Amylose and Amylopectin in Selected Malaysian Foods and its Relationship to Glycemic Index
(Amilosa dan Amilopektin dalam Makanan Malaysia Terpilih dan Kaitannya dengan Indeks Glisemik)

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ABSTRACT

The aim of this study was to determine the nutrient contents and to evaluate the relationship between amylose and amylopectin content to glycemic index of diet commonly eaten by Malaysian. The food samples consisted of nasi lemak, *fried rice, fried rice noodle, fried macaroni, sandwich sardine, doughnut, curry puff and* roti canai *with dhal. Each sample was prepared based on standard recipe (except doughnut,* roti canai *with dhal and curry puff were bought from 3 different locations) in two different cycles. Moisture, ash, crude protein and crude fat were analyzed using proximate analysis whereas amount of carbohydrate was calculated "by difference". Total dietary fiber was analyzed using* AOAC 991.43. *The content of amylose and amylopectin were analyzed using colorimetric method and calculated "by difference", respectively. Our results showed that doughnut contained the highest carbohydrate (49.49 ± 1.24 g/100 g) while* nasi lemak *had the lowest carbohydrate (25.04 ± 0.56 g/100 g). Roti canai with dhal had the highest total dietary fiber content (3.89 ± 0.43 g/100 g). The highest amylose content was found in roti canai with dhal (11.75 ± 1.38%) while highest amylopectin content was in* nasi lemak *(94.19 ± 0.48%). The amylose content of tested samples ranged from 5 to 12%. In conclusion, results showed that there was no significant relationship between the ratio of amylose to amylopectin and glycemic index but negative trend existed which indicated increase in amylose content will lower the glycemic index of a food.*

Keywords: Amylopectin; amylose; glycemic index

ABSTRAK

Tujuan kajian ini dijalankan adalah untuk menentukan kandungan nutrien dan menilai hubungan antara kandungan amilosa dan amilopektin dengan nilai indeks glisemik dalam makanan kegemaran penduduk Malaysia. Sampel makanan terdiri daripada nasi lemak, nasi goreng, bihun goreng, makaroni goreng, sandwic sardin, donut, karipap dan roti canai dengan dhal. Setiap sampel dimasak berdasarkan resipi piawai (kecuali donut, roti canai dengan dhal dan karipap dibeli daripada tiga lokasi berlainan) pada dua kitaran berbeza. Kelembapan, abu, protein kasar dan lemak kasar dinilai melalui analisis proksimat manakala kandungan karbohidrat total dikira secara pembezaan. Gentian diet total ditentukan mengikut AOAC 991.43. Amilosa dan amilopektin pula dinilai masing-masing melalui kaedah kolorimetrik dan kaedah pembezaan. Hasil kajian menunjukkan donut mengandungi karbohidrat total tertinggi (49.49 ± 1.24 g/100 g) manakala nasi lemak mempunyai kandungan karbohidrat total yang terendah iaitu sebanyak 25.04 ± 0.56 g/100 g. Roti canai dengan dhal mempunyai kandungan gentian diet total paling tinggi (3.89 ± 0.43 g/100 g). Kandungan amilosa yang tertinggi ditunjukkan dalam roti canai dengan dhal (11.75 ± 1.38%) manakala untuk kandungan amilopektin tertinggi pula ialah nasi lemak (94.19 ± 0.48%). Julat kandungan amilosa dalam kajian adalah di antara 5 hingga 12%. Kesimpulannya, hasil kajian mendapati tidak terdapat hubungan signifikan di antara nisbah amilosa kepada amilopektin dengan nilai indeks glisemik tetapi tren negatif wujud di mana peningkatan kandungan amilosa menyebabkan penurunan indeks glisemik dalam sesuatu makanan.

Kata kunci: Amilopektin; amilosa; indeks glisemik

INTRODUCTION

Carbohydrate is the main proportion of food consumed daily and the main energy source that provides approximately 40-80% of total daily energy requirement in human (FAO/WHO 1998). However, starch is the main carbohydrate source in a variety of diet (Chung et al. 2006). According to Recommended Nutrient Intake Malaysia (NCCFN 2005), carbohydrate should comprise 55-70% of daily energy intake. Thus, it is of importance to know more on this macronutrient especially good control of glycemic response plays role in preventing a varied disease indirectly.

