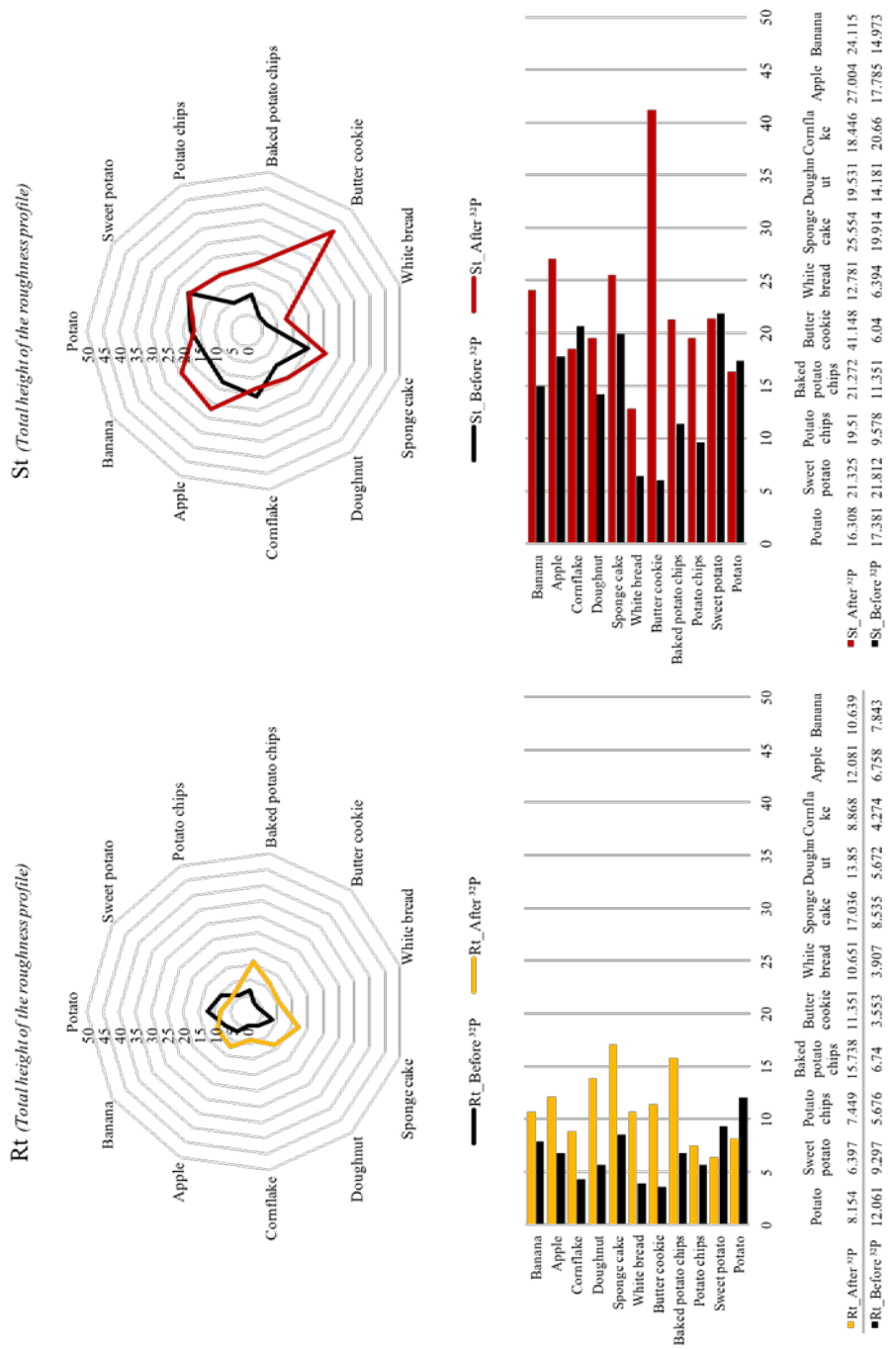


Fig 2. CLSM Parameter Analysis

(Variation of surface roughness in sequence (before & after) based on individual parameter values obtained from CLSM))

In-depth surface, roughness measurement by parameters was described with before and after induction. Descriptions for every parameter were followed by the title. Rader graphs represented the degree of parameter variation by test samples. The following histogram presented the equivalent. Numerical results have been provided in the bottom of each figure.

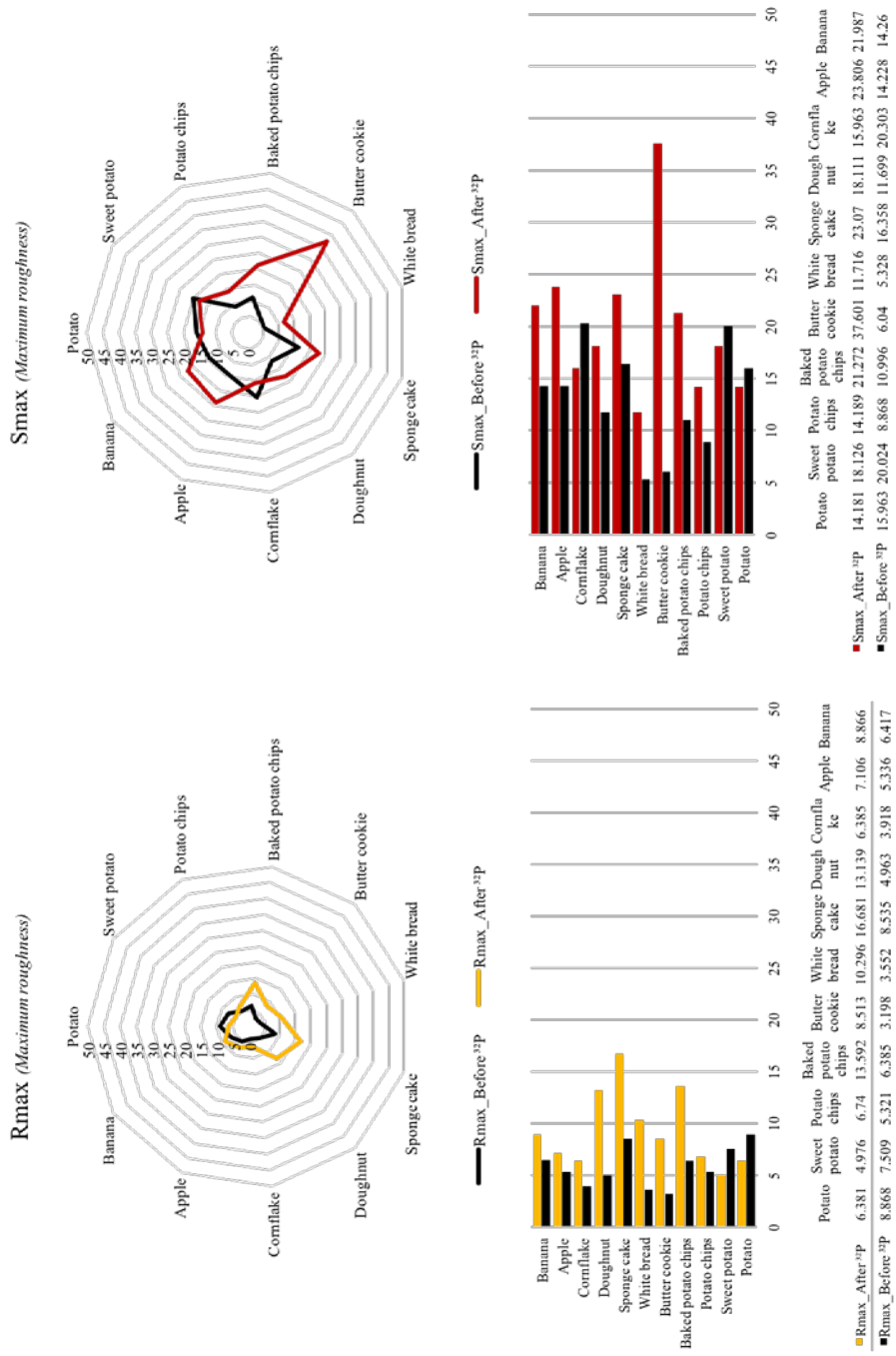
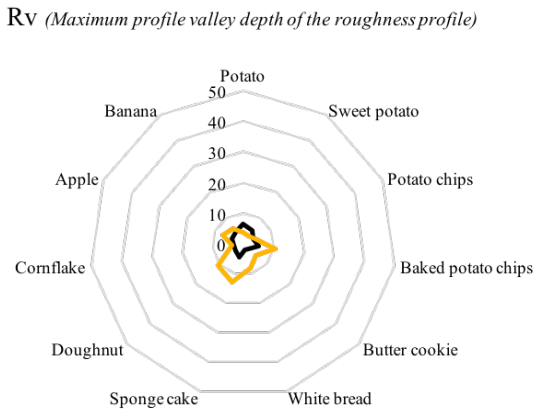
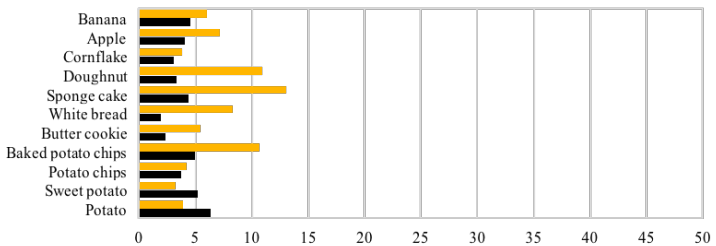


