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Resistant Starch Contents and the *in Vitro* Starch Digestibility of Thai Starchy Foods

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ABSTRACT

Samples from 29 starchy foods and 11 cultivars of banana were analyzed for their contents of resistant starch (RS), digestible starch and total starch, as well as the *in vitro* starch digestibility. The RS analysis was based on á-amylase and amyloglucosidase hydrolysis followed by colorimetric assay of the glucose released. The results showed that the main RS sources were found in the legume group (10.3 ± 1.2% to 22.9 ± 0.0%), glass noodle products (9.1 ± 0.8% to 11.3 ± 1.5%) and bananas (52.2 ± 4.1% to 61.4 ± 2.3%). For the rice group, the effect of processing methods on the retrograded RS formation was investigated. Khanomjean produced with fermentation had a higher RS content (8.5 ± 1.1%) than that of the cooked white rice of the high-amylose cultivar. With the rice snacks, the process for puffing kaotung and kaokreupvor by frying and roasting produced a higher RS content (2.6 ± 0.0% and 2.9 ± 0.3%, respectively) than in the raw samples. Among the rice noodles, vermicelli contained a higher RS content than the others. Moreover, a gelatinization effect on the RS contents was clearly evident, with the gelatinized cassava starch (2.2 ± 0.0%) having a lower RS content than the commercial starch (44.6 ± 0.3%), whereas the commercial RS content was high (58.5%). Finally, the test on *in vitro* starch digestibility showed that the legume samples, particularly with red beans, had the lowest rate of starch digestibility compared to the other samples.

Key words: starchy foods, bananas, resistant starch, amylose, rate of *in vitro* starch digestibility

INTRODUCTION

At present, consumers are showing increasing interest in choosing healthy functional food products. Resistant starch 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 (King, 2004; Ohr, 2004). As it is resistant to human digestive enzymes, the slow release of glucose results in a reduced energy intake by the intestinal cells, which is evident by the low glycemic index of the non-digested starch. This can help to improve glucose regulation in diabetes and facilitate better weight control for the obese (Higgins *et al*., 2004). The non-digested starch in the large intestine is fermented by colonic microflora producing short chain fatty acids that encourage the growth of beneficial bacteria, indicating a prebiotic functionality. This may lead to healthier colon cells and reduce the development of colon cancer. In addition, a diet high in RS can reduce blood cholesterol and triglyceride levels, because of higher excretion rates of cholesterol and bile acids.

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Overall, increasing the RS content in the diet has the potential to provide several significant health benefits and add value to food products (Alexander, 1995; Wursch,1999; Croghan, 2004).

RS is defined as the sum of the starch and the products of starch degradation that are not absorbed in the small intestine of a healthy individual (Englyst *et al.*, 1992). There are four types of RS. Type I represents physically inaccessible starch which is locked in the plant cell walls of some foodstuffs, such as partially milled grains, seeds and legumes. Type II RS is characterized by native granular starches found in food containing uncooked starch, such as bananas, raw potatoes and beans. The RS contents in reference banana flour samples determined by three laboratories averaged 52.1 % dry matter and lentil flour had 8.2% (Goni *et al.*, 1996). Type III RS is made up of retrograded starch or crystalline non-granular starch, like the starch found in cooked and cooled potatoes, bread crust, corn flakes and retrograded high-amylose maize starch. Type IV refers to specific chemically and thermally-modified or repolymerized starches (Englyst *et al.*,1992; Eerlingen and Delcour, 1995).

Starch is a nutritionally-available carbohydrate composed of the two glucose polymers, amylose and amylopectin (Champagne, 1996). Amylose comprises 17 to 30% of the common starches and has long linear glucose molecules. It is known that the amylose content is positively correlated with the RS content. Also starchy foods with a high amylose or RS content have been shown to exhibit a slow rate of digestibility, providing reduced calorific value (Eerlingen and Declour, 1995). However, starch origin and starch characteristics, as well as the ingredients and processing methods for starchy foods, are of great importance in altering the rate of hydrolysis *in vitro* and *in vivo*. Aside from the RS content, the glycemic index has been established as an important indicator of starch digestibility (Jenkins *et al.*,1981; Englyst *et al.*,1996).

