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Comparison of Resistant Starch Content and Survival of *Lactobacillus spp.* on Four Different Sources of Resistant Starch

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Abstract. Four different sources of resistant starch (RS) including black cowpea (*Vigna unguiculata*; black cowpea flour), green banana (*Musa sapientum;* green banana flour), retrograded flour of high amylose rice (*Oryza sativa*; retrograded rice flour), and commercial resistant starch (standard RS) were used to evaluate resistant starch content, chemical compositions, and the growth of *Lactobacillus acidophilus* TISTR 1338 and *Lactobacillus delbrueckii* subsp. *bulgaricus* TISTR 895. The black cowpea flour had significantly lowest amounts of resistant starch content (6.31%), but highest in protein content (27.28%) whereas the highest level of RS was found in RS standard (38.41%) followed by green banana flour (26.59 %). In the study on the growth of *Lactobacillus acidophilus* TISTR 1338 and *Lactobacillus delbrueckii* subsp. *bulgaricus* TISTR 895, the results indicated that the green banana and retrograded flour could protect the cell and promote survival of *L. bulgaricus* TISTR 895, but *L. acidophilus* TISTR 1338.

Keywords: Resistant starch, *L. acidophilus*, Green banana flour, Prebiotic

1. Introduction

Currently, resistant starch (RS) plays a major role in the health food industry, because it behaves with properties similar to soluble and insoluble dietary fiber in the gastrointestinal tract [1]. RS was defined as the starch and the products of starch degradation that are not absorbed in the small intestine of healthy individuals [2]. RS can be classified into four types; RS1, a physically inaccessible resistant starch, is found in whole or partially intact seeds, legumes, and unprocessed whole grains. RS2 occurs in the native granular form such as unripe bananas, and raw potatoes. RS3 is formed when starch is gelatinized and cooled to induce a retrogradation. RS4 (standard RS in this study) is formed by chemical modification of the starch [3, 4, 5]. Retrograded rice flour, formed through autoclaving and cooling cycle of starch, is stable when heating above 100 ℃, because retrograded starch (amylose) melts at 155 ℃ [6]. Resistant starch could stimulate fermentation of lactic acid bacteria, comprising multiple strains from the Lactobacilli and Bifidobacteria, in the large intestine and the small intestine, which might be helpful in the overall maintenance of colonic health and protective effect against colorectal cancers [7]. However, there are varieties of natural sources of resistant starch and each source contains different types and levels of RS content, but only few studies have investigated on the effect of RS sources on the growth of lactic acid bacteria. Therefore, the present study was carried out to determine the content of RS, chemical compositions of four types of resistant starch and to evaluate the viable of *Lactobacillus spp.* on media containing RS from different sources, which can provide some information about the health benefit of RS to human and to develop new food based products.

2. Materials and Methods

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2.1. Materials and Cultures

Four different sources of RS used in this study included black cowpea (*Vigna unguiculata* L. Linn., ABB group, Kluai Namwa), green banana (*Musa sapientum* L. Linn., ABB group, Kluai Namwa), retrograded high-amylose rice flour (*Oryza sativa* L**.** cultivar Leung 11). All samples were purchased from local market in Mahasarakham province, Thailand. The commercial or standard RS was purchased from Megazyme International Ireland Limited (Ireland). Fructooligosaccharides (FOS) and glucose standards were obtained from Sigma Chemical Co (St. Louis, MO., USA). Pure freeze-dried probiotic cultures (*Lactobacillus acidophilus* TISTR 1338 and *Lactobacillus delbrueckii* subsp. *bulgaricus* TISTR 895) were obtained from Thailand Institute of Scientific and Technological Research (Pathumthani, Thailand). Other chemicals and solvents were all of analytical grade.

2.2. Sample Preparations

2.2.1. Cowpea Flour Preparation

Cowpea seeds were decorticated according to the methods of [8] with some modifications. One kg of cowpea seeds was soaked in 3L of tap water in a wide plastic bowl over night, and then seed coats were removed manually. The decorticated cowpea were dried at 50 ℃ in a tray-dry oven to approximately 10% moisture content and then ground and sifted through 100 mesh screen and stored at 5 ℃ in a sealed plastic.