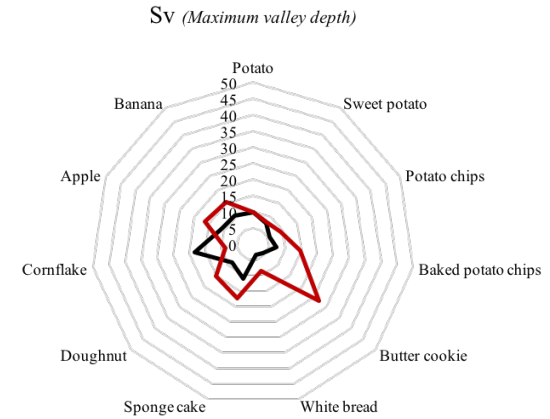
Fig 2. Continued

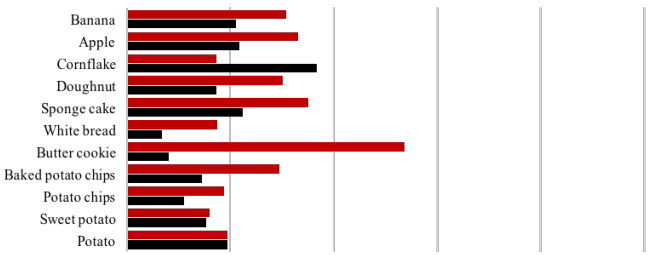
Fig 2. Continued





	Potato	Sweet potato	Potato chips	Baked potato chips	Butter cookie	White bread	Sponge cake	Dough nut	Cornfla ke	Apple	Banana
Rv_After ³²P	3.865	3.238	4.24	10.655	5.473	8.279	13.019	10.9	3.814	7.17	6.055
Rv_Before ³²P	6.384	5.217	3.724	4.993	2.333	1.927	4.373	3.321	3.066	4.056	4.528



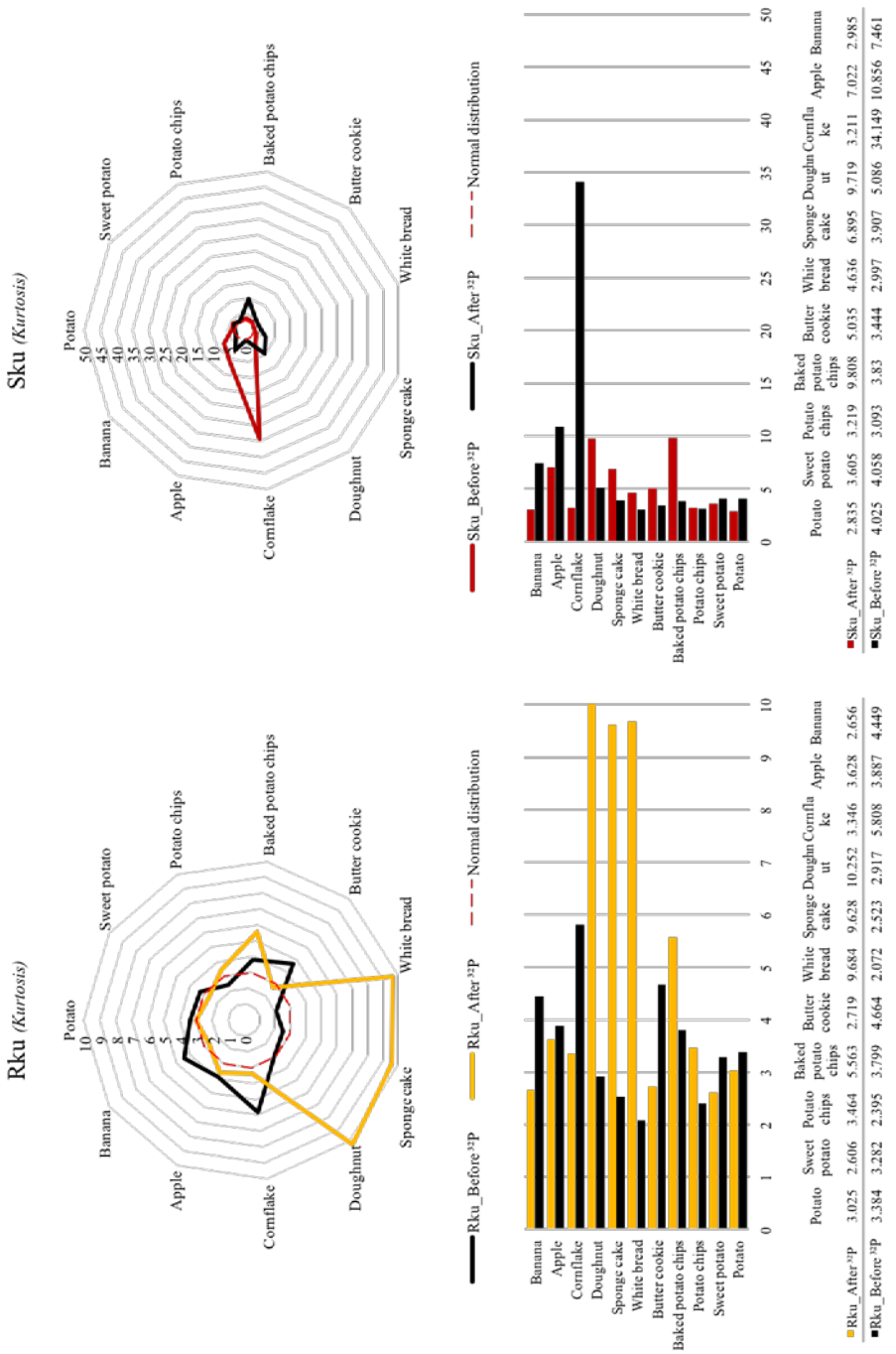


	Potato	Sweet potato	Potato chips	Baked potato chips	Butter cookie	White bread	Sponge cake	Doughnut	Cornfla ke	Apple	Banana
Sv_After ³²P	9.749	7.997	9.4	14.713	26.782	8.698	17.569	15.093	8.691	16.522	15.427
Sv_Before ³²P	9.755	7.688	5.498	7.272	4.086	3.374	11.202	8.686	18.344	10.849	10.516

	Potato	Sweet potato	Potato chips	Baked potato chips	Butter cookie	White bread	Sponge cake	Dough nut	Cornflake	Apple	Banana
Rsk_After ³²P	-0.255	0.018	0.479	0.245	-0.214	1.678	2.125	1.267	-0.6	0.714	0.233
Rsk_Before ³²P	0.492	0.004	0.281	0.812	1.092	0.064	0.249	0.232	1.4	0.153	0.747

	Potato	Sweet potato	Potato chips	Baked potato chips	Butter cookie	White bread	Sponge cake	Dough nut	Cornflake	Apple	Banana
Ssk_After ³²P	-0.04	-0.083	0.143	1.239	0.596	0.83	1.205	1.156	-0.076	1.355	0.132
Ssk_Before ³²P	0.612	0.119	0.27	0.631	0.665	0.208	0.214	0.906	3.137	1.415	1.174

Fig 2. Continued

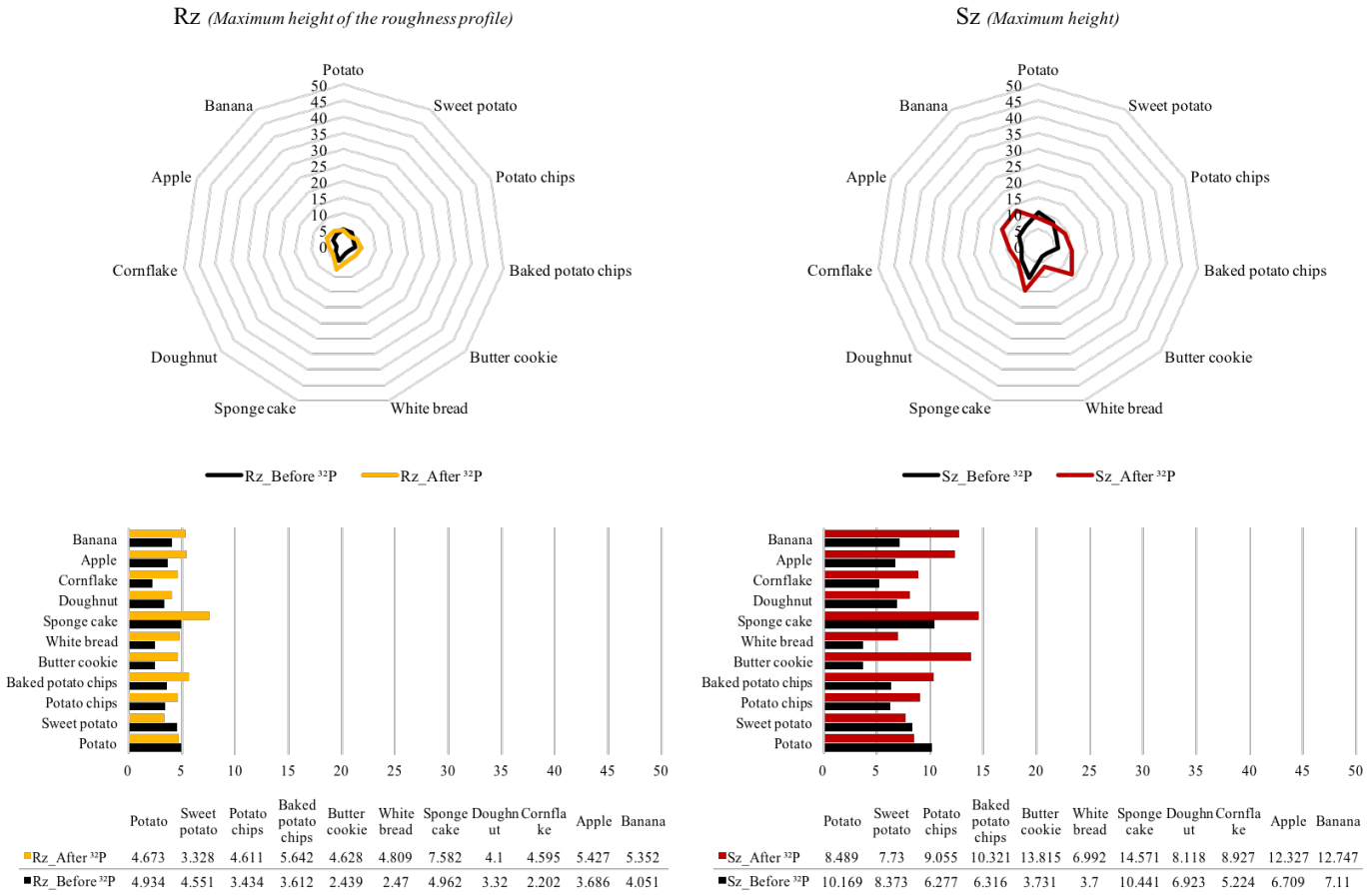


	Potato	Sweet potato	Potato chips	Baked potato chips	Butter cookie	White bread	Sponge cake	Doughnut	Cornflake	Apple	Banana
Rku_After ³²P	3.025	2.606	3.464	5.563	2.719	9.684	9.628	10.252	3.346	3.628	2.656
Rku_Before ³²P	3.384	3.282	2.395	3.799	4.664	2.072	2.523	2.917	5.808	3.887	4.449

