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ASSESSMENT OF PREBIOTIC PROPERTY OF SOME VIETNAM'S AGRO PRODUCTS AND THEIR APPLICABILITY

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SUMMARY

In nature, prebiotics occur not only in fruits and vegetables but also in the seeds from fruits. This study focused on surveying the prebiotic property of the kernel fibers from eight Vietnam's agro products which were jackfruit, avocado, rambutan, longan, durian, tropical almond, sesame, and lotus seeds. Kernels were investigated for total sugar, soluble protein, and fat contents. The highest total sugar content was found in lotus seed kernel (37.46%) but in kernels from sesame and tropical almond the value only was 2.42 and 3.07% respectively. Rambutan seeds had the highest fatty (54.3%) and soluble protein content (4.49%) while in kernel from durian had the lowest fatty (19.66%) and dissolved protein content (0.69%) among the studied seeds. Kernel powders were defatted, digested by enzymes: α – amylase, glucoamylase and neutrase, then extracted by 96% ethanol to obtain soluble fibers. The prebiotic potential of these fibers was investigated by the growth stimulation of four probiotic strains: *Bifidobacterium animalis* AP1.2, *Bifidobacterium bifidum* CNTP 6599, *Lactobacillus casei* PK2 and *Lactobacillus fermentum* SBV2. The highest increase of viable cells, compared to the control, was 0.52 lg (CFU/ml) found in medium supplemented with kernel fiber from longan, fermented by *L. casei* PK2. This fiber also stimulated the growth of four probiotics. However, the soluble fiber content obtained from longan seeds is the lowest among the types surveyed (0.02%). The fiber in jackfruit, avocado or lotus seeds also has a positive prebiotic index, except for *L. fermentum* SBV2, in which jackfruit and lotus seeds are still commonly used in Vietnamese life.

Keywords: Bifidobacterium, Lactobacillus, prebiotics, probiotics, soluble fiber

INTRODUCTION

Prebiotics are polysaccharides and oligosaccharides that cannot be digested and absorbed in the small intestine but can be selectively fermented by probiotic bacteria in the large intestine. By the fermentation of prebiotics, probiotic bacteria improve the host's health by enhancing the absorption of minerals such as Ca, Mg, and Fe, producing compounds capable of preventing colon cancer and reducing the numbers of pathogenic microorganisms (Gibson, Roberfroid, 1995). Prebiotics occur naturally in variety of fruits and vegetables such as asparagus, onion, cereals, garlic, artichoke, chicory, banana. Moreover, prebiotics are found in seed kernel from fruits. Recently, some studies on extraction and prebiotic activity evaluation of seed fiber from fruits: mango, jackfruit, avocado, cranberry have been reported (Barbosa – Martín *et al*., 2016; Majeed *et al*., 2018; Wichienchot *et al*., 2011).

In Vietnam, the annual yields of agroproducts such as avocado, jackfruit, durian, longan are huge. Therefore, the seed weight rates from 9.5% (rambutan) to 20 – 25% (jackfruit and durian) of the fruit weight (Andri et al., 2020) make these seeds as plentiful by – products. In the old time of our country habit, some of the fruit kernel types were consumed as snack food. This leads us to an idea to investigate these materials for potential prebiotic properties. In this study, soluble fibers extracted from kernels of jackfruit, avocado, rambutan, longan, durian, tropical almond (*Terminalia catappa*), sesame and lotus seeds were investigated prebiotic potential by growing of four probiotic strains belonged to popularly accepted probiotic genera *Bifidobacteria* and *Lactobacilli*, which were *Bifidobacterium animalis* AP1.2, *Bifidobacterium bifidum* CNTP6599, *Lactobacillus casei* PK2 and *Lactobacillus fermentum* SBV2.

MATERIALS AND METHODS

Materials

Eight types of fruits and seeds purchased from the local market. Kernels were dried, then cut and ground by blender. The seed powders were stored at 4ºC until for use.

Four strains *B. animalis* AP1.2, *B. bifidum* CNTP 6599, *L. casei* PK2 and *L. fermentum* SBV2 were obtained from FIRI's collection. These strains were component of commercially available FIRI-Probiotics supplement product. The *Bifidobacterium* strains required 0.05% L-cystein HCl in medium for growth.