The concept of glycemic index was first proposed by Jenkins et al. (1981). It is a system that ranks foods, particularly carbohydrate-based, on their actual postprandial blood glucose response, compared to a

reference food. According to Vosloo (2005), the blood glucose response to food is determined by the glycemic index of the particular food. There are many factors that influence postprandial blood glucose response (Augustin et al. 2002), the food itself and individual physiological factors (Kirwan et al. 2001). According to FAO/WHO (1998), factors that affect postprandial glycemic response include the amount of carbohydrate, natural monosaccharide components, natural starch, food processing and cooking method and the presence of other food components. Amylose and amylopectin content of a food are one of the factors that affect blood glucose response. It is inversely correlated to glycemic index (GI) (Behall & Howe 1995).

This study was conducted to determine the nutrient contents and to evaluate the relationship between amylose and amylopectin content of commonly consumed foods by Malaysian to glycemic index.

MATERIALS AND METHODS

SAMPLES SELECTION AND PREPARATION

Eight foods commonly consumed by Malaysian that had been determined its GI value in the previous study (Nik Shanita 2005) were selected as sample in this study. The food samples consisted of *nasi lemak*, fried rice, fried rice noodle, fried macaroni, sandwich sardine, doughnut, curry puff and *roti canai* with dhal. Each sample was cooked based on standard recipe (Nik Shanita 2005) except doughnut (bought from three different Dunkin Doughnut outlets), roti canai with dhal and curry puff (bought from food stall, cafeteria and restaurant in Kuala Lumpur). Each sample was prepared in duplicate from 2 different cycles providing a total of 4 replicates.

The food samples were thoroughly homogenized using a kitchen mixer (National, Malaysia) at speed no. 2 for 5 min. Moisture content was analyzed using on air oven (Carbolite, England) in prior (AOAC 1995). One portion of about 60 to 70 g homogenized samples was dried in the oven at 105ºC and stored in the refrigerator at 4ºC for Total Dietary Fibre (TDF) and amylose analysis. The other portion was stored in Biomedical Freezer (-30ºC) for proximate analysis.

Proximate composition. All food samples were analyzed in duplicate for moisture, ash, crude fat and crude protein by the AOAC methods (AOAC 1995). Total carbohydrate content was calculated by difference (Southgate 1991) as follows:

$$\% \text{ Total carbohydrate} = 100\% - (\% \text{ moisture} + \% \text{ ash} + \% \text{ crude protein} + \% \text{ crude fat})$$

Total dietary fiber. Total dietary fiber was analysed using the AOAC method 991.43 (AOAC 1995). For samples which contain more than 10% of fat, defatted analysis was carried out by adding samples with 25 parts (V/W)

petroleum ether. The mixture was centrifudged using Universal 30RF (Tuttilingen, Germany) and organic solvents was discarded. Later, samples were dried overnight at 70ºC in the air oven. For TDF analysis, samples were accurately weighed (1 g) in duplicates into tall form beakers. About 40 mL Mes-Tris buffer solution (pH 8.2) was added into the beakers and the mixture was stirred using magnetic stirrer until all samples completely dispersed in solution. This was followed by adding the 50 μL heat stable alpha amylase and stirred at low speed. The beakers were then covered with aluminium foils and incubated for 35 min (95-100ºC) with continous agitation. Each assay was run with two blanks in order to measure any contribution from reagents to residue. All samples beakers were then removed from hot water bath and cool to 60ºC before adding 100 μL of protease solution to the samples. Incubate the samples in shaking water bath for 30 min at 60°C. About 5 mL of 0.561 HCl was then added into the samples in order to adjust the pH to 4.1 – 4.8. About 200 μL amyloglucosidase was added into the solution and incubated in shaking water bath at 60ºC for 30 min with constant agitation. About 225 mL of 95% ethanol (preheated to 60ºC) was added to each beaker. Samples were left at room temperature for 60 min to allow the formation of precipitation. Samples were then filtered through crucible containing celite. The filtered residue was washed twice with 10 mL of distilled water, 95% ethanol and acetone. Crucibles containing residue were dried overnight in oven at 103ºC. One residue was analysed for protein and the second one was analysed for total ash. Calculations of total dietary fiber was based on the below formula: % TDF = [R sample – P sample – A sample – B) /SW] × 100, where TDF = Total Dietary Fiber. R is the average residue weight (mg), P is the average protein weight (mg), A is the average ash weight (mg), SW is the average sample weight(mg) and B is the (R blank – P blank –A blank).