	Potato	Sweet potato	Potato chips	Baked potato chips	Butter cookie	White bread	Sponge cake	Doughnut	Cornflake	Apple	Banana
Sku_After ³²P	2.835	3.605	3.219	9.808	5.035	4.636	6.895	9.719	3.211	7.022	2.985
Sku_Before ³²P	4.025	4.058	3.093	3.83	3.444	2.997	3.907	5.086	34.149	10.856	7.461

Fig 2. Continued

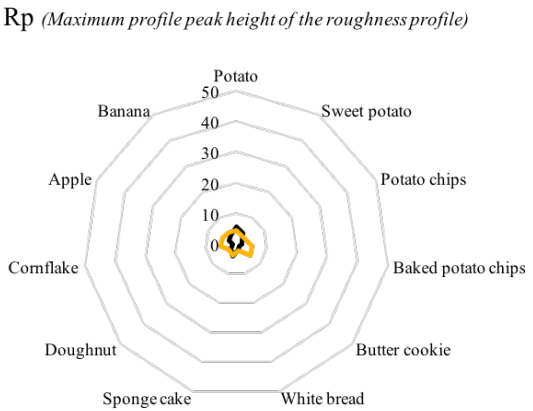
Fig 2. Continued

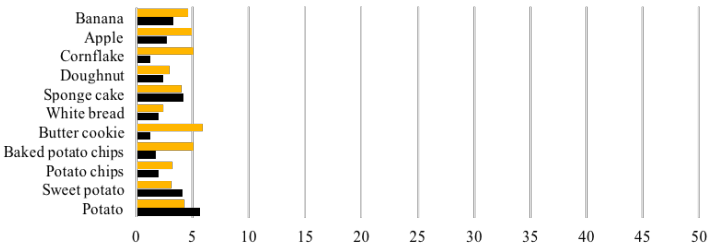


	Potato	Sweet potato	Potato chips	Baked potato chips	Butter cookie	White bread	Sponge cake	Doughnut	Cornflake	Apple	Banana
Rz_After ³²P	4.673	3.328	4.611	5.642	4.628	4.809	7.582	4.1	4.595	5.427	5.352
Rz_Before ³²P	4.934	4.551	3.434	3.612	2.439	2.47	4.962	3.32	2.202	3.686	4.051

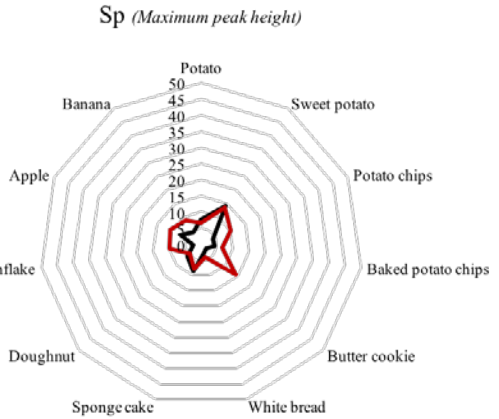
	Potato	Sweet potato	Potato chips	Baked potato chips	Butter cookie	White bread	Sponge cake	Doughnut	Cornflake	Apple	Banana
Sz_After ³²P	8.489	7.73	9.055	10.321	13.815	6.992	14.571	8.118	8.927	12.327	12.747
Sz_Before ³²P	10.169	8.373	6.277	6.316	3.731	3.7	10.441	6.923	5.224	6.709	7.11

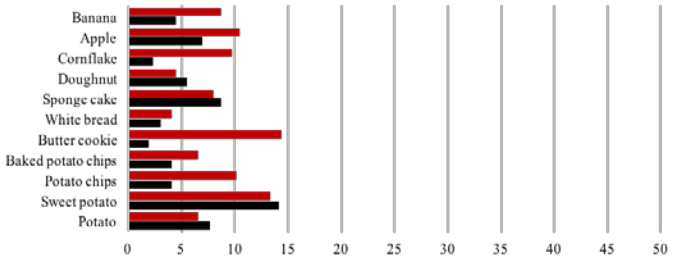
Fig 2. Continued





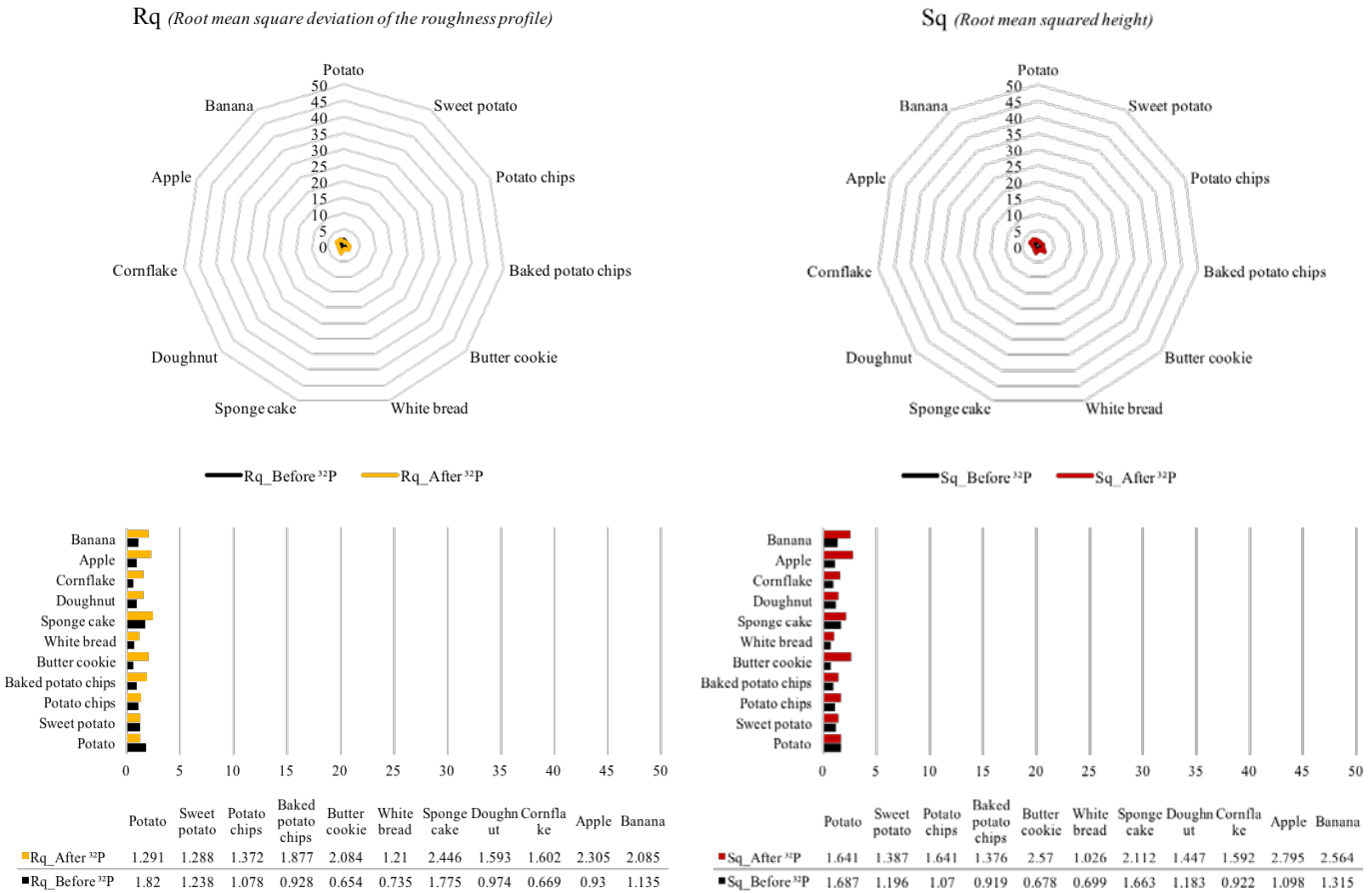
	Potato	Sweet potato	Potato chips	Baked potato chips	Butter cookie	White bread	Sponge cake	Dough nut	Cornfla ke	Apple	Banana
Rp_After ³²P	4.289	3.16	3.21	5.083	5.878	2.372	4.018	2.949	5.054	4.91	4.584
Rp_Before ³²P	5.677	4.08	1.951	1.747	1.22	1.98	4.161	2.351	1.209	2.703	3.315





	Potato	Sweet potato	Potato chips	Baked potato chips	Butter cookie	White bread	Sponge cake	Doughn ut	Cornfla ke	Apple	Banana
Sp_After ³²P	6.559	13.328	10.11	6.559	14.366	4.083	7.986	4.439	9.755	10.482	8.688
Sp_Before ³²P	7.627	14.125	4.079	4.079	1.954	3.019	8.713	5.496	2.316	6.936	4.456