Determination of fatty content

The fatty content was determined by solvent extraction method described by Abdelazim *et al*. (2013) with modification. The dried kernel powders (30 g) were defatted by mixing with 120 ml of *n* – hexane at room temperature for overnight and then filtered through filter paper. The obtained solids were washed twice with distilled water and then either used for fiber extraction or dried to constant mass to calculate the fat content as follows as:

Fat content (%) = (the weight of dry sample – the weight of defatted dry solid)/ the weight of dry sample] *100

Soluble fiber extraction

Soluble fibers were extracted and determined according to Lee *et al*. (1992), with modification. The defatted seed powders were added to 8 times volume (w/v) of 0.1 M phosphate buffer solution, pH 6. The mixtures were digested by incubating with (0.1% of dry materials) enzymes: termamyl, at 90ºC for 30 min; neutrase, at pH 7.5 (adjusted by NaOH 0.1N) and glucoamylase at pH 4.5 (adjusted by HCl 0.1 N), both at 60ºC for 30 min. The enzymatic digestion was terminated by heating at 100ºC for 10 min. After filtrating by six layers cheesecloth, the liquids were centrifuged at 10000 rpm, 20ºC for 5 mins. The supernatants were used to both determinate dissolved protein amount and extract soluble fiber.

For extraction, the filtrates were mixing with 4 times volume (v/v) of 96% ethanol. The precipitates, obtained from centrifugation at 10000 rpm, 10ºC for 10 min, were washed twice with 96% ethanol then dried either stored at -20ºC until used for fiber fermentation test or calculated soluble fiber content according to following equation:

Soluble fiber content (%) = [(the weight of dry precipitate – the weight of dissolved protein)/ the weight of dry seed powder] *100

Fermentation of soluble fiber from kernels

The fermentation of prebiotic fiber by probiotic is an important aspect for determination of prebiotic activity of a fiber. It was performed by adding cell biomass at a rate of 10^6 CFU/ml of each strain to separate tubes containing MRS broth with 1% (w/v) either glucose, used as control, or one of the studied fibers in the same amount equivalent with glucose. The cultivation was done at 30ºC for 22 h (*Lactobacillus* strains) and in anaerobic condition, at 37ºC for 16 h (*Bifidobacterium* strains). After fermentation, the growth of probiotic is evaluated by the number of colony forming units on MRS agar plates, incubating at 30ºC or 37°C for 48 h, the results expressed as lg (CFU/ml).

Determination of total carbohydrate

The total carbohydrate amount was estimated by the phenol sulfuric acid method (DuBois *et al*., 1956).

Determination of dissolved protein concentration

The dissolved protein content was measured by the Lowry method (Waterborg *et al*., 1984).

Prebiotic index

Prebiotic index (I_{preb}) was calculated according to the description by Figueroa – Gonzalez *et al*. (2019). It is the ratio of probiotic growth in the prebiotic to probiotic growth in a control carbohydrate. A prebiotic index higher than 1 means that the carbohydrate has a positive effect on the probiotic growth. If the prebiotic index is near to 1, indicates a low effectiveness of the evaluated carbohydrate. The prebiotic index was calculated according to equation:

I_{preb} = CFU of probiotics in prebiotic carbohydrate/ CFU of probiotics in control carbohydrate

RESULTS AND DISCUSSION

Dietary characterization

Kernels were investigated for total sugar, soluble protein, and fat contents. The results, summarized in Table 1, indicated that kernels had high fat amount, constituted from 19.66 to 54.3% (w/w of dry materials), especially in kernels of rambutan and jackfruit, the highest value were 54.3 and 44.4%, respectively. The dissolved protein content of these kernels was relatively low, within the range of 0.69 and 4.49%.

The highest total sugar content was found in lotus seed kernel (37.46%) but in kernels from tropical almond and sesame, the value only was 2.42 and 3.07% respectively.

Table 1. Composition of kernels.

Seed kernels	Moisture (%)	Total sugar (%)	Soluble protein (%)	Fat (%)
Jackfruit	7.07	20.94	1.92	44.40
Avocado	9.59	6.06	2.16	25.44
Rambutan	4.78	25.20	4.49	54.30
Longan	5.28	27.46	3.56	33.70
Sesame	7.09	3.07	2.10	34.44
Trop. Almond	4.02	2.42	1.44	41.72
Lotus seed	5.81	37.46	4.11	43.22
Durian	5.4	19.95	0.69	19.66

Soluble fiber content

The soluble fiber content of kernels, obtained by extracting in 96% ethanol, in Table 2, stated that durian and avocado kernels had the highest soluble fiber content, were 2.24% and 1.89%, respectively. According to Barbosa – Martín *et al*. (2016), the crude fiber content was 4.98% in avocado seeds, while soluble fiber took up 23.16% of crude fiber weight. The lowest soluble fiber content was 0.02%, found in longan kernel.

