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Evaluation of the Acceptability of Instant Fiber Powder from Thai Vegetables Supplemented with Probiotic Bacteria

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Abstract— **several studies have confirmed the potential of Thai vegetables as good sources of dietary fiber. This research evaluated the survival of the Lactobacillus acidophilus FBRL-B07 and acceptability in dietary fiber crucial to dietary fiber production for application in the food industry. Thai vegetables (basil seed, malva nut, rice bran, garlic, eggplant) were washed with water, dried, ground to fine powder (particle size of 1.0 mm) and mixed into dietary fiber formulas in a ratio of 1:2:2 (w/w). The study examined three different dietary fiber formulae; Formula 1: mucilage of basil seed, powdered mucilage of malva nut, and powdered rice bran. Formula 2: mucilage of basil seed, powdered mucilage of malva nut, powdered garlic and Formula 3: mucilage of basil seed, powdered mucilage of malva nut, powdered Thai pea eggplant. A probiotic bacterium (L. acidophilus FBRL-B07; 109 CFU/ml) was added to each of the dietary fiber formulas. Evaluation of the dietary fiber formulas focused on their functional properties. Survival of the L. acidophilus FBRL-B07 was monitored during 1 month of storage at 4°C, 25°C and 37°C. Results indicated that storage temperatures of 4°C promoted survival of the L. acidophilus FBRL-B07 in each of the dietary fiber formulas. The highest ($p<0.01$) approximately 9 - 10 log CFU/g. Acceptability results, where 0.1% dietary fiber formulas were added to soft drinks, dietary fiber formulas 1 and 3 yielded a preference score of ($p>0.01$) which was not significant when compared with the control formula. Research findings supported the hypothesis that the production of instant fiber powder from Thai vegetables shows a great potential for application in the food industry, in soft drinks and dairy products such as ice cream.**

Keywords-dietary fiber; Thai vegetables; rice bran; probiotic; Lacbacillus acidophilus; prebiotic

I. INTRODUCTION

Dietary fiber (DF) is a group of food components, which are resistant to hydrolysis by human digestive enzymes. Dietary fiber consists of polysaccharides, oligosaccharides and lignin. [1], [2] The health benefits of dietary fiber have led to increased consumption of fiber-rich products. Fruit and vegetables are good sources of dietary fiber. [3], [4] The beneficial aspects of dietary fiber depend on their functional or physic-chemical properties. The principal physiological effect of fiber is its ability to swell when adsorbing water [5], which occurs due to the presence of carbohydrates with free polar groups, interaction with hydrophilic links or retention within the matrix. Among the vegetables commonly consumed in Thailand many studies indicate that basil seed, malva nut, rice bran, garlic, and eggplant provide the richest fiber sources (e.g. mucilage, cellulose. hemicelluloses, or pectin etc.). Some of these foods also contain other active substances that affect health, for example, there is a substance known as prebiotic found in garlic. [6] Thai pea eggplant has anti-free radicals, substances that catch food and fiber to help control sugar in people suffering from diabetes. [7], [8]

Probiotics are defined as “living microorganisms, which upon ingestion in certain numbers exert health effects beyond inherent basic nutrition”. [9] Lactic acid bacteria (LAB), predominantly selected from the genera *Lactobacillus* and *Bifidobacterium*, constitutes a significant proportion of probiotic cultures in nutritional supplements, pharmaceuticals and functional foods. There is significant scientific evidence, based mainly on in vitro studies and on clinical trials using animals, suggesting potentially beneficial effects in probiotic microorganisms. These include the metabolism of lactose, control of gastrointestinal infections, suppression of cancer, reduction of serum cholesterol, and immune stimulation. [10]

Prebiotics are non-digestible dietary ingredients that benefit the host by selectively stimulating the growth and/or activity of beneficial bacteria in the colon. [11] Perhaps the most frequently studied examples are inulin-type fructans and fructo-oligosaccharides. The positive effect of combining probiotic and prebiotic agents is termed “synbiotics”. Such combinations generally improve the survival of bacteria in the upper gastrointestinal tract and enhance their effect in the large bowel. These have also been increased focus on synbiotics, a combination of prebiotics and probiotics in a single product. A prebiotic is a polysaccharide compound that should not be digested by humans because once inside the upper gastrointestinal tract of a host, they can selectively stimulate the growth of one or more species of colonic bacteria.