As starchy foods are a main source of energy in the diets of Thai people, healthier starchy foods, providing beneficial functionalities for sustaining good health, should be recommended. Therefore, it is of interest to study the RS content in common starchy foods and the rate of *in vitro* digestibility. The objective of this study was to determine the content of total starch, digestible starch and RS (or non digestible starch) in 29 starchy food products and 11 cultivars of common and indigenous bananas. The *in vitro* digestibility rate of some starchy foods was examined. A comparison between the RS content and amylose was also investigated.

MATERIALS AND METHODS

Sample preparation

Twenty nine starchy foods, commonly consumed by Thai people (rice and rice products, dried noodle style products, legumes and others), were selected and purchased from a local market. The samples were ground and passed through a 100-mesh size sieve before analysis. Eleven cultivars of green and unripened banana fruits (*Musa sapientum* L.), aged from 90 to 120 days, were also collected from the Pakchong Research Station, Kasetsart University, Bangkok, Thailand. For processing into banana flour, the bananas were peeled and sliced into 1 mm thick pieces and placed on a stainless steel tray, dried in a hot air oven at 45°C for 8 h, then milled and passed through a 100-mesh size sieve before analysis.

Determination of total and resistant starch

The resistant starch (RS) content of the samples was determined by the direct method of Goni *et al.* (1996). Ground samples (100 mg) were incubated with a solution containing 20 mg pepsin (Merck No. 7190, 2000FIT-μ/g) at 40°C for 60 min to remove any protein. Tris-maleate solution containing 40 mg pancreatic α-amylase (Sigma

No. A-3176, 23 IU/mg) was added and incubated at 37°C for 16 h to hydrolyze digestible starch (DS). The hydrolysates were centrifuged and the residues were solubilized and incubated with amyloglucosidase (Boehringer No.102857) at 60°C for 45 min to hydrolyze the RS. The glucose content was measured using a glucose oxidase-peroxidase kit (Sigma No. G3660) and RS comprising fractions of RS type I, II and III was calculated as mg of glucose x 0.9.

According to the modified method of total starch determination of Goni *et al*. (1997), 50 mg ground samples were dispersed in 6 ml of 2M KOH and incubated for 30 min at room temperature. Solubilized starch was then hydrolyzed by adding 60 µl of amyloglucosidase by incubating at 60°C for 45 min in a shaking waterbath. After centrifugation (15 min, 4500 g), the glucose content in the supernatant was measured using a glucose oxidase-peroxidase kit and the total starch content was calculated as mg of glucose × 0.9.

Digestible starch (DS) was then calculated as the difference between the total starch and the RS or indigestible starch expressed as a percentage of the sample weight.

Determination of the apparent amylose content

The amylose content of the banana flour samples was determined by a colorimetric method. Standards of amylose (Sigma No. A0512) and amylopectin (Sigma No. A8515) and other chemical reagents from Merck, Germany were used, following the AACC method (2000).

***In vitro* starch digestibility test**

Ten starchy foods were analyzed for the *in vitro* rate of starch digestibility using the method of Goni *et al*. (1997). Ground food samples (50 mg) were boiled and incubated with a solution containing 20 mg pepsin at 40°C for 60 min to remove the protein and the volume was made up to 25 ml with a Tris-maleate buffer. To hydrolyze the digestible starch (DS), 5 ml of Tris-maleate solution containing 3.3 IU a-amylase was added and incubated at 37°C. One millilitre aliquot samples were taken from each tube every 30 min from 0 to 180 min and placed in a tube at 100°C to inactivate the enzyme. Then 60 µl amyloglucosidase was added to hydrolyze the digested starch into glucose at 60°C for 45 min. After centrifugation (15 min, 4500 g), the glucose content in the supernatant was measured using a glucose oxidase-peroxidase kit and the digestible starch was calculated as mg of glucose × 0.9**.** The rate of digestion was expressed in terms of the glucose released per 100g of sample hydrolyzed at different times (0, 30, 60, 120, 160 and 180 min).

Statistical analysis

The SPSS for Windows program, version 10, was employed to analyze the results obtained from at least two replications. Means and standard deviations for the contents of the total, digestible and resistant starch of the categorized samples were calculated and compared. A relationship between the amylose and the resistant starch of the banana samples was also investigated using linear regression analysis.