2.2.2. Unripe Banana Flour Preparation

Green banana fruits (approximately 105-120 days after bloom), were peeled and cut into 0.2cm slices and immediately rinsed in citric acid solution (0.3% w/v). The slices were dried at 50 ℃ in a trey-dry oven to obtain moisture content below 10% and then ground and sifted through 100-mesh screen and stored at 5 ℃ in sealed plastic containers until use.

2.2.3. Retrograded High-amylose Rice Flour Preparation

High-amylose rice flour (cultivar; Lueng 11) was cleaned, ground by a hammer mill, and screened through 100 mesh sieve. Retrograded high-amylose rice flour was prepared according to the methods of [9] and [10] with some modifications. The rice flour (150g) was dispersed in 300 mL of 10 mmol lactic acid in ethanol. The mixture was pressure-cooked in an autoclave at 121 ℃ for 60 min, and then incubated in water bath at 60 ℃ for 24 h. The precipitated flour was oven dried at 50 ℃ overnight, ground by the hammer mill and screened through 100 mesh sieve.

2.3. Resistant Starch Determination

Resistant starch content was determined by a Megazyme Resistant Starch Assay Kit [11].

2.4. Chemical Composition Determination

Proximate analysis of the samples, including moisture, ash, crude protein, crude fat and total carbohydrates were determined in triplicate, according to [12]. The moisture content was determined by further heating of the dried sample at 105 ℃ overnight until constant weight; the ash content was determined by weighing the incinerated residue obtained at 550 ℃ for 24 h; the crude protein content was determined by the Kjeldahl method; the crude fat content was determined by Soxhlet extraction with petroleum ether as a solvent; the total carbohydrates content were calculated by difference: Total carbohydrates (%) = 100 - (% crude protein + % crude fat + % ash)

2.5. Simulated Fermentation of RS Sources in Vitro

2.5.1. Activated Culture

Pure freeze-dried probiotic cultures were activated by inoculating in the MRS-broth at 37 ℃ for 48 h under anaerobic conditions and stored at 4 ℃ between transfers. The cultures were sub cultured twice in the MRS agar plate to maintain freshness before use.

2.5.2. Simulated Fermentations of RS Sources in Vitro

L. acidophilus TISTR 1338 and *L. bulgaricus* TISTR 895 were grown overnight in a MRS broth using a shaker incubator at 37 ℃. Cells were measured optical density (OD) using a spectrophotometer under 600 nm, and the viable counts were determined by plating on MRS agar. Viable cell counts were calculated as averages of all colony-forming units per gram of sample (CFU g^{-1}). The initial counts of cultures for *L.*

acidophilus TISTR 1338 and *L. bulgaricus* TISTR 895 were approximately 10^9 and 10^8 CFU g^{-1} respectively. Cells were added 1% of each RS and incubated at 37 ℃ for 3 days under anaerobic conditions. Each samples were carried in triplicate.

2.5.3. Viable Cell Counts

Viable cell counts were enumerated every 24 h for 3 days on a MRS agar plate. The plates were incubated at 37 ℃ for 48 h and calculated as colony-forming units per gram of sample with results expressed as $\log_{10}$ values.

2.6. Statistical Analysis

The experimental data were analyzed using an analysis of variance (ANOVA). Duncan's multiple range tests was used to establish the multiple comparisons of the mean values; mean values were considered at 95% confidence level ($p \leq 0.05$).

3. Results and Discussion

3.1. Resistant Starch Contents

RS content of different RS sources are shown in Table1. The commercial RS had the highest level RS content (38.41%), whereas the RS contents of retrograded rice flour, green banana, and black bean were 26.59, 16.45 and 6.31%, respectively. The result was similar to that reported by [13] in cowpea seeds.

Table 1: Chemical compositions (%) and RS content of four different sources of resistant starch