Effect of soluble fibers from kernels on the growth of probiotic strains

Selectively stimulating the growth of beneficial intestinal bacteria is one of the required criteria of prebiotics. Therefore, in the study, the soluble fibers were supplemented into medium as a carbon source, fermented by 4 probiotics: *B. animalis* AP1.2, *B. bifidum* CNTP 6599, *L. casei* PK2 and *L. fermentum* SBV2. After fermentation, the increase of viable cells in tested medium compared to control medium added to glucose, represented the stimulation of probiotic growth.

Effect of soluble kernel fibers on the growth of Bifidobacterium animalis AP1.2

The highest cell density of *B. animalis* AP1.2 strain was found in medium supplemented with jackfruit kernel fiber, reached at 8.36 lg (CFU/ml). In mediums added to soluble fibers from avocado, longan or lotus seeds, the viable cells were slightly higher than those in control medium, with the Δlg(CFU/ml) being 0.15, 0.12 and 0.11, respectively.

In contrast, soluble fibers from other kernels inhibited the growth of strain AP1.2, especially fibers from rambutan and tropical almond kernels, the viable cells decreased, 1.18 and 1.02 lg (CFU/ml) respectively, compared to the control. This indicated that *B. animalis* AP1.2 didn't use the fibers as a carbon source.

Table 2. The soluble fiber content of kernels.

Seed kernels	Soluble fiber (%)
Jackfruit	1.17
Avocado	1.89
Rambutan	0.87
Longan	0.02
Sesame	0.25
Trop. Almond	0.87
Lotus seed	1.18
Durian	2.24

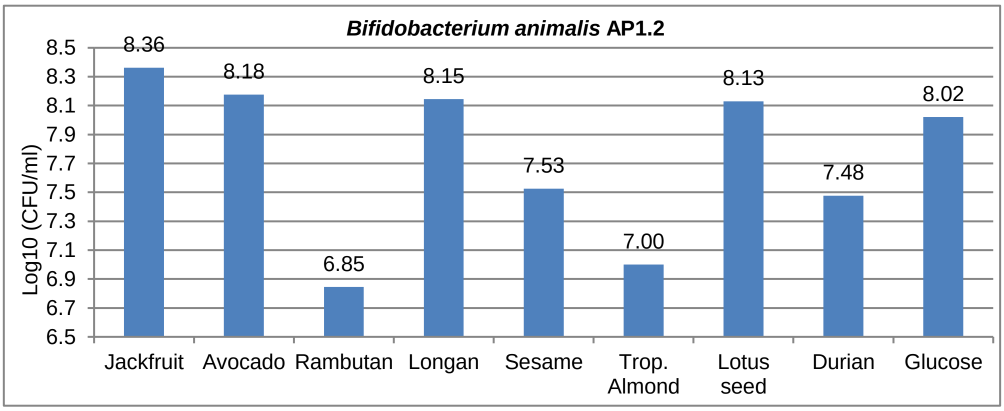

Figure 1. The growth of *B. animalis* AP1.2 on medium supplemented with different kernel soluble fibers.

Effect of soluble kernel fibers on the growth of Bifidobacterium bifidum CNTP6599

The results in Figure 2 indicated that the soluble fibers from most tested kernels, except sesame seeds, stimulated the growth of *B. bifidum* CNTP6599. Specifically, the highest increase of viable cells was recorded in the medium supplemented with kernel fibers from longan, rambutan and lotus seed which the Δlg (CFU/ml) values were 0.44, 0.41 and 0.41, respectively.

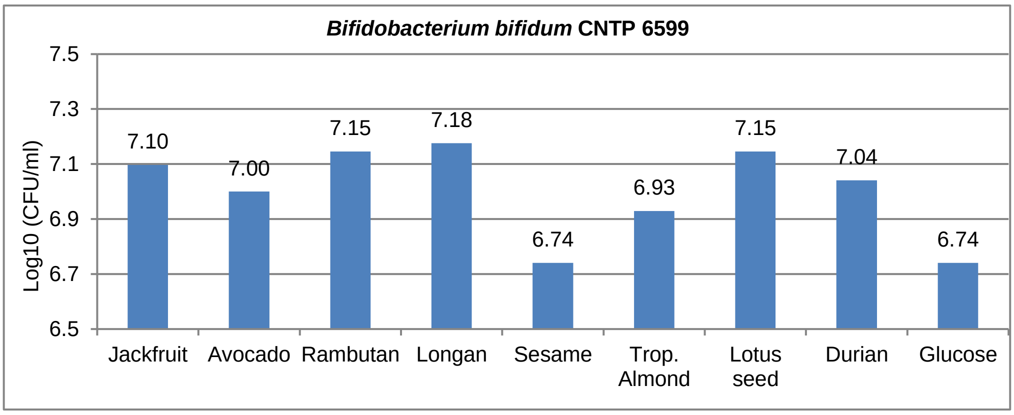

Figure 2. The growth of *B. bifidum* CNTP6599 on medium supplemented with different kernel soluble fibers.