Fig 2. Continued



	Potato	Sweet potato	Potato chips	Baked potato chips	Butter cookie	White bread	Sponge cake	Doughnut	Cornflake	Apple	Banana
Rq_After ³²P	1.291	1.288	1.372	1.877	2.084	1.21	2.446	1.593	1.602	2.305	2.085
Rq_Before ³²P	1.82	1.238	1.078	0.928	0.654	0.735	1.775	0.974	0.669	0.93	1.135

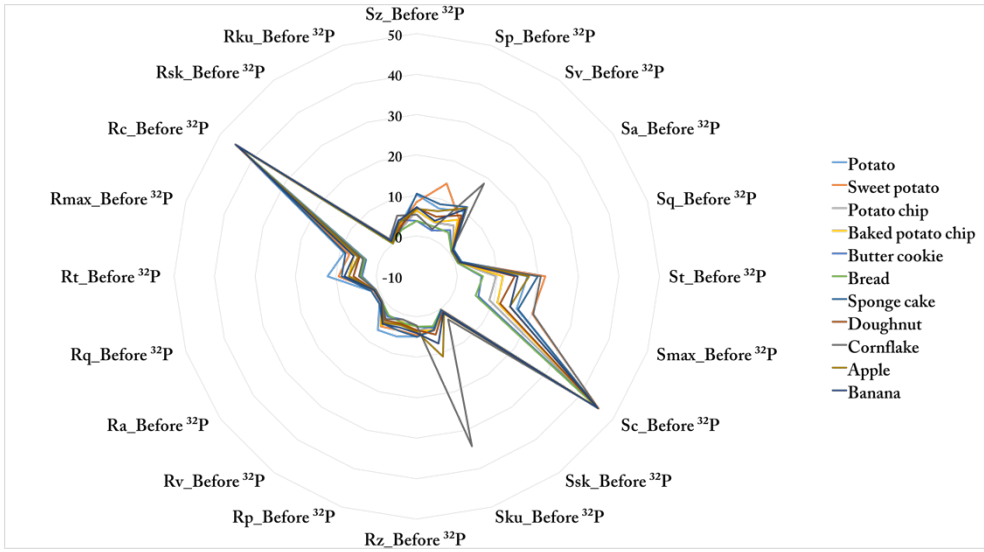
	Potato	Sweet potato	Potato chips	Baked potato chips	Butter cookie	White bread	Sponge cake	Doughnut	Cornflake	Apple	Banana
Sq_After ³²P	1.641	1.387	1.641	1.376	2.57	1.026	2.112	1.447	1.592	2.795	2.564
Sq_Before ³²P	1.687	1.196	1.07	0.919	0.678	0.699	1.663	1.183	0.922	1.098	1.315

Fig 2. Continued

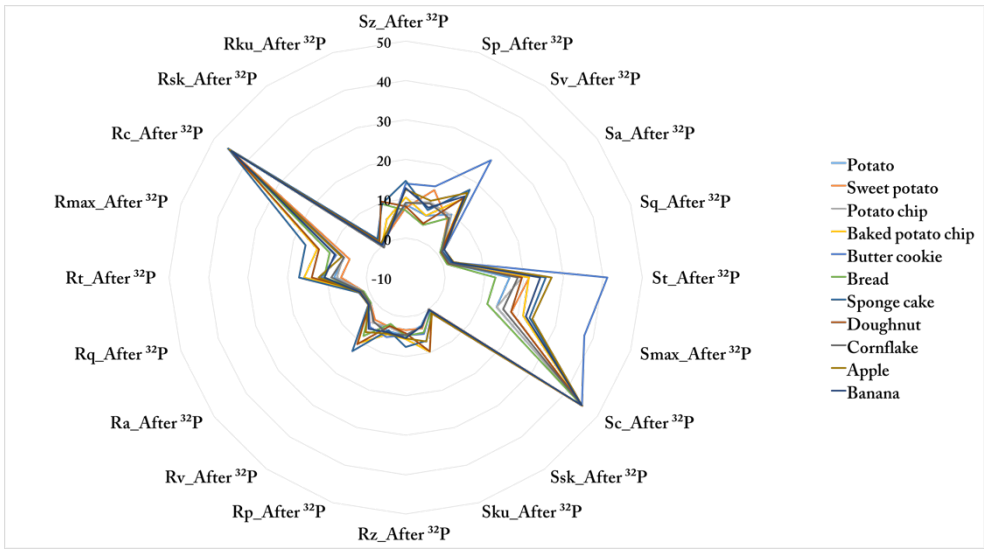
	Potato	Sweet potato	Potato chips	Baked potato chips	Butter cookie	White bread	Sponge cake	Doughnut	Cornflake	Apple	Banana
Rc_After ³²P	44.281	44.821	45.742	44.992	45.202	44.846	45.671	44.992	45.317	45.189	44.709
Rc_Before ³²P	44.694	45.266	45.227	44.722	45.326	44.908	44.202	45.155	45.453	44.962	45.168

	Potato	Sweet potato	Potato chips	Baked potato chips	Butter cookie	White bread	Sponge cake	Doughnut	Cornflake	Apple	Banana
Sc_After ³²P	45.202	45.315	45.227	45.202	45.227	45.265	45.252	45.278	45.227	45.303	45.215
Sc_Before ³²P	45.227	45.591	45.227	45.227	45.302	45.29	45.34	45.202	45.415	45.353	45.453

Fig 2. Continued



Before

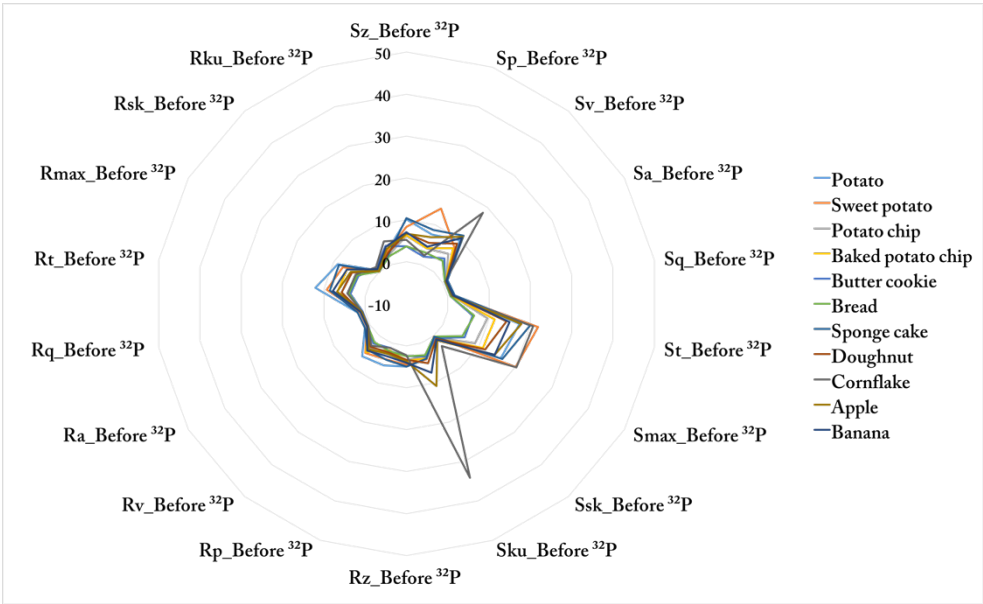


After

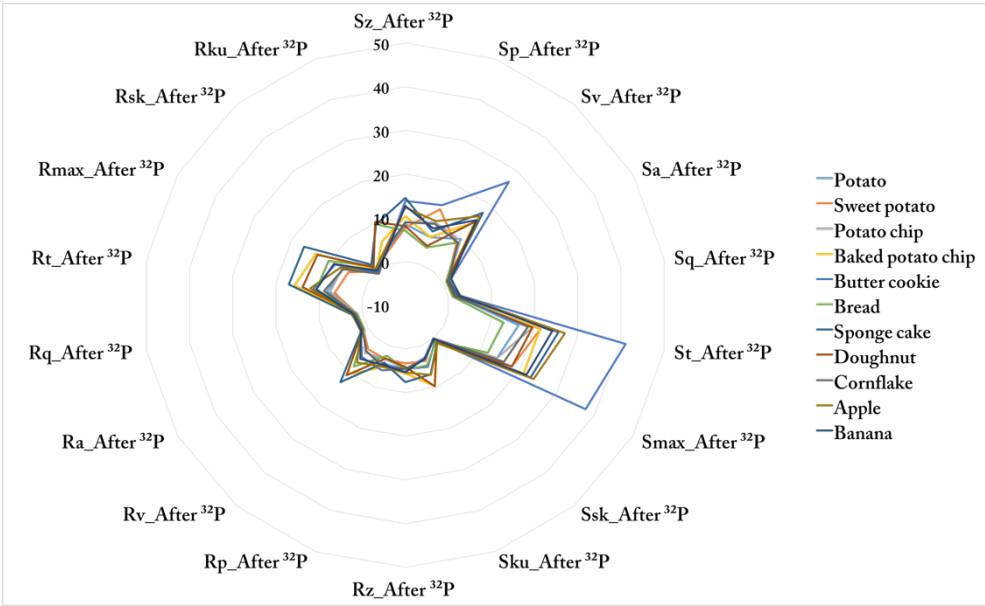
Fig 3. QDA Profile Comparison of CLSM Parameter in Test Foods (before and after)

Both 2D and 3D characterized surface parameters' measurements by food samples were provided. Upper radar graph presented preinduction of samples, and bottom graph presented the induced sample.