Samples	Moisture	Ash	Crude fat	Crude protein	Carbohydrate	RS content
Black cowpea	2.70 ± 0.09^{f}	3.09 ± 0.03^{a}	1.79 ± 0.10^{a}	27.28 ± 0.68^{a}	65.15 ± 0.64^{d}	6.31 ± 0.08^{d}
Green banana	4.77 ± 0.37^{e}	2.58 ± 0.49^{b}	0.45 ± 0.10^{b}	2.77 ± 0.45^{c}	89.43 ± 0.79^{b}	26.59 ± 1.65^{b}
Retrograded rice	5.58 ± 0.07^{d}	0.77 ± 0.07^{c}	1.44 ± 0.11^{a}	7.07 ± 0.29^{b}	85.14 ± 0.48^{c}	16.45 ± 2.93^{c}
Standard RS	6.11 ± 0.16^{c}	0.14 ± 0.02^{d}	0.31 ± 0.05^{b}	0.23 ± 0.00^{d}	93.21 ± 0.20^{a}	38.41 ± 0.57^{a}
FOS	6.56 ± 0.09^{b}	0.04 ± 0.01^{d}	$0.26 \pm 0.05^{a,b}$	0.24 ± 0.00^{d}	92.90 ± 0.05^{a}	-
Glucose	8.96 ± 0.01^{a}	0.00 ± 0.00^{d}	0.91 ± 0.94^{b}	0.23 ± 0.00^{d}	89.90 ± 0.93^{b}	-

Values are means ± SD, n=3

Values in the same column with different superscripts are significantly different ($p \leq 0.05$)

3.2. Chemical Compositions of Resistant Starch Sources

The proximate compositions and RS content of different food sources, FOS, and glucose are presented in Table 1. It was found that glucose indicated the highest level of moisture content (8.96%) whilst that of FOS, standard RS, retrograded rice flour, green banana flour and black cowpea flour were 6.56, 6.11, 5.58, 4.11 and 2.70%, respectively ($p \leq 0.05$). Black cowpea flour and green banana flour contained higher level of ash content (3.09 and 2.58%, respectively) than other sources. The highest crude fat contents were found in the black cowpea flour (1.79%) and retrograded rice flour (1.44%), whilst the highest concentration of protein was detected in the black cowpea flour (27.28%). Similar results were reported by [14] who reported that protein content in cowpea seeds was 24.8%. Carbohydrate was the main composition of all samples in this study ranging between 65.15 and 93.21%.

3.3. Viable Cell Counts

Viable cell counts of *L. acidophilus* TISTR 1338 and *L. bulgaricus* TISTR 895 of each RS source are presented in Table 2. The viable populations of *L. acidophilus* TISTR 1338 grown in each sample at 0 h (initial) were not significant ($p \geq 0.05$), except in commercial RS, ranging from 9.25 to 9.28 log CFU g^{-1}. After incubated in different RS sources for 24 h, the viable populations of *L. acidophilus* TISTR 1338 decreased significantly, except those incubated in glucose was increased ($p \leq 0.05$). After fermentation for 48 h, it was observed that the viable populations of *L. acidophilus* TISTR 1338 grown in green banana flour, retrograded flour, standard RS, and FOS increased, compared with 24 h, whereas that grown in the cowpea and glucose further decreased.

The populations of *L. bulgaricus* TISTR 895 inoculated to different RS sources (at 0 h, initial) were not significant ($p \geq 0.05$). After fermentation for 48 h, It was found that the viable cells still were not significant (p ≥0.05). However, when the viable cells incubated between 24 and 48 h were compared, the results indicated that retrograded rice flour, green banana flour, and glucose had viable cells of *L. bulgaricus* TISTR 895 increased (0.18, 0.16 and 0.01 log $CFUg^{-1}$, respectively). The results showed that different sources of resistant starch had different influence on the growth and viability of *L. acidophilus* (probiotic bacteria) and *L. bulgaricus*. Fig. 1 shows the relative change (%) of viable cell counts of *L. acidophilus* TISTR 1338 and *L.bulgaricus* TISTR 895, compared the cell counts of each food source at 48 with 0 h). The results indicated that the viable cell counts of all food sources were reduced. The highest reduction was found with *L. acidophilus* TISTR1338 in retrograded rice flour (25.03%). Contrarily, the green banana flour and retrograded rice flour could protect the *L.bulgaricus* TISTR 895, as a result in these two food sources yielded a lower relative changes. These may due to the differences of chemical compositions of RS, as sources of chemical nutrients, such as nitrogen and carbon source. RS, as a prebiotic, affected the growth and viability of cell, as well as provides some metabolites that positively influenced consumer health such as short chain fatty acids. The chemical compositions and resistant starch contents is important on growth of probiotic bacteria. Generally, food with high RS content (green banana flour, retrograded rice flour, and RS standard) would be better to promote the survival of probiotic bacteria.

