https://doi.org/ 10.33472/AFJBS.6.9.2024.1439-1444

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

The effect of transglucosidase concentrations and digestion times on the oligosaccharide, physical properties, and in vitro digestibility of the modified rice flour and starch

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Article History
Volume 6,Issue 9, 2024
Received: 26-03-2024
Accepted : 28-04-2024
doi: 10.33472/AFJBS.6.9.2024.1439-1444

Abstract— Rice flour and starch are carbohydrates used in food and beverage products. They generally contain more rapidly digestible starch than slowly digestible and resistant starch, which can produce high blood glucose levels after rice digestion, modified using the transglucosidase (TGD) enzyme. Therefore, by employing a combined production process, this research investigates the effect of enzyme concentrations and digestion times on the sugar profile and physical properties of the modified rice flour and starch. After liquefaction, the TGD enzyme added 0.7-1.4% of the dry substance for 12-24 hours. Then dry to powder form through a spray dryer. The result shows an interaction between the concentration enzyme and incubation time on the yield of branched oligosaccharides ($p<0.01$). In the optimized condition (use TGD 1.4% TDS for 12 hours), starch provides glucose, DP2, DP3, and a glycemic index (GI) of 9.96, 12.23, 10.12 g/100g, and 85.04, respectively. In flour, the corresponding values are 13.19, 17.66, 14.35 g/100g, and 81.54. The physical properties affect color (L*, a*, b*), especially b*, which represents the yellow color in the product. Starch and flour provide values of 2.33 and 2.63, respectively. Additionally, solubility affects the flour but is non-significant in starch; both are soluble in water at over 90 percent. In conclusion, it was found that using different enzyme concentrations, digestion times, and types of carbohydrates influenced the type and quantity of slowly digestible carbohydrates, glycemic index (GI) and properties of products.

Index Terms— ***rice starch, enzymatic modify starch, Isomalto-oligosaccharides (IMOs), transglucosidase***

I. INTRODUCTION

According to the IDF Diabetes Atlas 2021, 95 percent of diabetes patients have type 2 diabetics, primarily due to obesity and food consumption, and the trend is expected to continue upward. [1] Currently, 1 in 11 people has diabetes without realizing it, and every 6 seconds, a person dies of diabetes [1]. For this reason, rice and cassava are carbohydrate sources mainly developed for healthy products [2]. Although most of the starch in rice or cassava is rapid digestion starch (RDS). It may additionally change into slow digestion starch (SDS), resistant starch (RS) [3], or sugars [4] like fructo-oligosaccharides (FOS) and isomalto-oligosaccharides (IMOs), which are sweeteners suitable for diabetics or those who want to control blood sugar levels.

Recently, modified starch using the transglucosidase (TGD) enzyme, Isomalto-oligosaccharides has been quite an interesting low-calorie sugar with the use of enzymatic modification methods to change starch to oligosaccharides and produce healthy products that are available both domestically and internationally. The key benefit is its prebiotic property [5], which promotes the growth of Bifidobacterium, which is one of the probiotics that benefit the body. Thailand imports isomalto-oligosaccharides from

Japan, China, the United States, Korea, Australia, and Canada, produced from wheat, barley, corn, peas, red beans, oats, cassava, rice, and potatoes. Therefore, it is possible to use raw materials in Thailand, such as rice or cassava, in production. Waste residual from rice milling can be used as a raw material to produce this high-value product.

Industrial production consists of three steps. Firstly, starch is liquefied using α-amylase. Secondly, saccharification occurs through the action of β-amylase, resulting in the

generation of maltose and glucose. The third step involves the transfer of maltose and glucose using transglucosidase to form isomaltose. The source of transferases affects the type and content of isomalto-oligosaccharides. Additionally, the kind of bond and the concentration of oligosaccharides depend on the substrate used. This research aims to study the interaction between enzyme concentration and incubation time on the yield of branched oligosaccharides, such as isomaltose [α-D-Glcp-(1,6)-α-D-Glcp], panose [α-D-Glcp-(1,6)-α-D-Glcp-(1,4)-D- Glcp], isomaltotriose [α-D-Glcp-(1,6)-α-D-Glcp-(1,6)-D-Glcp], and other branched oligosaccharides, by employing a combined production process. This is done through a comparative study of two different applications: starch, which contains more than 90% carbohydrates, and flour from polished white rice, containing carbohydrates, proteins, fats, and minerals. This approach aims to produce modified starch that still retains other nutritional components. The physical
and usability in healthy food and beverage products, have been measured.