Sz, maximum height of the surface; Sp, maximum peak height; Sv, maximum valley depth; Sa, arithmetic mean height; Sq, root mean square height; St, total height; Smax, maximum roughness depth; Sc, mean height of the roughness profile elements; Ssk, skewness; Sku, kurtosis; Rz, maximum height of the surface; Rp, maximum peak height; Rv, maximum valley depth; Ra, arithmetic mean height; Rq, root mean square height; Rt, total height; Rmax, maximum roughness depth; Rc, mean height of the roughness profile elements; Rsk, skewness; Rku, kurtosis.



Before (without Rc & Sc)



After (without Rc & Sc)

Fig 3. Continued

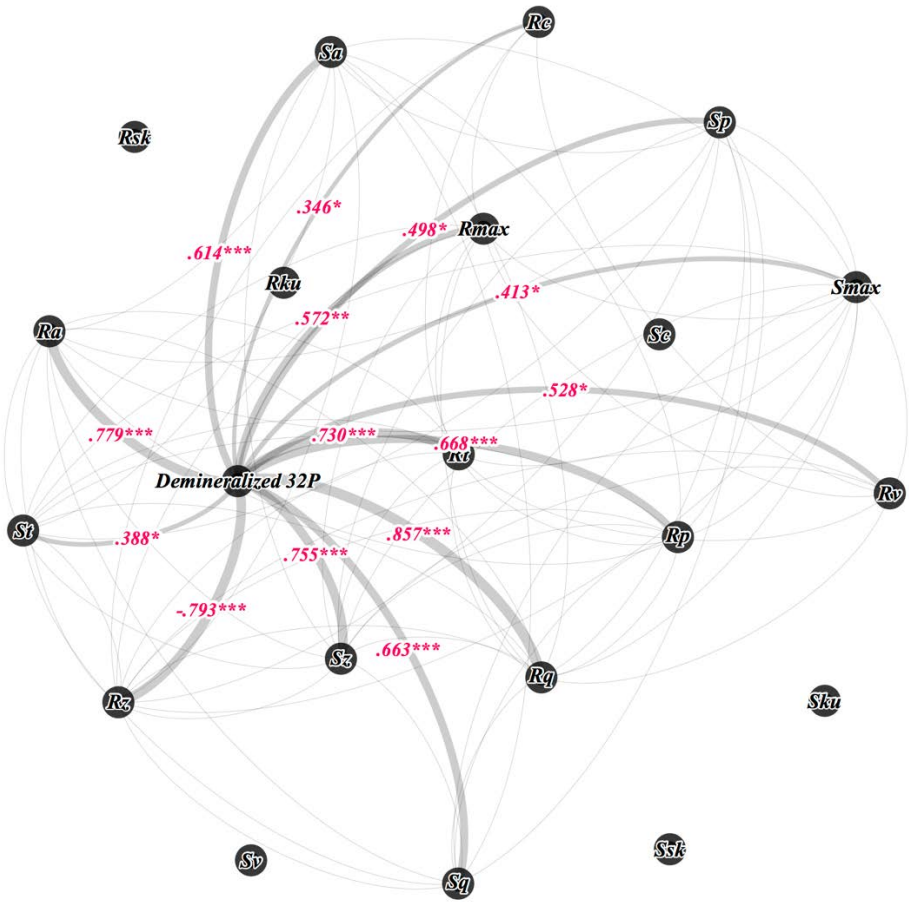


Fig 4. Pearson Correlation Coefficients (r) of CLSM Parameters and Demineralized ^{32}P

Fruchterman Reingold model based on network visualization

Every node except Demineralized ^{32}P indicates individual parameters from CLSM.

The thickness of the edge and annotated numeric values represent the degree of r-value from Pearson correlation result and only applied to the correlation between Demineralized quantity ^{32}P and CLSM parameters. Thin edges between b) non- demineralized ^{32}P nodes indicates the significant correlation between parameters ($p < 0.05$).

$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

3.4 Modeling of Caries-associated Variables

Table 11 showed the Pearson correlation coefficients (r) of caries-associated variables. Total starch, hydrolyzed starch, titratable acidity, reducing sugar and TPA (hardness, cohesiveness, chewiness and adhesiveness) were significant at $p < 0.001$, moisture, pH, total sugar and springiness presented $p < 0.01$ significance and total viable cell count of *S.mutans* were significant at $p < 0.1$.

Fig. 5 represented the scatterplot matrices by Pearson correlation visualization of carries-associated variables. Scatterplot matrices showed all the pairwise scatter plots of the variables on a single view. Negative correlations are represented as negative gradient ellipses form and positive correlations as positive.

Table 12 showed the stepwise regression analysis of caries-associate variables. Hydrolyzed starch, adhesiveness, total viable cells of *S. mutans*, moisture content and titratable acidity affected cariogenic potentiality at $p < 0.001$, reducing sugar presented $p < 0.01$ significance, springiness at $p < 0.05$ and an adjusted R^2 at 0.904 showed $p < 0.05$ significance.

Fig. 6 showed stepwise regression of caries-associate variables in the scattering form. An adjusted r^2 value was also provided in a single format. X axis represents the resulting model from the stepwise regression analysis. Y axis indicates r value for significance which represented in both positive and negative correlation coefficients. An adjusted r^2 is represented as a red line.

Table 11. Pearson Correlation Coefficients (r) of Caries-associated Variables and Demineralized ^{32}P

Caries-associated variables		**r**
Moisture content		-0.296**
Total starch		0.700***
Hydrolyzed starch		-0.735***
pH		-0.278**
Titratable acidity		0.515***
Total sugar		0.246**
Reducing sugar content		-0.653***
TPA (Texture Profile Analysis)	Hardness	0.366***
	Springiness	-0.315**
	Cohesiveness	0.337***
	Chewiness	0.340***
	Adhesiveness	0.699***
Total viable cell count of *S.mutans*		0.172*

*p < 0.1 ; **p < 0.01 ; ***p < 0.001.

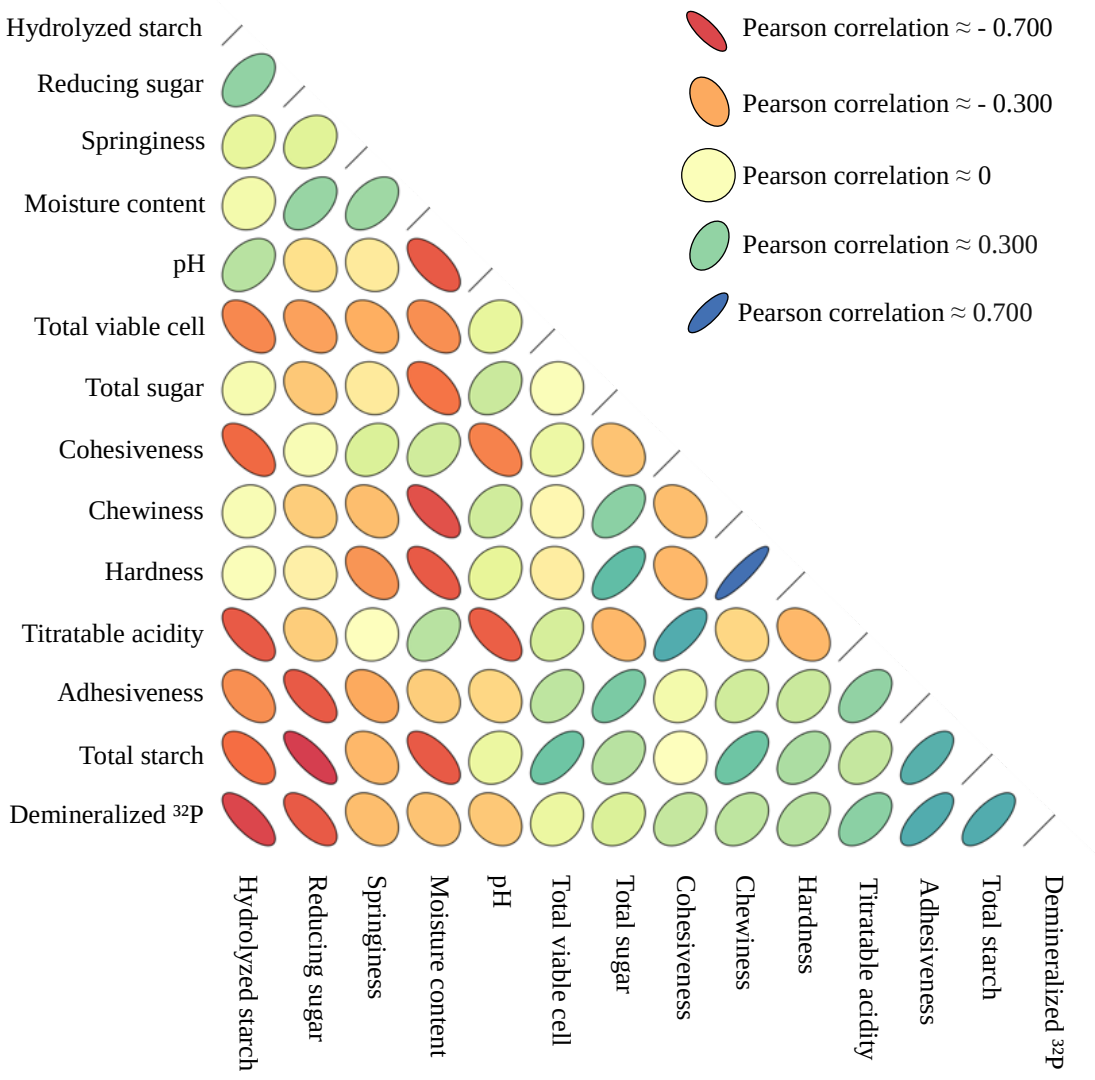


Fig 5. Scatterplot Matrices

Pearson correlation visualization of carries-associated variables. Scatterplot matrices show all the pairwise scatter plots of the variables on a single view. Negative correlations are represented as negative gradient ellipses form and positive correlations as positive.