Amylose. Amylose content was estimated by the iodine colorimetric method of Mohana (Mohana et al. 2007). The dried sample was ground to pass through a 60 mesh (British standard screen).

Apparent amylose. Sample (100 mg) was weighed accurately and dissolved in ethanol (1 mL, 95%) and NaOH (1 N, 9.2 mL) and left overnight and made to volume (100 mL) in a volumetric flask. An aliquot (5 mL) of this solution was then added with acetic acid (1 N, 1 mL) and iodine solution (2 mL, 0.2% I_2 in 2% KI) and the volume made up to 100 mL with distilled water and mixed. After 20 minutes, the absorbance was measured at 620 nm using blank with 5 mL 0.09 N NaOH, 1 mL acetic acid and 2 mL iodine solution and made to 100 mL in total volume (Juliano et al. 1981). The above analysis was carried out in duplicates. A standard curve was plotted for mixtures of amylose and amylopectin from potato containing 0, 10, 25, 50, 75, and 100% amylose (McGrance et al. 1998).

Total amylose. To determine the total amylose content, the dried sample was defatted prior to analysis. Lipid extraction procedure is similar as described in TDF analysis.

Amylopectin. Amylopectin in tested food was calculated by difference (Juan et al. 2006) using following formula:

$$\text{Amylopectin (\%)} = 100\% - \text{amylose (\%)}$$

Statistical Analyses. The results collected were analyzed using SPSS version 12.0 and expressed as means ± standard deviation (SD). Pearson Correlation analysis was performed to determine the relationship between the ratio of amylose to amylopectin in tested food to glycemic index value.

RESULTS AND DISCUSSION

The proximate composition of tested food is presented in Table 1 and expressed on wet weight basis. The moisture content was generally high in all tested foods, ranged between 23 and 63%. The moisture content of *nasi lemak* was the highest, 62.78 ± 0.04%. It may due to its recipe of preparation. *Nasi lemak* comprised coconut milk rice served together with cucumber, sambal chili, steamed egg, groundnuts and anchovies where most ingredients were high in moisture than other foods that prepared either by pan frying or deep frying except sandwich sardine. According to Krokida et al. (2000), frying temperature affects moisture loss from food. The higher the temperature, the more moisture lost because frying process caused the moisture to leak while heat and fat will go into the food. Temperature for deep frying is much higher than pan frying. Hence, fried macaroni, fried rice and fried bihun prepared from pan frying generally contained more moisture than curry puff and doughnut that were prepared by deep frying. This also explains why curry puff and doughnut had the highest fat. The more the moisture lost due to high temperature, the more fats go into the food. Moreover, deep frying is a cooking method in which food is submerged in hot oil while pan frying uses lesser oil as compared to deep frying. Consequently, deep frying may cause extra oil immersed into the food. Hence, curry puff and doughnut contained the most fat. Crude fat ranged between 3 and 18% was obtained for the tested food.

Ash was very low in all foods, ranged between 0.6 and 2%. The highest ash content was recorded in doughnut (1.93 ± 0.09%). Crude protein ranged from 6 to 12% with sandwich sardine contained the highest protein (11.31 ± 0.65%). Sardine which is a rich source of protein explains why sandwich sardine contained the highest protein among the tested food. Total carbohydrate was relatively high, ranged between 25 and 50%. Doughnut had the highest total carbohydrate (49.49 ± 1.24%). Total dietary fiber was very low in all foods, ranging between 1.8 and 3.9 g/100g. *Roti canai* with dhal showed the highest total dietary fiber value (3.89 ± 0.43%).

The amylose and amylopectin content is presented in Table 2 and expressed on dry weight basis. The amylose content ranged between 5 and 12%. Juliano (1992) suggested the classification of amylose content in rice as waxy (0-5%), very low (5-12%), low (12-20%), intermediate (20-25%) and high (25-33%). Thus, the tested foods which were rice-based including fried rice, nasi lemak and fried bihun were classified as very low in amylose. Roti canai with dhal recorded the highest amylose content (11.75 ± 1.38%) followed by curry puff (10.77 ± 0.48%). *Roti canai* with dhal contained the highest amylose content due to the addition of dhal sauce which contributed the most starch to this food. Dhal sauce was prepared from lentil, a legume group which is high in amylose, ranged between 24 to 65% (Hoover & Sosulski 1991). Curry puff contained the second highest amylose after *roti canai* with dhal was similar to the observation by Hoover and Sosulski (1991) and Elliasson and Gudmundsson (1996).