RESULTS AND DISSCUSION

Resistant starch content of starchy foods

The RS content, digestible starch (DS) and total starch (TS) of the 29 starchy food products are shown in Table 1. The RS values obtained were different between the raw and processed samples of rice and rice products. The polished raw rice of the *Saohai* cultivar had the highest amount of RS (34.8 ± 1.4 %), which might be attributed to the nature of RS type I and II and its high amylose molecule content. In contrast, the low amylose rice, *Dookmali* cultivar, had an RS content of only 11.6 ± 2.8%. This study also found a processing effect on the RS content; fermentation as well as frying and roasting resulted in a higher

Table 1 Contents of resistant starch, digestible starch and total starch of selected starchy food products (g/100g dry weight)[1].

Samples	Total starch	Digestible starch[2]	Resistant starch
Rice and rice products			
Polished hard rice (Saohai cultivar), raw	52.0 ± 3.2	17.2 ± 4.5	34.8 ± 1.4
Polished soft rice (Dookmali cultivar), raw	67.5 ± 13.9	55.9 ± 16.7	11.6 ± 2.8
Brown rice, raw	66.6 ± 2.5	62.3 ± 2.5	4.2 ± 0.0
White rice, cooked	64.7 ± 1.6	57.6 ± 1.6	7.1 ± 0.0
Khanomjean, cooked	41.9 ± 0.0	33.3 ± 1.7	8.5 ± 1.1
Kaotung, dried	4.7 ± 0.1	3.2 ± 0.0	1.5 ± 0.0
Kaotung, fried	75.9 ± 0.5	73.2 ± 0.5	2.6 ± 0.0
Kaokreupvor, dried	28.4 ± 5.9	28.1 ± 5.9	0.3 ± 0.0
Kaokreupvor, roasted	54.6 ± 0.0	52.6 ± 0.1	2.9 ± 0.3
Kaokreup, dried	44.2 ± 0.0	42.1 ± 0.2	2.2 ± 0.3
Kaokreup, fried	44.9 ± 0.8	42.9 ± 0.6	2.0 ± 0.1
Extruded rice snack	4.2 ± 0.1	3.9 ± 0.0	0.4 ± 0.0
Noodle type products, uncooked			
Glass noodles	59.0 ± 0.9	47.7 ± 0.6	11.3 ± 1.5
Instant glass noodles	60.7 ± 0.3	51.6 ± 1.1	9.1 ± 0.8
White rice noodles	67.0 ± 3.7	64.0 ± 4.1	3.0 ± 0.4
Brown rice noodles	73.7 ± 0.7	71.5 ± 2.0	2.2 ± 1.3
Instant rice noodles	57.1 ± 0.0	54.7 ± 0.1	2.4 ± 0.1
Vermicelli	58.5 ± 1.6	54.1 ± 0.4	4.4 ± 1.2
Rice sheet	41.3 ± 0.4	38.9 ± 0.5	2.4 ± 0.0
Legumes, raw			
Mungbeans	44.3 ± 2.0	21.4 ± 2.0	22.9 ± 0.0
Mungbean starch, commercial	99.1 ± 3.9	48.8 ± 2.7	50.3 ± 1.2
Black beans	39.8 ± 5.4	21.5 ± 5.2	18.3 ± 0.2
Small red beans	40.2 ± 0.1	23.2 ± 0.6	17.0 ± 0.5
Red beans	33.5 ± 3.2	23.2 ± 4.4	10.3 ± 1.2
Other			
Job’s tears	66.5 ± 0.6	60.1 ± 0.6	6.4 ± 0.0
Tapioca (cassava) pearls	44.7 ± 0.2	40.1 ± 1.2	4.5 ± 1.0
Cassava starch, commercial	94.6 ± 0.3	50.0 ± 0.0	44.6 ± 0.3
Gelatinized cassava starch (50% solid)	5.7 ± 0.1	3.5 ± 0.1	2.2 ± 0.0
Resistant starch (Hi maize), commercial	77.6 ± 0.1	19.1 ± 0.0	58.5 ± 0.0

[1] Values are the means of at least two analyses.

[2] Calculated as the difference between total starch and resistant starch content.

RS content when compared to the non-treated samples. Khanomjean or fermented rice noodles had an RS value of 8.5 ± 1.1%, which was higher than that of the cooked rice (7.1 ± 0.0%). This indicated that rice fermentation for khanomjean-making could account for the formation of a stable starch structure, particularly of amylose molecules. Unlike the study by Sagum and Arcot (2000) on processing methods that lead to a high rice starch digestibility, it showed that boiling and pressure cooking produced low RS values of 1.0 to 2.2% and 0.8 to 2.8%, respectively, compared to that of the raw rice samples (8.6 to 12.9%).