Table 12. Summary of Stepwise Regression Analysis of Caries-associate Variables (dependent variable: demineralized ^{32}P)

Caries-associated variables	Model						
	1	2	3	4	5	6	7
Hydrolyzed starch	-0.735***	-0.522***	-0.653***	-0.739***	-0.579***	-0.548***	-0.537***
Adhesiveness		0.453***	0.499***	0.434***	0.298***	0.244***	0.203***
Total viable cell			0.316**	0.502***	0.592***	0.587***	0.601***
Moisture content				-0.350***	-0.586***	-0.543***	-0.516***
Titratable acidity					0.377**	0.375***	0.391***
Reducing sugar						-0.123***	-0.148**
Springiness							-0.087*
N	110	110	110	110	110	110	110
F	126.786	124.662	121.180	165.530	184.460	164.207	147.845
Significance	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Adjust R^2	0.536***	0.694***	0.768***	0.858***	0.894***	0.900**	0.904*

*p < 0.05 ; **p < 0.01 ; ***p < 0.001.

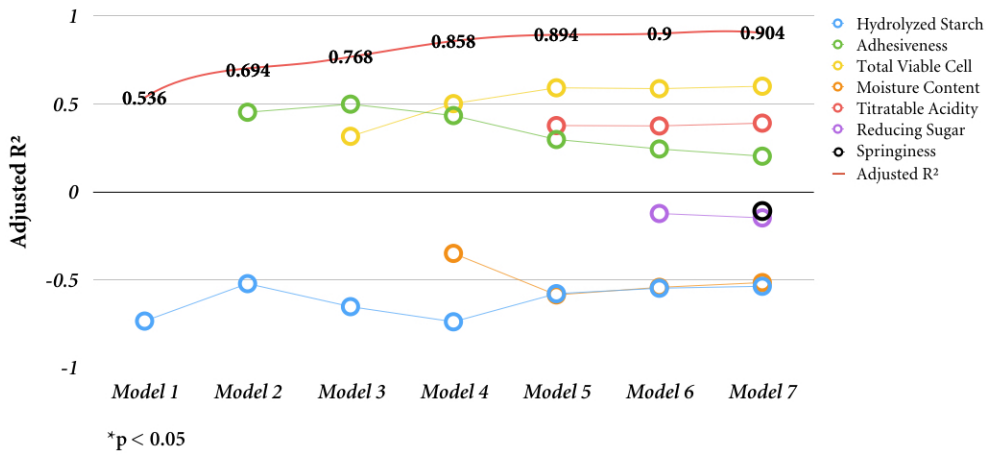


Fig 6. Stepwise Regression of Caries-associate Variables

X axis represents the final model from the stepwise regression analysis. Y axis indicates r value for significance which represented in both positive and negative correlation coefficients. A red line represents an adjusted r^2.

4. Discussion

Dental caries development is often referred as a multifactorial disease which reflects the status of our body system. This study concentrates on the diet, as an environmental aspect. The course of demineralization and remineralization process is mostly caused by diet. These consecutive burgeoning and receding, commonly known as a buffer, occurs based on varied physiochemical conditions. This study covers the multi-dimensional approach to validate interrelation between caries-associated variable factors.

Both quantity and frequency of sucrose intake have been recognized as a major promoter of dental caries (Bradshaw & Lynch, 2013). In a larger scope, fermentable carbohydrates are also reflected as a crucial mediator of dental caries development (Lingström *et al.*, 1994). However, the subject of starch-induced dental caries and whether it is independent or dependent (with sucrose) intermediary is an ongoing debate. Moreover, the demineralization effect of starch in oral biofilm has been remained in uncharted territory (Aires *et al.*, 2008). Some prior research disclosed a substantial increase of starch intake between-meal and the stickiness degree of starch within human oral throughout history (Bibby *et al.*, 1951; Bibby & Ludwig, 1957; Caldwell, 1970; Gustafson *et al.*, 1953; Mundorff *et al.*, 1990). Kashket *et al.* (1991) have dedicated to the retention of food particles on the teeth rather marking whole-mouth, soft tissues, and space within them.

Starch is naturally present in a number of different foods as heterogeneous form and refined to variable grades by diverse manufacturers in the food

industry. A rapid solidification in food texture is increased by adding sugar to starch, adhesiveness. Therefore, starchy foods such as cakes, biscuits, and sugary cereals may be a particular concern (Moynihan, 2002; Rugg-Gunn, 1990). Potato chips, cereal, cookies, and bread as part of high starch content foods showed more retention compared to low starch content foods. Within retained food mass, it showed an accumulation process of the starch breakdown products delivered with a large amount of retained starch, hence exhibited a cariogenic potential (Lingström *et al.*, 1993). It was observed that the level of cariogenic properties and demineralization process by frequent intake of starchy foods resembled intake of sucrose products. In short, stickiness property and prolonged retention caused by starch, induced demineralization process in enamel and dentin. This demineralizing effect was previously observed by Brudevold *et al*. (1985 & 1988), who suggested that heat-processed starch may have the potential of demineralization depend on gelatinization.

The degree of starch hydrolysis relies on the prime structure which presence in compact and semi-crystalline granules at the hydrolysis stage of raw starch. The molecular chains in the starch granule yields a hydrolysate of polysaccharides with lower molecular weight shorten during the hydrolysis of an aqueous suspension of starch (Martinez-Anaya & Rouzaud, 1997). This considerably increases their alteration by gelatinization (Holm *et al.*, 1988). A complete enzymatic hydrolysis of starch for total starch content measurement requires a disruption of this crystalline structure for a reasonable amount of time, otherwise undervalued results may be obtained

(Englyst *et al.*, 1982). Mainly, maltose (M_2), maltotriose (M_3), dextrin and low molecular weight carbohydrates diffuse into plaque by salivary α-amylase, during the course of rapid starch degradation (Brudevold *et al.*, 1985; Jacobsen *et al.*, 1972). The higher degradation of starch indicated by longer retention and salivary α-amylase. Such reaction act as a determinant, both in metabolic response and fermentability within the plaque. Its availability is affected by the physiochemical distinction of product including texture. By delivering moderate temperature and moisture condition, starch may partially gelatinize, which limits the amylase availability. To minimize the degradation of starch foods (by sensitive characteristics), the entire starch essay should be performed within a day. Also purified starch as a sample is required for the enzyme assessment, assay conditions, and technical efficacy (Hall, 2003). Table 5 showed the degree of hydrolysis of starch foods by latent time. Generally, as reaction time due to the degree of hydrolysis increased, and prominent difference occurred between 0 minutes and 90 minutes. After 180 minutes, an average degree of hydrolysis was 81.8% in test foods, and potato was the highest group which was completely gelatinized along with white bread. This research viewed that foods with high amount of starch are more susceptible to dental caries due to a longer retention time of residuals. Not only the prolonged retention caused by the starch, the stickiness property of entrapped food particles became the critical factor to increase cariogenic properties of starch food products.

It is commonly known that the dentin is highly susceptible to time spent

below critical pH, especially if it's prolonged. Frequent fermentable carbohydrates (i.e. sucrose) consumption has been suggested as a high cariogenic potential through plaque pH expression and acid production in plaque suspensions *in vivo* (Birkhed *et al.*, 1978; Edgar *et al.*, 1975; Jensen & Schachtele, 1983). Also, different intra-oral test systems have been implemented as an assessment of food's cariogenic potentiality and dietary carbohydrates on enamel and dentin demineralization (Lingström *et al.*, 1994).

Earlier studies have shown that boiled potato and potato chips give rise to great pH fall in dental plaque (Lingström *et al.*, 1993). Generally, increased amount of acid-producing micro-organisms and the plaque during frequent intake of fermentable carbohydrates were accounted, specifically to starch and sucrose exposure. An observation of different source of cariogenic potentials including starch and starchy products have supported this phenomenon (Rugg-Gunn *et al.*, 1986). By boiling, extrusion cooking, or popping, the starch granule was gelatinized, with partial degradation, thereby prone to salivary α-amylase. Preceding animal plaque-pH studies have shown a rather high cariogenic potential of starch foods (Grenby, 1996). Bread showed plaque pH rise in 30 minutes which represented overall similar result to similar reports (Rugg-Gunn *et al.*, 1981). Lingström *et al.* (1994) stated that bread manufactured with wheat or barley initiated a pH drop in 15 minutes (Mundorff-Shrestha *et al.*, 1994; Utreja *et al.*, 2009). All baked wheat products have shown cariogenic potential throughout a group of studies (Mortazavi & Noin, 2011). Luke *et al.* (1999) discovered dental caries risk of white bread.

Edgar grouped frequently consumed sugar content foods into high-cariogenic and low-cariogenic based on pH within the plaque. A rapid pH decrease has been presented in high-cariogenic food groups, including cereal, doughnuts, biscuit and etc. Edgar also claimed that the higher acidity induced by specific foods within oral can stimulate salivary flow, clearance of sugars and other fermentable carbohydrates, which could possibly trigger dental caries ($pH < 5.5$) and dental erosion ($pH < 4.0$). Cariogenic potential also depends on the neutralizing capacity of the oral buffer system. Test food groups including potato, chips & cookie, bread, cereal, fruit were assorted by average pH & titratable acidity (Table 5). Apple had comparatively high cariogenic potential among test foods. An analysis between caries scores and caries-associated variable factors has shown that pH and reducing sugar in test foods affected cariogenic potentials significantly ($p < 0.001$).