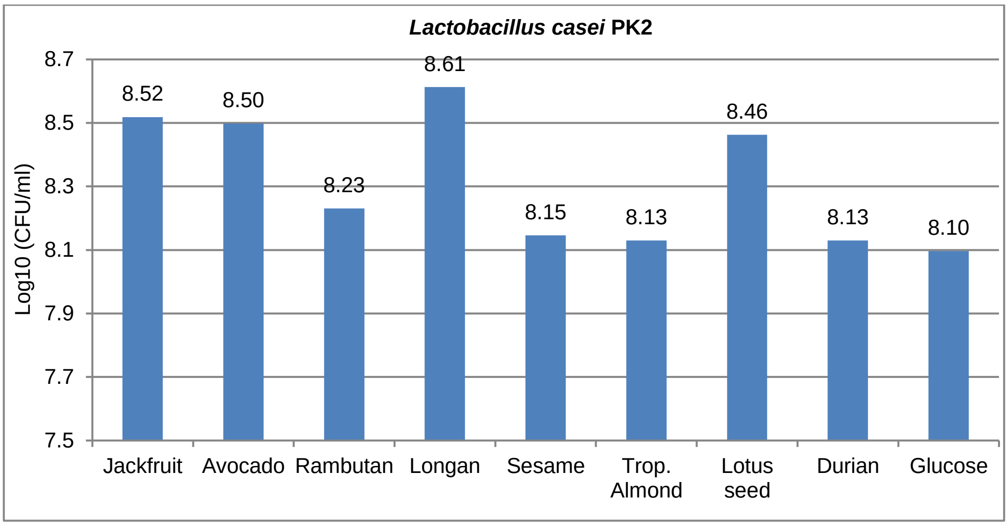

Figure 3. The growth of *L. casei* PK2 on medium supplemented with different kernel soluble fibers.

***Effect of soluble kernel fibers on the growth of Lactobacillus casei* PK2**

L. casei PK2 could use all tested kernel fibers as carbon source, shown in Figure 3. However, there were differences in fermentation of these kernel fibers, expressed in terms of cell density. In particularly, it fermented well kernel fibers from jackfruit, avocado and longan. In medium added to these kernel fibers, the viable cells of *L. casei* PK2 was dramatically higher (from 0.4 to 0.52 lg (CFU/ml) than that cultured in control medium.

Soluble kernel fibers from rambutan, tropical almond, durian and sesame seeds stimulated not significantly the growth of *L. casei* PK2, with the Δlg (CFU/ml) value being within the range of 0.03 and 0.13.

***Effect of soluble kernel fibers on the growth of Lactobacillus fermentum* SBV2**

Among fiber sources, only the logan kernel fiber dramatically promote the growth of strain SBV2, compared to control, the Δlg (CFU/ml) value was 0.5. Conversely, the kernel fibers from jackfruit, avocado, rambutan and lotus seed were not suitable for this strain’s fermentation.