II. METHODS AND MATERIALS

A. *Materials*

Rice starch was obtained from Cho Heng Rice Vermicelli Factory Co., ltd. Rice flour was obtained from Chia Meng Marketing Co., ltd. It was packed in a plastic bag and kept at room temperature until analyzed. The enzyme including Clarease® and optimax® from Genencor®, α-amylase produced by Aspergillus oryzae, and Transglucosidase L, was purchased from Amano Enzyme Inc., Nagoya Japan. The standard sugar composed of glucose and maltose, was purchased from Sigma-Aldrich Company Ltd. Also, isomaltose, maltotriose, isomaltotriose, and panose were purchased from Megazyme Ltd.

B. *Methods*

Rice starch and flour have an enzymatic modification including three steps to compare the effect of two commercial tranglucosidases on yield and The Physicochemical properties of oligosaccharides.

1st Step: Liquefaction using α-amylase from *Bacillus-licheniformis.*

Weigh starch at 35% w/v and add enzyme α-amylase, stir well, then heat to 90–95 °C, stir all the time, and collect samples every 5 minutes to determine the dextrose equivalent (DE)

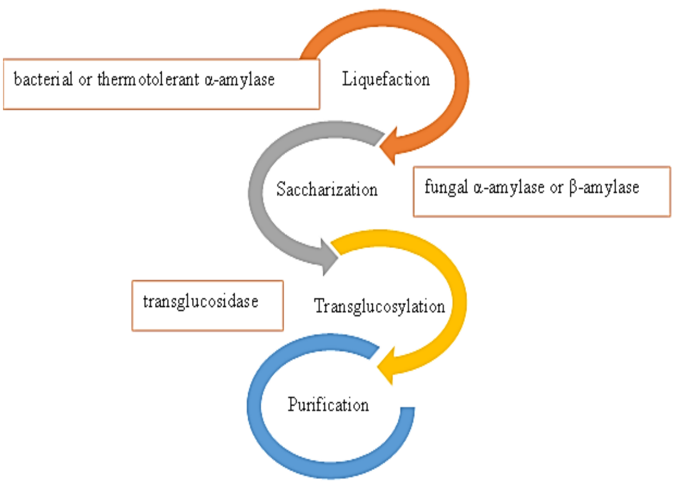

Fig. 1 IMOs critical process

2nd Step: Saccharification using α-amylase from *Aspergillus oryzae* and Pullulanase from *Bacillus-licheniformis.*

3rd Step: Transglucosylation using enzymes Transglucosidase,TGD L Amano from Aspergillus niger

The combined processes of saccharification and transglucosylation [3] are designed to generate maltose and glucose as substrates, which the TGD enzyme subsequently transforms into isomalto-oligosaccharides. Two variable factors, time (12-24 hours) and doses (0.7-1.4% per tds), are systematically manipulated within a factorial design framework.

The pH of the hydrolyzed starch or flour was adjusted to 5.2. Subsequently, α-amylase from Aspergillus oryzae (0.5 kg/tds), Pullulanase from Bacillus licheniformis (1 kg/tds), and TGD were added, following an experimental design approach. The treatments were incubated in a shaking incubator at 60°C for 12-24 hours. The process was inhibited by adjusting the pH to 3.0 and heating it to 100°C for 5 minutes; then it was made to powder using Buchi mini spray dryer B-290, inlet temperature 140 C°, outlet temperature 95-100 C°, pump 6 ml/min, nozzle cleaner four times/min.

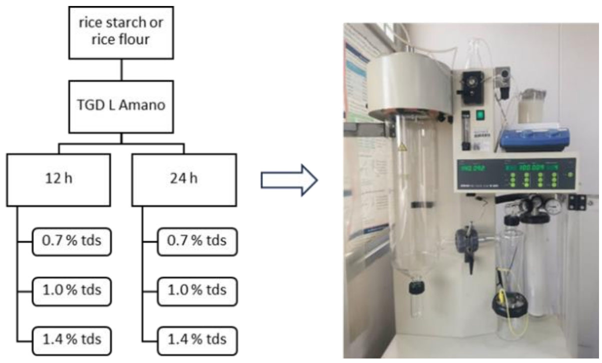

Fig. 2 Factorial design framework.

C. *Analytical Methods*

1) *Proximate composition of rice flour and starch*

Rice flour and starch were analyzed composition by milling, which was passed through a 120-mesh sieve and stored in aluminum foil bags. Then, moisture, protein, fat, ash, and carbohydrate content were analyzed by AOAC (1995) [6].

2) *Dextrose equivalent (DE) value*

Dextrose equivalent is the amount of reducing sugars in modified starch. It is used as an indicator in the liquefaction step to produce limit dextrin. Analyze the Somogyi-Nelson Method to calculate reducing sugar and convert it to Dextrose Equivalent (DE) value by the Lane-Eynon formula [7].]. A glucose standard of 1000 mg/L was created to create a standard curve.