Whether the food is dietary cariogenic, anti-cariogenic or cariostatic may determine the following properties; consumption frequency of sugar and other fermentable carbohydrates, food form, nutrient composition, retention time, the potential of salivary stimulation, and combinations of foods (König, 2000; Lingström *et al.*, 2000). Sugar, along with other fermentable carbohydrates is closely associated with high caries risk foods and its cariogenic potential has been extensively reviewed (König, 2000; Kashket *et al.*, 1991 & 1996; Lingström *et al.*, 2000; Touger-Decker & van Loveren, 2003). An inescapable relationship between sugar and dental caries is

associated with physicochemical features of food, including texture, solubility, particle size, retentiveness, and particularly stimulation of salivary flow and chemical changes, which affects the clearance time. The formation is also a critical component in terms of fermentable carbohydrates' cariogenic potential since it directly affects the duration of exposure and retention, ultimately to acid production and demineralization (Kashket *et al.*, 1991 & 1996; Lingström *et al.*, 2000). The cariogenicity of foods, such as refined carbohydrates may also depend on several variables (salivary flow, contact time of the product with the plaque, bacterial constituents of the plaque, and individual plaque control), which maneuver the period and frequency of "demineralizing mode" over the plaque. Also, the cariogenic potential of food, incorporating refined carbohydrates depends on such variables as saliva flow, contact time of the product with the plaque, bacterial constituents of the plaque, and individual plaque control measures; all of which help determine how long and how often the plaque is in a "demineralizing mode." Typically, intake of high-sugar content foods forms dextran by *S. mutans* which provide sticky substance. In consequence, acid production leads to an inability to be neutralized by buffer capacity, causing the oral cavity. Therefore, relatively high total sugar and reducing sugar in test foods are considered to be cariogenic than other foods.

Simple carbohydrates -sucrose, fructose, lactose, galactose have been considered as cariogenic. When these sugars situate in plaque, they easily converted to fermentable sugars which contributes dental caries as a major factor as mentioned above. The demineralizing potential of sugar was

observed by Brudevold *et al*. (1988) which leads to the direct relationship between acid production and enamel decalcification. In particular, fructose, glucose and sucrose caused the most demineralization, lactose and maltose follow as moderate while galactose showed the least causation. A sucrose baked in forms of bread, cookies with starch was even more cariogenic because of their slow clearance time inside oral and the acid production continued even after the food has been cleared. The role of fruit sugars (i.e. glucose, fructose, and sucrose) in the initiation of caries has been disputed throughout the prior research (Sheiham, 2001). Some data suggested that the consuming unnecessary amount of fruit (apples and grapes) might affect the level of dental caries (Grobler & Blignaut, 1989), which was supported by Edgar (1993), who suggested that in terms of cariogenic potentiality, fruits exhibit properties which didn't exonerate them were they to be consumed at a frequency similar to other foods (Beighton *et al*., 2004). Although the quantity of sugar intake may affect the total amount of acid production, this suggests that certain fruits might consist comparative acidogenic potential to sucrose (Hussein *et al.*, 1996; Pollard, 1995). Through the rat experiment, Mundorff *et al*. (1990) showed that fruit diets including grapes, apples and bananas denoted noteworthy caries potential. However, the question is whether free sugar constituents (i.e. glucose, fructose, and sucrose) pose a cariogenic effect when consumed as a whole. Intrinsic sugars inside fruits in natural state have been suggested as non-cariogenic, since the absence of fermentation induced by dental plaque bacteria. On the other hand, extrinsic sugars have shown cariogenic potential, due to the process of fermentation

into demineralizing organic acids (Beighton *et al*., 2004). A wide range concentrations of sugar were observed over different kinds of fruits at a different stage of ripeness and etc. Due to the disaccharide derived polymer formation, some certain fruits presented the greater amount of acid production than 10% sucrose.

In terms of controlling dental caries, potential substitutes for sucrose such as coupling sugar has been suggested. The present study reports the effects of one of the coupling sugar preparations derived from starch, which is rendered free of sucrose, and the effects of sucrose on various metabolic activities of *S. mutans*. This includes acid production, glucan synthesis, cellular aggregation, cellular adherence to glass surfaces, and *in vitro* plaque formation (Ikeda *et al*., 1978). Present research focused on the synergistic effect of starch and sucrose combination related to amylase activity, microbiological and biochemical composition thru biofilm foundation, acidogenicity, and the relationship between the mixture and the demineralization of deciduous dental enamel (Ribeiro *et al.*, 2005).

As for interfering substance, determined by the glucose or reducing sugars, any materials, which increase or decrease the starch content from its original value have shown relevant outcome. Through the procedure, low molecular weight carbohydrates may act as a hindrance which can be demoted by pre-extraction (80% ethanol (v/v)) before the residual and starch analysis. Using enzyme untreated sample blank and subtracted value of glucose released by hydrolysis is a viable method for measuring free glucose, which requires sufficient purity in subject’s enzyme to prevent the hydrolysis of non-starch

carbohydrates. In a short term, when the substance interference come across through the study, the type of sample which needs to be analyzed should be considered. For instance, the mature grain will have a potential inference such as sugar remains within the analysis. As for the byproduct feeds (i.e. bakery waste), carbohydrate interference may be an issue.

It is important to identify the physical properties of food regarding the retention or breakdown in oral, since its commonly recognized as a cariogenic determinant. Furthermore, an inquisition about the odds altering the physical properties of food also needs to be answered (Bourne, 2002; Caldwell, 1970). A texturometer imitates the mouth's biting action (occlusion) using multiple compression and examine force (g) as y-axis and time (second) as x-axis to determine the textural properties of foods. The characterization of food's textural identity involves several other parameters to quantify multiple textural attributes in one instance and control the consistency of production unit. Primary factors, a mechanical boundary involves hardness, cohesiveness, springiness or elasticity, adhesiveness, and secondary factor includes chewiness, fracturability or brittleness, gumminess. Resilience is classified as a tertiary parameter which measures how well a product "fights to regain its original height". Chewiness and gumminess are rather expressed through calculation which utilizes the primary factor as dependent. Hardness indicates the degree of rigidity by measuring the peak force in first compression (unit: N). Adhesiveness represents attractive forces between the surface of the food and the surface of other materials with which the food comes into contact

(unit: J). Cohesiveness is the attractive forces between the surface of the food and the surface of other materials with which the food comes into contact. Springiness is the ratio of the height the sample springs back (D_2) after the first compression (D_1) compared to the maximum deformation. If the division presents the value lower than 1, the springiness (elasticity) is little. As a product of hardness, cohesiveness and gumminess require a disintegration of a semi-solid food product to a state ready for swallowing. It applies to semi-solid products only if they have no springiness and undergoes permanent deformation throughout the process. Chewiness is somewhat similar to gumminess in terms of derivation, however, includes springiness and represents the energy required for chewing a solid food.. Though the texture profiling may bear some limitation such as handling pressure sensitive subjects, it is the most general recreation of dental occlusion to explain the composition of starchy foods.

In the case of corn starch (amylopectin) becomes substantial and cohesive as granules inflate in boiling condition with 8% of starch solids. When granules start to broke, the viscosity decreases while the cohesiveness and the clarity of the solution remain within the texture. This behavior is generally reflected on where the fraction of amylopectin is in dominance. Through the process of cooling the amylose modifies the suspension of products and transfer it to nontransparent, solid gel. This depends on the molecular weight of amylose. If it's too high the gelatinization may not take place due to gradual water resistance. On the other hand, if it's too low the portion of the amylose may come out to make a temporary bond. Therefore, adhesiveness

refers as an existence of amylose and amylopectin properties. The dispersions can be altered by the modification of starch, which is primary to decrease the starch component's molecular weight allowing a higher solids content through formulation.

However, the subject of this study is a solid type of starch foods *in vitro*. Which means viscosity cannot be applied in this circumstance, thus require soluble substance such as saliva *in vivo*. The hardness of test food groups showed the highest mean of 9050.9 by cereal group and the lowest mean of 557.5 by potato group. It was found that banana has the lowest correlative value ($p < 0.05$) between texture profile, including hardness and chewiness while cereal presented the highest. For this reason, a low degree of properties within hardness · chewiness and high value in adhesiveness considered to represent relatively longer residual time in oral, causing dental caries in a result.

Acidogenicity test is the only indirect measurement between the quantification of cariogenicity which measures plaque responses. Generally, plaque pH studies presented an excellent reproducibility, though its cost-effectiveness and the degree of complexity have always been an issue. As an alternative, this research introduced a method which overcomes the compromises, as a reproducible and reliable approach *in vitro*. Instead of human dental, a hydroxyapatite polyacrylamide discs were utilized, then the amount of ^{32}P released from the radioisotope PAHA was measured. Measuring microhardness of PAHA with several concentrations (20%, 50% and 70%) using microhardness tester was unmanageable since concentration

degree at below 60% was too fragile, and over 60% was too hard. Therefore, a polyacrylamide gel with 60% concentration was the best candidate. After the irradiation, the cariogenicity was quantified by the amount of ^{32}P was released. An OD measurement was performed with viable cells of *S. mutans* contained saliva (count per 1 ml saliva). To establish the pertinent incubation time, the variation of OD was calibrated maximum value between 90 to 150 minutes. Table 9 showed a released ^{32}P from PAHA in different sugar concentrations. Through one-way ANOVA analysis, the average amount of released ^{32}P at different sugar concentrations (0.5%, 2%, 5%, and 10%) showed the difference and 10% sucrose as a control showed the minimum standard deviation. According to the Scheffe's test, the average amount of released ^{32}P in sugar concentration at 5% and 10% showed the significant difference ($p < 0.05$). Since this study adopted a 10% sucrose as a standard, the 10% sugar solution was reproducible when PAHA sample was arbitrarily evaluated ($p < 0.05$). A comparable analysis based on a volume of ^{32}P released from PAHA radioisotope-labeled in test and the standard subject has substantiated to be inconsistent.

Table 10 showed the amount of dissolved PAHA in starch samples. The values have presented both in arithmetic mean and ratios to sucrose control. All experiments performed in both control and test foods, the results proved to be reproducible ($p < 0.05$).