TABLE 1. Percentage proximate composition and total dietary fiber values of tested food

Sample	% Moisture	% Ash	% Crude protein	% Crude fat	% Total carbohydrate	Total dietary fiber (g/100g)
Sandwich sardine	47.69 ± 0.59	1.92 ± 0.02	11.31 ± 0.65	5.04 ± 0.34	34.04 ± 1.60	2.70 ± 0.62
Fried rice	52.64 ± 3.80	0.68 ± 0.05	7.35 ± 0.56	5.23 ± 0.76	34.09 ± 2.45	3.95 ± 1.76
Nasi lemak	62.78 ± 0.04	0.97 ± 0.04	7.46 ± 0.27	3.75 ± 0.28	25.04 ± 0.56	2.57 ± 0.56
Doughnut	23.68 ± 0.87	1.93 ± 0.09	7.44 ± 0.31	17.46 ± 0.77	49.49 ± 1.24	1.84 ± 0.29
Fried rice noodle	56.41 ± 2.02	0.85 ± 0.01	7.86 ± 0.34	5.27 ± 0.31	29.61 ± 1.34	2.35 ± 0.13
Fried macaroni	55.19 ± 3.60	0.67 ± 0.07	8.05 ± 0.44	3.55 ± 0.48	32.54 ± 2.61	2.52 ± 0.06
Roti canai with dhal	57.20 ± 0.99	1.21 ± 0.03	6.28 ± 0.28	2.47 ± 0.33	32.84 ± 1.62	3.89 ± 0.43
Curry puff	37.50 ± 3.37	1.43 ± 0.16	6.41 ± 0.22	15.84 ± 1.53	38.82 ± 2.21	2.37 ± 0.06

*mean ± standard deviation of analysis expressed on wet weight basis or as it is consumed

TABLE 2. Percent total amylose and amylopectin of tested food and its GI value

Sample	% Total amylose	% Amylopectin	Ratio total amylose: amylopectin	Glycemic index (Nik Shanita 2005)
Sandwrich sardine	6.34 ± 0.21	93.66 ± 0.21	0.07	73
Fried rice	8.99 ± 1.02	91.01 ± 1.02	0.10	59
Nasi lemak	5.81 ± 0.48	94.19 ± 0.48	0.06	66
Doughnut	8.40 ± 0.06	91.60 ± 0.06	0.09	57
Fried bihun	7.72 ± 0.30	92.28 ± 0.30	0.08	99
Fried macaroni	9.73 ± 0.03	90.27 ± 0.03	0.11	74
Roti canai with dhal	11.75 ± 1.38	88.26 ± 1.38	0.13	71
Curry puff	10.77 ± 0.48	89.23 ± 0.48	0.12	54

*mean ± standard deviation of analysis expressed on dry weight basis

They reported that legume starch had the highest amylose compared to cereal starch, pseudo cereal starch and tuber. The filling of curry puff was mainly potato and the amylose in potato was reported 20% (Young 1984). From the analysis, amylopectin was found greater than amylose in all tested food. This observation is in agreement with Yotsawimonwat et al. (2008) that amylopectin is the major component in most starch.

Figure 1 shows the comparison of total amylose and apparent amylose content. Total amylose in tested food ranged from 5-12% whereas apparent amylose was 2-8%. This finding was similar to studies by Hoover and Ratnayake (2002) where total amylose was higher than apparent amylose. Apparent amylose is the amount of amylose that do not involve in amylose-lipid complex. Amylose-lipid complex occur when lipid binds into the helical amylose (Godet et al. 1993) whereas total amylose is determined after lipid extraction (Kwas´niewska-Karolak et al. 2008). Lipid extraction liberated the amylose-lipid complex and thus increasing the total amount of amylose (Radhika et al. 2008).