Furthermore, the rice snacks produced by frying and roasting such as kaotung, kaokreupvor showed RS values of 2.6 ± 0.0% and 2.9 ± 0.3%, respectively, which were higher than the raw samples. The findings agreed with several studies on the retrograded RS content (type III) of different starches formed during processing (Garcia-Alonso *et al.*, 1999; Rendon-Villalobos *et al.*, 2002; Tovar *et al.*, 2002; Gonzalez-Soto *et al.*, 2006). However, the extruded rice snacks made with low amylose rice (*Doogmali* cultivar) had a very low RS value of 0.4 ± 0.0%.

Among the dried noodle-type products, uncooked glass noodles and instant glass noodles showed the highest RS contents of 11.3 ± 1.5% and 9.1 ± 0.8%, respectively. The high amylose content (~40%) in mungbean starch would contribute to the formation of high RS (Tavor *et al.*, 1999), especially when the bean flour was processed into glass noodle products as observed in this study. Previous studies on the properties of rice starch differing in amylose content indicated that the RS contents increased with increasing amylose (Frei *et al.*, 2003; Hu *et al.*, 2004). When rice noodle products were analyzed, the white rice noodles, brown rice noodles, instant rice noodles, vermicelli and rice sheet had an RS content of 3.0 ± 0.4%, 2.2 ± 1.3%, 2.4 ± 0.1%, 4.4 ± 1.2% and 2.4 ± 0.1%, respectively. These values were lower than the RS content observed in the glass noodle products.

The five cultivars of common beans such as mungbean, black bean, small red bean and red bean showed a very high RS content of 22.9± 0.0%, 18.3 ± 0.2% 17.0 ± 0.5%, 10.3 ± 1.2%, respectively, whereas mungbean starch had 50.3 ± 1.2%. A study by Osario-Diaz, *et al.* (2002) pointed out that the starch-lipid and starch-protein complex structures formed in beans during processing would resist enzymatic digestion. Considering the RS values of glass noodle products made from mungbean starch, it indicated that the high RS in mungbean was likely to produce the high RS content observed in the glass noodle products. In addition, the samples of job's tears, tapioca pearls, commercial cassava starch, gelatinized cassava starch and commercial RS had RS contents of 6.4 ± 0.0%, 4.5 ± 1.0%, 44.6 ± 0.3%, 2.2 ± 0.0% and 58.5 ± 0.0%, respectively. This result indicated that the process of gelatinization and retrogradation of the cassava starch caused a low content of RS type III to be observed when compared to the RS of native starch containing RS type II.

Resistant starch and anylose contents of different cultivars of banana

Eleven cultivars of common and indigenous bananas were selected for analysis of their RS content compared with their apparent amylose content. Table 2 shows the contents of total starch, digestible starch and RS as well as the amylose content of the banana flour samples. The common cultivars of Kluai Namwa, Kluai Hom, Kluai Kai, Kluai Lepmurnang and Kluai Hugmuk are sold in general markets and commonly consumed by Thai people. The RS content observed with these common banana cultivars ranged between 52.2 ± 4.1% and 61.4 ± 2.3% on a dry matter basis. Kluai Hugmuk had the highest value of 61.4 ± 2.3% and the second highest was Kluai Hom with 57.7 ± 1.1%. The indigenous cultivars of Kluai Hin, Kluai

Table 2 Contents of resistant starch, digestible starch and total starch of banana flour samples (g/100g dry weight)[1].