This research project’s primary objective was to study the survival of the *L. acidophilus* FBRL-B07 in naturally occurring dietary fiber contained in Thai vegetables in order to assess and their functional properties and consider possible future benefits and applications of dietary fiber formulas.

II. Materials And Methods

A. Materials

Thai vegetables consisting of basil seed, garlic and eggplant were obtained locally in Maha Sarakham, Thailand at a fresh vegetable market; the rice bran came from Borabu District also located within Maha Sarakham province. The Central Market of Thailand in Pathumthani supplied the malva nut and the corn oil (Golden Drop) was imported through Sime Darby Edible Products Limited in Singapore. Lastly, the Lactobacilli MRS broth (Criterion Dehydrated Culture Media Ca93455) was imported from Santa Maria, USA.

B. Methods

1) Fiber fraction

This study examined three different dietary fiber formulae derived from Thai vegetables. Formula 1: mucilage of basil seed, powdered mucilage of malva nut, powdered rice bran, dietary fiber; Formula 2: mucilage of basil seed, powdered mucilage of malva nut, powdered garlic and dietary fiber and Formula 3: mucilage of basil seed, powdered mucilage of malva nut and powdered Thai pea eggplant. The Thai vegetables were washed with water and tray dried at 55°C for 12 hours and ground to a fine powder (particle size of 1.0 mm) using a hammer mill before mixing the dietary fiber from Thai vegetables in a ratio of 1:2:2 (w/w).

2) Bacterial strain and culture conditions

The microorganism used in the study was *Lactobacillus acidophilus* FBRL-B07 from the (Foodborne Pathogens and Biofilm Research Laboratory, FBRL, Maha Sarakham, Thailand). The strain was maintained in a MRS broth at 37°C for a period of 24 hours and stored at 4°C between transfers. The culture was sub cultured twice in the MRS agar plate to maintain freshness before use.

3) Preparation of cells immobilization within dietary fiber

L. acidophilus FBRL-B07 was grown overnight in a MRS broth using a shaker incubator (Labtech LSI-1005R, Namyangiu, Kyonggi-Do, Korea) at 37°C. Cells were harvested from the suspension by centrifugation (Sigma 6-16K, Gottingen, Germany) 2500 g for 10 minutes at 4°C and adjusted to an optical density (OD_{600}) of 1.0 - 1.2 (10^9 logCFU/ml) with sterile distilled water. The cell suspensions of *L. acidophilus* FBRL-B07 were mixed with each of the dietary fiber formulas in ratio 1:80 (w/v) then stirred at 1200 rpm (VELP® Scientifica, Milano, Italy) for 30 minutes. The samples were freeze-dried (Heto PowerDry PL3000 Freeze Dryer, Czech Republic) at - 45°C after that the samples were contained in capsules and then packed in a foil bag to be stored at 4°C 25°C and 37°C.

4) Viable cell counts

Viable cell counts were enumerated every 10 days for 1 month on a MRS agar plate. The plates were incubated at 37°C for 48 hours and calculated as colony-forming units per milliliter with results expressed as $\log_{10}$ values.

5) Acceptability

In order to measure potential acceptability, untrained panels consisting of thirty panelists (student and staff members from the Department of Food Technology and Nutrition, Mahasarakham University) tested soft drinks supplemented with dietary fiber product. Soft drinks were brewed in boiling water and then cooled. At 50°C they were added to each dietary fiber formulas at 0.1%. The samples were served at 5°C to 10°C in 30-ml plastic cups, the lids coded with random three-digit codes. [12] Each sample was presented at one time. Panelists were asked to evaluate their overall liking of each sample on a 9 - point hedonic scale (9=like extremely, 5=neither like nor dislike, 1=dislike extremely).

6) Statistical Analysis

Results were expressed as mean values standard deviations. To assess for differences in the composition and functional characteristics between the different treatments, a multiple sample comparison was performed using SPSS software (version 12.0 for windows, SPSS Inc, Chicago, USA) Multivariate analysis of variance (ANOVA), followed by Scheffe's multiple comparison test, was performed to contrast the groups. The level of significance used was $P< 0.01$.

III. Results And Discussion

A. Viable cell counts

The viable populations of *L. acidophilus* FBRL-B07 on the dietary fiber demonstrated the survival of the *L. acidophilus* FBRL-B07 of each dietary fiber formulas at 4°C. Survival of *L. acidophilus* FBRL-B07 in the control formula each day of storage were statistically significant (*$p<0.01$*). The results showed that survival of *L. acidophilus* FBRL-B07 at 0 d. in the control formula had survived 8.8 logCFU/g before cell viability begins to rapidly decrease until 30 d. at 4.8 logCFU/g.