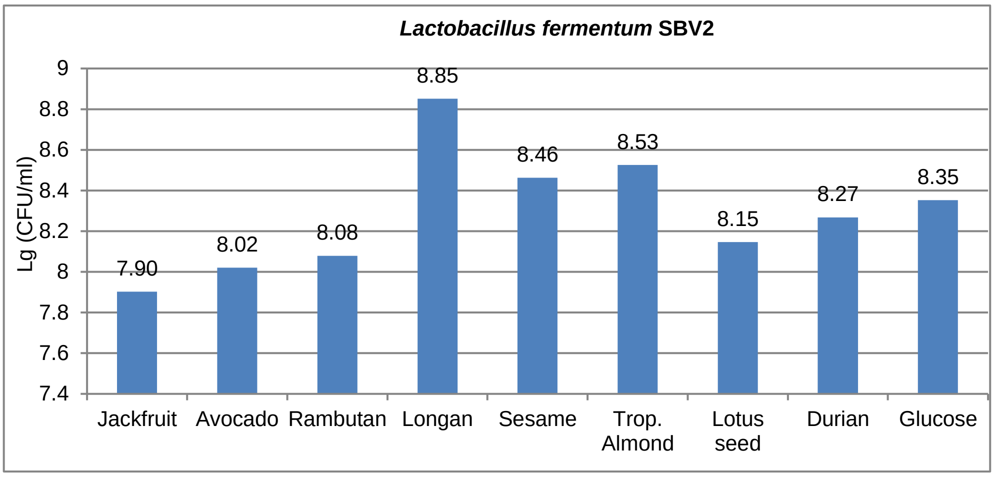

Figure 4. The growth of *L. fermentum* SBV2 on medium supplemented with different kernel soluble fibers.

Prebiotic index

Prebiotic index reflects the ability of a given substrate to support the growth of an organism compared to the growth on a non – prebiotic substrate, such as glucose or any other sugar used as control (Figueroa-Gonzalez et al., 2019). The result in Figure 5 showed that the longan kernel fiber had high prebiotic index for all probiotic strains, especially for *L. casei* PK2 and *L. fermentum* SBV2, were 3.28 and 3.16 respectively. The jackfruit kernel fiber also had positive prebiotic index for probiotics, except *L. fermentum* SBV2 strain, which had negative effect.

The prebiotic index of kernel fibers from tropical almond, durian and sesame seeds was very low for all tested probiotic strains, especially for *B. animalis* AP1.2, was 0.10, 0.29 and 0.32, respectively.

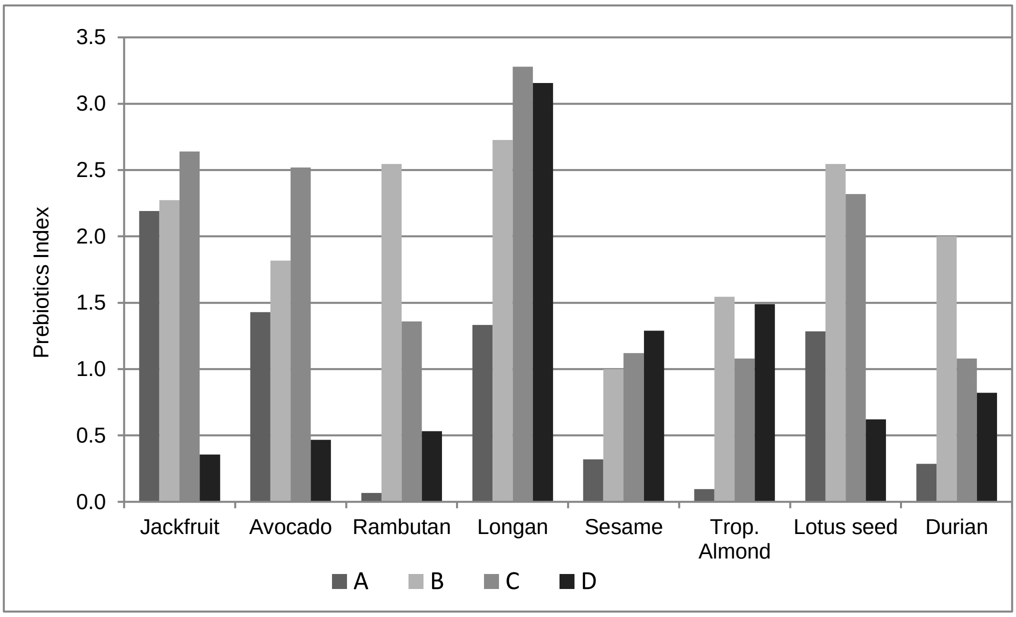

Figure 5. The prebiotic index value of kernel fibers for *B. animalis* AP1.2. (A), *B. bifidum* CNTP6599 (B), *L. casei* PK2 (C), and *L. fermentum* SBV2 (D).

CONCLUSION

Among eight types of fiber source, the longan kernel had the lowest soluble fiber content (0.02%) but its fiber stimulated significantly growth of the tested probiotic strains. The avocado, jackfruit and lotus kernel fiber also affected positively on the growth of *B. animalis* AP1.2, *B. bifidum* CNTP 6599, *L. casei* PK2 but *L. fermentum* SBV2. Strains AP1.2 and SBV2 fermented selectively given fibers, whereas strains CNTP 6599 and PK2 could use the majority of studied kernel fibers as carbon source. Therefore, fruit kernels could be consumed as snack food that serve as a cheap prebiotic source and supply other nutrients at the same time.

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