Samples	Starch composition			
	Total starch	Digestible [2] starch	Resistant starch	Amylose molecules
Common cultivars				
Kluai Namwa	79.7 ± 1.1	23.0 ± 4.8	56.6 ± 5.8	25.8 ± 2.7
Kluai Hom	91.0 ± 3.1	33.3 ± 1.9	57.7 ± 1.1	37.1 ± 0.5
Kluai Kai	80.5 ± 0.3	28.2 ± 4.5	52.2 ± 4.1	24.7 ± 2.5
Kluai Lepmurnang	72.1 ± 3.4	15.1 ± 3.2	57.0 ± 0.2	31.2 ± 0.8
Kluai Hugmuk	72.3 ± 1.8	10.9 ± 0.5	61.4 ± 2.3	27.3 ± 2.8
Indigenous cultivars				
Kluai Hin	72.7 ± 1.4	4.6 ± 0.6	68.1 ± 2.0	29.9 ± 0.6
Kluai Ngachaeng	75.5 ± 1.1	11.0 ± 0.5	64.6 ± 1.7	35.5 ± 0.1
Kluai Lepchaengkut	88.7 ± 1.1	38.0 ± 0.7	50.7 ± 0.3	35.9 ± 0.1
Kluai Nangphaya	91.0 ± 0.7	24.1 ± 3.9	66.8 ± 4.6	34.8 ± 0.2
Kluai Phamahagkuk	91.4 ± 0.0	31.3 ± 0.7	60.1 ± 0.7	32.2 ± 0.5
Kluai Thepparod	82.4 ± 0.7	23.9 ± 0.0	58.5 ± 0.7	33.2 ± 0.1

[1] Values are means of at least two analyses.

[2] Calculated as the difference between total starch and resistant starch content.

Ngachaeng, Kluai Lepchaengkut, Kluai Nangphaya, Kluai Phamahagkuk and Kluai Thepparod are generally rarely consumed by Thai people. The RS content observed in these banana cultivars ranged between 50.7 ± 0.3% and 68.1± 2.0% on a dry matter basis and most of them were obviously higher than that of the common cultivars. The highest value of 68.1 ± 2.0% was recorded in Kluai Hin and the second highest was Kluai Nangphaya of 66.8 ± 4.6%. This finding agreed with studies on the RS analysis of banana flour, that recorded values from 51.3% to 53.1% (Englyst *et al*., 1992; Goni *et al*., 1996).

Moreover this study found a high content of apparent amylose in the banana flour when compared to that of cereals and tubers according to reports by Swinkels (1985) and Champagne (1996). The amylose content of the common bananas such as Kluai Namwa, Kluai Hom, Kluai Kai, Kluai Lepmurnang and Kluai Hugmuk ranged between 24.7 ± 2.5% and 37.1 ± 0.5% and most values were slightly lower than those observed in the indigenous bananas. Kluai Hin, Kluai Ngachaeng, Kluai Lepchaengkut, Kluai Nangphaya, Kluai Phamahagkuk and Kluai Thepparod had an amylose content of 29.9 ± 0.6%, 35.5 ± 0.1%, 35.9 ± 0.1% 34.8 ± 0.2% 32.2 ± 0.5% and 33.2± 0.1%, respectively. Furthermore, analysis showed no significant linear relationship between the apparent amylose and the RS contents in the banana samples ($r^2 = 0.03$) (Figure 1), whereas a study by Eerlingen and Delcour (1995) indicated that amylose content and RS yield were positively correlated, since the formation of highly-resistant starch involved a crystallization of amylose, particularly in different cultivars of rice.

In vitro starch digestibility of some starchy food samples

Figure 2 shows profiles of the *in vitro* enzymatic starch digestibility of a selection of starchy food samples compared to that of the commercial cassava starch. Small kidney bean and mungbeans showed the lowest rate of starch digestibility during a period of 30 to 180 min compared to the other starchy food samples. The high amylose content in the bean starches and the composition of the RS-I and RS-II resulted in a very low rate of starch digestibility in the observed samples (Osorio-Diaz *et al.*, 2002), whereas commercial cassava starch exhibited greater starch digestibility than the other samples, as it contained a very pure starch with low fat and protein content.

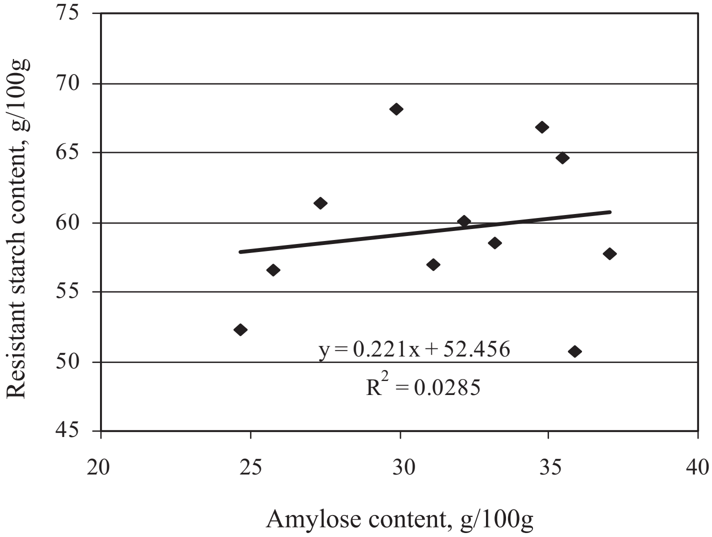

Figure 1 Linear correlation between apparent amylose and resistant starch in the banana samples.