Fig. 1 indicated that the amount of ^{32}P released was less in baked potato case in comparative to 10% sucrose control. Its surface roughness had less deviation toward than the control. A number of released ^{32}P in simple sugar

composed cereal showed higher amount than control. These samples accelerated the growth of *S. mutans,* which demineralized the radioisotope-labeled PAHA surface. An inconsistency has been identified with subjects which showed the higher acid production than the 10% sucrose; this anomaly has been observed in previous research (Andlaw, 1960; Bibby & Mundorff, 1975; Khanna & Bibby, 1966; Soni & Bibby, 1961). The pre-processed food subjects induced PAHA (Lee *et al.*, 2015) was analyzed with surface areal amplitude parameters through CLSM. Such fields and subjects including biomaterials and dental implants regularly covered the diverse surface roughness parameters as limited amount of consideration (Al-Omari *et al.*, 2001; Elias *et al.*, 2008), though this application wasn't submitted to oral biology and studies including cariogenic potential. A mean height of the surface roughness (*Ra* & *Sa*) and statistically stable root mean square height (*Rq* & *Sq*) were predominant due to its conventional usage and ease of applicability. The nature of arithmetic mean height and root square mean height stands as an overall average measure of texture finishing. This explains the fundamental characteristics of these parameters' insensitiveness in discerning peaks, valleys, disregards the spatial structure, moreover variety of general topographies. The study supported that other than parameters indicating a mean height of the surface have shown distinctive aspect of starchy foods. Parameters such as skewness (*Ssk*) and kurtosis (*Sku*) presented predominance of peaks and valleys and presence of inordinate peaks and valleys correspondingly.

This study covered multi-dimensional approach to validate interrelation

between caries-associated variable factors using CLSM. Simulating organic oral model with PAHA induced then incubated with starch content substance and *S. mutans* respectively. CLSM values depended exquisitely on arithmetic mean height (*Ra: 2D, Sa: 3D*). Surface mean height (*Sa*) and roughness profile mean height (*Ra*) is a commonly used parameter in various field of research. Practically the magnitude of a single injury (i.e. maximum μm of valley and peak) in roughness becomes minimal therefore stabilized results are expected (Whitehouse, 1982 & 2002).

However, this research was based on organic, physiochemical phenomenon, which implies non-equally distributed variance of surface roughness is necessary. Although the precedential usage of Ra and Sa may represent average absolute values, a significant tendency of demineralization or remineralization in the comparative analysis was barely observed. Each figure from CLSM results with before and after demineralized difference (Δ) proves the point since Ra and Sa parameter showed the subtlest gradient among other parameters. Whereas, most other criteria showed significant slopes. Also, apple showed an abrupt, steep surface alteration. However, due to normalization of arithmetic mean evaluation in few instance of injury, a single parameter virtually disregarded this variation, while total height of surface roughness (*St, Rt*) expressed the erosion on the surface clearly. It is considered that in order to acquire comparative trends of demineralization and remineralization, an analysis between full scopes of given parameters from CLSM including skewness, kurtosis, valley depth and etc. are worth to take account (Hansson & Hansson, 2011).

The main limitation of using *in vitro* methods lies in false-premise which the results could possibly simulate *in vivo*, especially in a single agent. The present investigation embedded this drawback to devise a feasible method (West *et al.,* 1998). This study adopted a method which quantifies ^{32}P loss from neutron-activated PAHA and has been shown to give reproducible results in earlier experiments (Asp, 1995; Khanna & Bibby, 1966; Ribeiro *et al.*, 2005; Sheiham, 2001). This method may prove its validity for measuring demineralization *in vitro*.

Although this research does not represent clinical significance nor *in vivo* study of the cariogenic source, the result clearly illustrated the prospect of starch's cariogenicity *in vitro*. Further studies will cover the *in vivo* perspective using *S. mutans* in human saliva.

5. Conclusions

Starch is considered as nutritionally superior to other low-molecular-carbohydrates because of its slow degradation and absorption. Yet, current studies reflect incoherent claims about the cariogenic potentiality of starch. In terms of cariogenic potential, starch in foods was less considered as contributing factor than sugar or other aspects, against its general usage in dietary carbohydrates.

Test subjects analyzed by traditional physicochemical and microbial methods including starch content, hydrolyzed starch, pH, titratable acidity, total sugar, reducing sugar, TPA, and viable cell count after inoculation of *S.mutans*. This multivariate analysis indicated that as the proportion of starch hydrolysis increases the cariogenic potentiality decreased. Compared to 10% sucrose control, while titratable acidity and reducing sugar presented a higher tendency to demineralization, pH showed the least drift. With Pearson correlation coefficient (r) of caries-associated variables including total starch, hydrolyzed starch, titratable acidity, reducing sugar, and TPA results including hardness, cohesiveness, chewiness, adhesiveness was significant at $p < 0.001$ and pH, total sugar and springiness (TPA) presented $p < 0.05$ significance. Hydrolyzed starch, adhesiveness, total viable cells of *S. mutans*, moisture content and titratable acidity affected cariogenic potentiality at $p < 0.001$, reducing sugar presented $p < 0.01$ significance, springiness significant at $p < 0.05$ and an adjusted R^2 at 0.904 showed $p < 0.05$ significance, analyzing caries-associated variable factors by stepwise regression analysis.

The results suggested the possible cariogenic potential of starch and the the legitimacy of measuring ^{32}P released from radioisotope-labeled PAHA disc with a liquid scintillation counter, CLSM, and SEM as measuring demineralized quantity and degree by foods. Without compromising in cost and degree of intricacy, PAHA disc model has verified its reproducibility and reliability *in vitro*.

Our diet, specifically a varied physicochemical and microbial properties of food is closely associated with oral health. In this research, such variables including the residual period in oral, physical properties of food, intra-oral acid production, caries preventive effect of certain food ingredients, oral bacteria constitute, amount and types of sugar were evaluated through multiple methods. This indicates a solitary variable cannot determine the cariogenic potential of starchy food, and the development of dental caries is multivariate *in vitro*.

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국문초록

전분식품의 치아우식유발력에 관한 연구

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전분은 널리 사용되는 식이 탄수화물로서, 단당류 및 이당류와 같은 저분자 탄수화물보다 느린 분해력과 흡수력을 가지고 있지만 치아우식유발력 측면에서 전분에 대한 연구결과는 일관성을 나타내지 않고 있다. 따라서, 본 연구에서는 전분식품을 대상으로 치아우식유발에 영향을 미치는 다양한 인자(물리·화학적 특성 및 미생물학적 특성) 간의 상관 관계를 분석하고, 재현도와 신뢰도가 높은 방사능 수산화인회석 시편 (radioisotope polyacrylamide hydroxy-apatite disc, PAHA)을 사용한 ^{32}P 방사성동위원소측정법으로 탈회량을 정량화하여 치아우식유발력을 검증하고자 하였다.

총 11가지 전분식품은 5개 실험군으로 분류하여 물리·화학적 특성 및 미생물학적 특성 (총 전분 함량, 수분 함량, 가수분해전분, pH, 적정 산도, 총 당, 환원당, 기계적인 물성, *S. mutans* 접종 후 총 생균수)을 분석하고, 폴리아크릴아마이드 수산화인회석 시편의 ^{32}P 방사성동위원소 탈회량은 액체섬광계수(liquid scintillation count), 탈회 정도는 공초점레이저주사현미경 (CLSM)과 주사전자현미경 (SEM)으로 측정하였다.

치아우식유발에 영향을 미치는 이화학적 특성의 일반 성분을 분석한 결과, 수분 함량은 32.3%이었고, 총 전분 함량은 67.4%이었으며, 가수분해전분은 9.3%이었고, pH는 5.8이었으며, 적정 산도는 0.38%이었고, 총 당은 245.1 ㎎/g 이었으며, 환원당은 17.5 ㎎/g이었고, 경도는 2409.0이었으며, 탄력성은 0.57 이었고, 응집성은 0.43이었으며, 검성은 621.5이었고, 점착성은 -38.8 이었으며,

S. mutans 접종 후 총 생균수는 2.22×10^6/㎖ 이었다.

치아우식유발에 영향을 미치는 물리화학적 특성 및 미생물학적 특성과 폴리아크릴아마이드 수산화인회석 시편 모델에서 산출된 탈회량과 상관관계를 분석한 결과, 총 전분 함량, 가수분해전분, 적정 산도, 환원당과 기계적 물성 중 경도 · 응집성 · 씹힘성 · 점착성 ($p < 0.001$), 수분 함량, pH, 총 당, 기계적 물성 중 탄력성 ($p < 0.01$)이 통계적으로 유의한 것으로 분석되었다. 단계적 회귀분석결과 가수분해전분, 점착성, *S. mutans* 접종 후 생균수, 수분 함량, 적정 산도는 유의수준 0.001에서, 환원당은 유의수준 0.01에서, 탄력성은 유의수준 0.05에서 유의한 것으로 분석되었다 (수정결정계수; adjusted $R^2 = 0.904$, $p < 0.05$).

전분함유식품의 우식유발력을 평가하기 위해 치아우식유발에 영향을 미치는 이화학적 특성과 폴리아크릴아마이드 수산화인회석 시편 모델에서 산출된 탈회량을 다변량분석으로 분석한 결과, 폴리아크릴아마이드 수산화인회석 시편 (^{32}P 방사성동위원소)의 탈회량은 생체외 실험 (*in vitro*)으로 수집된 이화학적 인자들과 유의한 양상을 나타내었다 ($p < 0.05$).

전분함량이 높은 식품은 구강 내에서 타액 α-amylase에 의해 빠르게 가수분해되어 맥아당, 말토트리오스, 덱스트린, 포도당으로 전환되어, 큰 전분입자보다 빠르게 플라그 속으로 확산되기 때문에, 구강 내 잔류 시간이 길고, 치아에 부착성이 높아 치아우식유발력이 높을 것으로 검토되었다.

주요어: 우식유발력, 공초점주사레이저현미경, 폴리아크릴아마이드 수산화인회석, 환원당, 전분, 물성분석

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