Table 2: Viable cell counts of L. acidophilus TISTR 1338 and L. bulgaricus TISTR 895grown on different types of resistant starch, FOS, and glucose

Samples	Viable cell counts (log CFU g^{-1})		
	0 h	24 h	48 h
***L. acidophilus* TISTR 1338**			
black cowpea flour	9.27 ± 0.01^{a}	8.65 ± 0.22^{b}	8.13 ± 0.16^{c}
Green banana flour	9.28 ± 0.03^{a}	7.24 ± 0.03^{c}	7.90 ± 0.27^{c}
retrograded rice flour	9.28 ± 0.12^{a}	6.82 ± 0.02^{d}	6.96 ± 0.03^{d}
Standard RS	9.25 ± 0.04^{b}	6.43 ± 0.00^{f}	8.54 ± 0.13^{b}
FOS	$9.27 \pm 0.11^{a,b}$	$7.11 \pm 0.08^{c,d}$	8.43 ± 0.03^{b}
Glucose	9.28 ± 0.10^{a}	9.53 ± 0.46^{a}	9.19 ± 0.04^{a}
***L.bulgaricus* TISTR 895**			
black cowpea flour	8.68 ± 0.01	8.69 ± 0.15^{a}	8.10 ± 0.02
Green banana flour	8.66 ± 0.01	$8.52 \pm 0.05^{a,b}$	8.67 ± 0.01
Retrograded rice flour	8.66 ± 0.02	8.36 ± 0.01^{b}	8.55 ± 0.74
Standard RS	8.67 ± 0.01	8.31 ± 0.10^{b}	8.27 ± 0.06
FOS	8.66 ± 0.02	8.74 ± 0.21^{a}	8.60 ± 0.11
Glucose	8.66 ± 0.03	8.63 ± 0.16^{a}	8.64 ± 0.25

Values are means ± SD, n=3, Values in the same column with different superscripts are significantly different ($p \leq 0.05$)

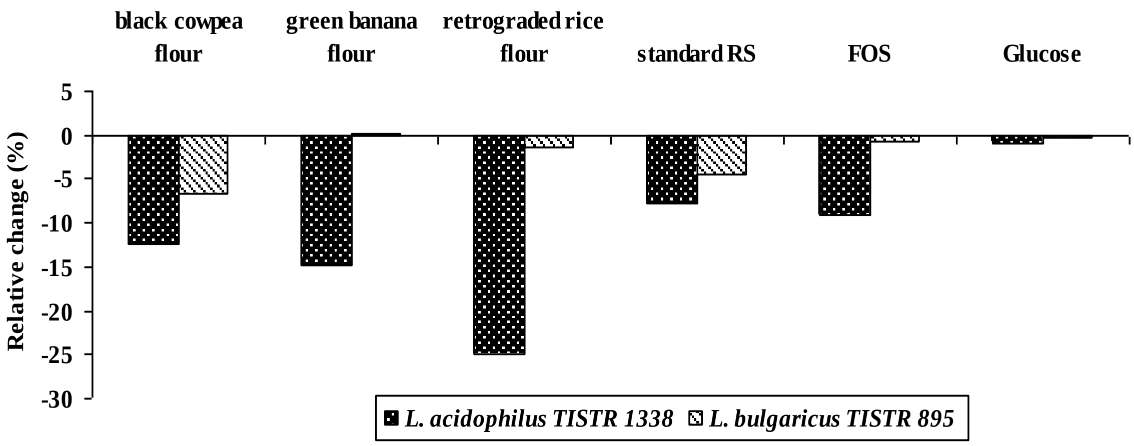

Fig.1: Relative change (%) of viable cell counts of *L. acidophilus* TISTR 1338 and *L.bulgaricus* TISTR 895 (calculated by related the viable cell counts at 48 h with cell counts at 0 h

4. Conclusions

Different sources of resistant starch had different the resistant starch contents ($p \leq 0.05$). The black cowpea flour had lowest level of resistant starch (6.31%), whereas the highest values were 38.41% found in standard RS and 26.59% in green banana flour. After fermentation with different types of food sources, the natural RS sources i.e. green banana flour and retrograded rice flour could protect the cell and promote survival of *L.bulgaricus* TISTR 895, but *L. acidophilus* TISTR 1338. The study suggested that it is likely to employ resistant starch from natural source in food containing probiotic products, especially in fermented foods and to develop new food based products.

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