Survival of *L. acidophilus* FBRL-B07 in dietary fiber formulas 1, 2, and 3 showed that on 0 d. of storage measurements taken were 10.4, 10.3, and 9.5 logCFU/g, respectively, and then slowly decreased until at 30 d. of storage measures were 10.1, 9.4, and 9.1 logCFU/g, respectively each day of storage at 10, 20 and 30. The survival of *L. acidophilus* FBRL-B07 in each fiber formula and each days of storage had statistical significance (*$p<0.01$*). Meanwhile survival of *L. acidophilus* FBRL-B07 was compared to the survival of bacteria in each fiber formula on day 0 of storage. Results showed that dietary fiber formulas 1 and 2 were the highest (*$p<0.01$*), followed by dietary fiber formula 3 and the control formula, respectively. At 10, 20, and 30 d. of storage, the survival of *L. acidophilus* FBRL-B07 in dietary fiber formula 1 was still highest, followed by dietary fiber formula 2, 3 and the control formula, respectively. The survival of the *L. acidophilus* FBRL-B07 in each dietary fiber formula at 25°C and 37°C could be explained in the same trend, which at 25°C and 37°C of storage temperature that the survival of microorganisms depends on the temperature, atmospheric pressure, light and humidity. [4] The survival of *L. acidophilus* FBRL-B07 in the control formula and each of the dietary fibers at 25°C rapidly decreased until at 30 d. of storage they were measured at 4.4, 5.7, 5.7, and 9.1 logCFU/g, respectively. Meanwhile survival of *L. acidophilus* FBRL-B07 in the control formula and each dietary fiber at 37°C rapidly decreased until at 30 d. of storage they were measured at 3.1, 5.7, 5.6, and 5.9 logCFU/g, respectively.

In terms of explaining the results, the temperature of storage is very important after freeze-drying. Generally, storage at low temperature will promote the survival of probiotic bacteria. The addition dietary fiber can protect the cell of probiotic bacteria during freeze-drying and promote the survival of the bacteria during storage.

TABLE I. SURVIVAL OF THE *LACTOBACILLUS ACIDOPHILUS* FBRL-B07 OF EACH DIETARY FIBER FORMULAS* AT 4∘C

Days	*L. acidophilus* FBRL-B07 (log CFU/g)			
	Control formula	Dietary fiber formula 1	Dietary fiber formula 2	Dietary fiber formula 3
0	8. 8±0 .00^{a,C}	10.4 ±0 .15^{a,A}	10.3 ± 0.08^{a,A}	9.5 ±0 .12^{a,B}
10	7.8 ± 0.06^{b,D}	10.1 ± 0.06^{b,A}	9.5 ± 0.14^{b,B}	9.0 ± 0.13^{b,C}
20	6.5 ± 0.04^{c,D}	10.1 ± 0.00^{ab,A}	9.5 ± 0.07^{b,B}	9.0 ± 0.08^{b,C}
30	4.8 ± 0.02^{d,D}	10.1 ± 0.06^{b,A}	9.4 ± 0.02^{b,B}	9.1 ± 0.01^{b,C}

$^{a,b,c,\dots}$ values in the same column sharing a common letter are not significantly different (p<0.01)

$^{A,B,C,\dots}$ values in the same row sharing a common letter are not significantly different (p<0.01)

* Dietary fiber formula 1: mucilage of basil seed, powdered mucilage of malva nut, powdered rice bran

Dietary fiber formula 2: mucilage of basil seed, powdered mucilage of malva nut, powdered garlic

Dietary fiber formula 3: mucilage of basil seed, powdered mucilage of malva nut, powdered Thai pea eggplant

TABLE II. SURVIVAL OF THE *LACTOBACILLUS ACIDOPHILUS* FBRL-B07 OF EACH DIETARY FIBER FORMULAS* AT 25∘C

Days	*L. acidophilus* FBRL-B07 (log CFU/g)			
	Control formula	Dietary fiber formula 1	Dietary fiber formula 2	Dietary fiber formula 3
0	8.8 ±0 .00^{a,C}	10.4 ± 0.15^{a,A}	10.3 ±0 .08^{a,A}	9.5 ±0 .12^{a,B}
10	7. 0± 0.03^{b,A}	5.8 ± 0.06^{b,B}	5.7 ± 0.05^{b,B}	5.8 ± 0.02^{b,B}
20	5.6 ± 0.09^{c,NS}	5. 8± 0.00^{b,NS}	5.4 ± 0.32^{b,NS}	5.4 ± 0.51^{b,NS}
30	4.4 ± 0.03^{d,B}	5.7 ± 0.00^{b,A}	5. 7± 0.26^{b,A}	5.9 ± 0.12^{b,A}