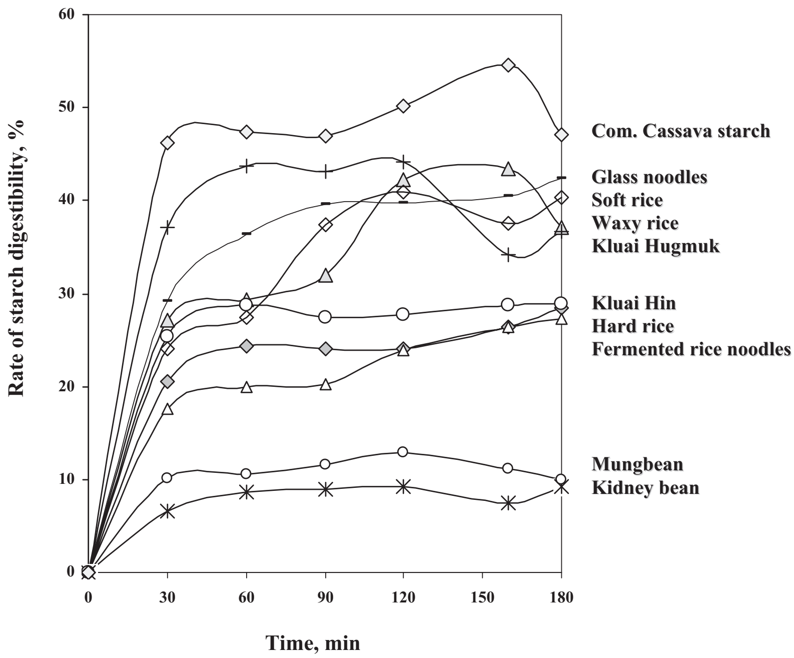

Figure 2 Rate of the *in vitro* enzymatic starch digestibility of the selected starchy foods.

Among the varieties of cereal grains, waxy rice had the highest rate of starch digestibility when compared to the other varieties of amylose rice. This result indicated that the amylopectin molecules, comprising almost 99% of the waxy starch granules, took up water into the amorphous regions at a fast rate and the digestion rate was enhanced (Wang and Wang, 2001). In other comparisons of bananas, the Kluai Hin indigenous type showed a lower rate of digestibility than Kluai Hugmuk, commonly consumed by Thai people.

Furthermore, fermented rice noodles (khanomjean) showed a low rate of starch digestibility relatively similar to that of the bean samples. Especially, glass noodles, had a digestibility rate as high as the waxy rice flour. This finding indicated that glass noodle manufacturers might replace some modified starches with mungbean starch in order to reduce the cost of raw materials. Therefore, an expectation of slow digestibility rate of the glass noodles which should relate to that of the mung bean samples was not observed in this study.

CONCLUSIONS

This present study found notable differences in the RS content in the starchy foods based on different plant origins and processing methods**.** The main RS sources were found in bananas (52.2 ± 4.1% to 61.4 ± 2.3%), legumes (10.3 ± 1.2% to 22.9 ± 0.0%) and glass noodle products (9.1 ± 0.8% to 11.3 ± 1.5%). The rice products were affected by the processing method as indicated by the formation of retrograded RS-III. Khanomjean produced by the fermentation of milled rice had a higher RS content (8.5 ± 1.1%) than that of the cooked white rice of the high amylose cultivar. Traditional Thai rice snacks, such as kaotung and kaokreupvor, had quite a low RS content compared to extruded snacks made with either rice kernels or flour. However frying and roasting the rice snacks seemed to enhance the RS-III content. Among the noodle products, vermicelli contained a higher RS than the other noodles. In addition, gelatinization of the starch had an effect on the RS content, as was shown in the cassava starch samples. This finding clearly indicated that the RS content in the banana samples was not statistically related to their amylose content. Finally, the different food sources had an impact on the *in vitro* starch digestibility and the legume group, in particular with the red beans, showed a very low rate of starch digestibility**.**

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