According to Table 2, *roti canai* with dhal (0.13) showed the highest ratio of amylose to amylopectin, followed by curry puff (0.12), fried macaroni (0.11), fried rice (0.10), doughnut (0.09), fried bihun (0.08), sandwich (0.07) and *nasi lemak* (0.06). The glycemic index value of each tested food that was reported by Nik Shanita (2005) and total amylose and amylopectin content are presented in Table 2. Results showed that there was no significant relationship ($p \geq 0.05$) between the ratio of amylose to amylopectin and glycemic index but negative trend existed which indicated increase in amylose content will lower the glycemic index value of a food where $r = -0.27$). Previous studies showed that the amylose content in a meal required at least 50% to significantly reduce plasma glucose and insulin response (Behall & Hallfrisch 2002; Behall & Scholfield 2005). However, the amylose content of tested foods was less than 50% thus it may explain why no significant relationship obtained in this study. Nonetheless, the negative trend showed that amylose does affect the GI value and similar to observation by Frei et al. (2003) and Hu et al. (2004) which high amylose rice produce
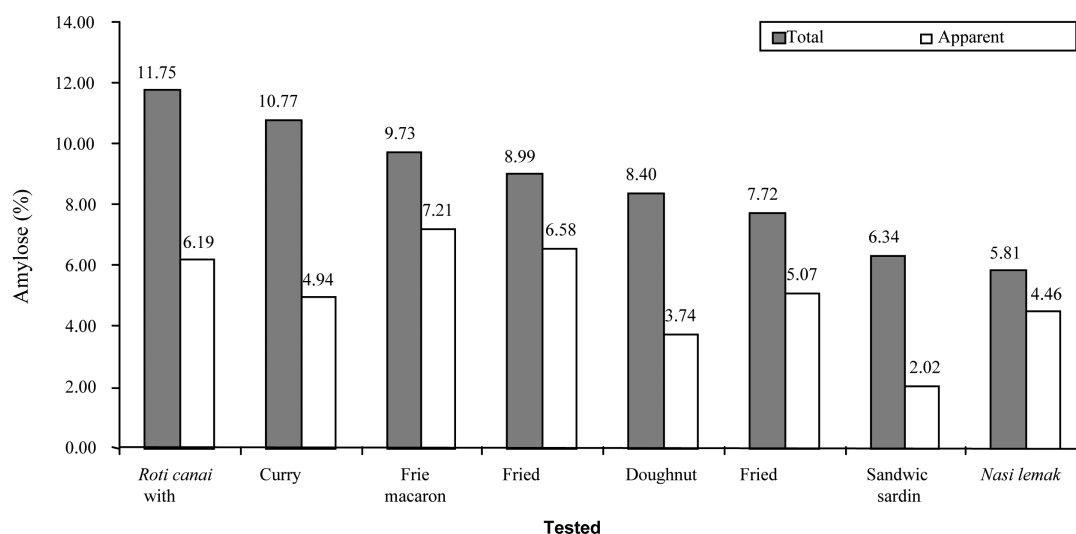

FIGURE 1. Comparison of total amylose to apparent amylose in tested food

lower GI value. This is due to high amylose slowed the digestion rate. There is greater hydrogen bonding between glucose units in amylose molecule than amylopectin thus less exposure to enzymatic digestion (Behall & Hallfrisch 2002). In addition, the larger size of amylopectin provides more open and wide surface for enzymatic attack as compared to smaller amylose (Bennion & Schuele 2000; McWilliams 2001). The presence of amylose-lipid content also reduces the amylose digestion rate (Hu et al. 2004).

Besides, there was no significant correlation (p≥0.05) between total dietary fiber of tested food to glycemic index where r = -0.079 but negative trend existed. High dietary fiber was believed to reduce the blood glucose response and thus lower the GI of a food (Barakatun Nisak et al. 2005). However, the results of this study showed that there was no relationship between total dietary fiber to GI (r = -0.079, p = 0.852). It is because only soluble fiber was reported to significantly lower the postprandial glucose concentration than insoluble fiber (Nuttall 1993; Riccardi & Rivellese 1991). Gagné (2008) showed that high soluble fiber lowers the GI by slowing down the stomach emptying and halted pancreatic enzymatic action to starch molecule in the gut. However, the relationship of soluble dietary fiber cannot be examined in this study because soluble and insoluble fiber was not determined.

CONCLUSION

There was no significant correlation between the ratio of amylose to amylopectin and glycemic index. However, amylose does affect glycemic index of the food. Furthermore, it may need to be greater than 50% to significantly reduce blood glucose and insulin response as been proved in previous studies. Critical evaluation and analysis involving amylase, soluble and insoluble fibre content of more foods deserve further research. This is relevant for epidemiological studies to investigating the role of carbohydrate including glycemic index in non-communicable chronic diseases.

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