$^{a,b,c,\dots}$ values in the same column sharing a common letter are not significantly different (p<0.01)

$^{A,B,C,\dots}$ values in the same row sharing a common letter are not significantly different (p<0.01)

* Dietary fiber formula 1: mucilage of basil seed, powdered mucilage of malva nut, powdered rice bran

Dietary fiber formula 2: mucilage of basil seed, powdered mucilage of malva nut, powdered garlic

Dietary fiber formula 3: mucilage of basil seed, powdered mucilage of malva nut, powdered Thai pea eggplant

TABLE III. SURVIVAL OF THE *LACTOBACILLUS ACIDOPHILUS* FBRL-B07 OF EACH DIETARY FIBER FORMULAS* AT 37oC

days	*L. acidophilus* FBRL-B07 (log CFU/g)			
	Control formula	Dietary fiber formula 1	Dietary fiber formula 2	Dietary fiber formula 3
0	8.8 ±0 .00^{a,C}	10. 4± 0.15^{a,A}	10.3 ±0 .08^{a,A}	9.5 ±0 .12^{a,B}
10	5.4 ± 0.06^{b,C}	5.7 ± 0.00^{b,AB}	5.8 ± 0.04^{b,A}	5. 5± 0.09^{bc,BC}
20	4.2 ± 0.06^{c,B}	5.7 ± 0.10^{b,A}	5. 2± 0.36^{b,A}	5.0 ± 0.26^{c,AB}
30	3. 1± 0.12^{d,B}	5.7 ± 0.10^{b,A}	5. 6± 0.21^{b,A}	5. 9± 0.02^{b,A}

a,b,c,… values in the same column sharing a common letter are not significantly different (p<0.01)

A,B,C,… values in the same row sharing a common letter are not significantly different (p<0.01)

* Dietary fiber formula 1: mucilage of basil seed, powdered mucilage of malva nut, powdered rice bran

Dietary fiber formula 2: mucilage of basil seed, powdered mucilage of malva nut, powdered garlic

Dietary fiber formula 3: mucilage of basil seed, powdered mucilage of malva nut, powdered Thai pea eggplant

B. *Acceptability*

Soft drinks supplemented with 0.1% dietary fiber formulas 1 and 3 resulted in significantly better acceptability ratings from consumer testers ($p<0.01$). The addition of dietary fiber formula 2 to soft drink decreased its acceptability primarily because it had an unappealing odor due to its garlic component. The most important precursor for the flavor of garlic is allicin (allyl 2 - propenethiosulfinate) [13] results in a unique tangy and spicy taste.

TABLE IV. ACCEPTABILITY RESULTS (9-POINT HEDONIC SCALE) OF SOFT DRINK SUPPLEMENTED WITH DIFFERENT DIETARY FIBER FORMULAS* (0.1%).

Samples	Acceptability
Control formula	7.04±1.44^{a}
Dietary fiber formula 1	6.98±1.48^{a}
Dietary fiber formula 2	5.19±2.51^{b}
Dietary fiber formula 3	6.82±1.66^{a}

a,b Means within columns with different letters are statistically different (P < 0.01)

* Dietary fiber formula 1: mucilage of basil seed, powdered mucilage of malva nut, powdered rice bran

Dietary fiber formula 2: mucilage of basil seed, powdered mucilage of malva nut, powdered garlic

Dietary fiber formula 3: mucilage of basil seed, powdered mucilage of malva nut, powdered Thai pea eggplant

IV. CONCLUSION

Storage temperatures at 4°C promoted survival of the *L. acidophilus* FBRL-B07 in each of the dietary fiber formulas tested with the highest ($p<0.01$) approximately 9 - 10 logCFU/g. In terms of acceptability, where 0.1% dietary fiber formulas were added to soft drinks, dietary fiber formulas 1 and 3 preference scores were not significant ($p>0.01$) when compared with control formula. The research findings supported the hypothesis that the production of instant fiber powder derived from Thai vegetables shows a great potential for application in the food industry, i.e. soft drinks and a variety of dairy products like salad dressing and ice cream, etc.

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