DE = (% Reducing sugar x 100) / (% dry substrate)

3) High-Performance Liquid Chromatographic (HPLC)

Oligosaccharide in the modification process was adapted by Shodex application using Asahipak NH2P-50 4E columns (4.6 mm I.D. x 250 mm) with RI detector and column temperature 30 °C. The mobile phase was prepared by 65% (v/v) acetonitrile with pure water and then filtered through a 0.45-mm filter. The HPLC was performed at a flow rate of 0.6 mL/min and an injection volume of 10 ul [8]. Sugar or oligosaccharide profile reported as glucose (DP1), maltose,isomaltose (DP2), maltotriose, isomaltotriose and panose (DP3) [9-12].

4) Glycemic index (GI)

Weight 0.1 g of sample into a 50 mL centrifuge tube, followed by 0.9 mL of distilled water. Digestion with pepsin at 40 ◦C for 60 min, then add porcine pancreatic and incubate 37◦C for 30 min intervals until 180 min, stopping reaction by heating at 100 ◦C for 5 min. The supernatant 100 ul was added to amyloglucosidase and incubated at 60◦C for 45 min. They were measured by a microplate reader and incubated at 50◦C for 20 min with GOPOD reagent. The adapt method follows Goni, I.; Garcia-AIonso, A.; Saura-Calixto, F. A. (1997) [13].

Calculation GI = 39.71+ (0.549 x HI)

5) The Physicochemical properties

a) *Color was determined in the L* a* b* system using a colorimeter.*

b) *pH was determined by using a pH meter.*

c) *The water solubility index (WSI) and water absorption index (WAI) were determined using a hot air oven [14].*

d) *Water activity (Aw) was determined by using Water Activity (aw) Aqualab*

D. Statistical analysis

Proximate composition was compared using a completely randomized design (CRD) experiment. Results, presented as mean ± standard deviation, were evaluated by analysis of variance (ANOVA) employing the general linear model. Means were compared using Duncan’s new multiple range test (DMRT) at the 95% significance level. The factorial design, which explores the association among subjects, underwent evaluation through analysis of variance (ANOVA) within SPSS statistics version 25 for Windows. Outcomes are expressed as f-ratios, with significance levels indicated by superscripts at p-values of <0.05, 0.01, and 0.001, respectively.

III. RESULT AND DISCUSSION

A. Proximate composition of rice flour and starch

Rice flour and starch were analyzed using the AOAC (1995) method to determine the amount of starch, the initial substrate. Table 1 shows significant amounts of fat, ash, and carbohydrates. Rice flour contains 1.1711 g/100g, 0.4860 g/100g, and 90.7417 g/100g respectively. Rice starch contains 0.2052 g/100g, 0.5394 g/100g, and 93.2040 g/100g respectively. However, there is a non-significant amount of protein. According to the previous report [3,14-15] , rice flour demonstrates component values closely aligned with white rice. In contrast, rice starch exhibits lower quantities of fat and protein due to the partial removal of these components to attain a purer form of starch [16].

B. The effect of transglucosidase concentrations and digestion times on the oligosaccharide of the modified rice flour and starch

Plongbunjong.V et al. (2015) report that digestion time influences the oligosaccharide profile [17,18]. However, the catalytic activity is specific to the substrate molecule or suitable for unique reactions [19], which determine the value of oligosaccharides.According to previous research, we are interested in a study focused on critical factors, namely enzyme concentrations and digestion times, in the enzymatic modification of rice to produce modified flour and starch using the transglucosidase (TGD) enzyme. Two levels of time (12 h and 24 h) and three levels of enzyme concentration (0.7-1.4% TDS) were investigated.The results analyzed through ANOVA at a 95-99.99% significance level, revealed a significant joint influence of both time and concentration on the yield of various oligosaccharide molecules, including glucose, DP2, and DP3.

TABLE I

Proximate composition of rice flour and starch

Item	Rice flour	Rice starch
moisture (%ww)	$8.5692^a \pm 0.08$	$11.2786^b \pm 0.01$
Fat (%dw)	$1.1711^b \pm 0.21$	$0.2052^a \pm 0.07$
Ash (%dw)	$0.4860^a \pm 0.03$	$0.5394^b \pm 0.03$
Protein (%dw)	$7.6012^a \pm 0.36$	$6.0514^a \pm 0.06$
Carbohydrate (%dw)	$90.7417^a \pm 0.47$	$93.2040^b \pm 0.07$

Values are means ± standard deviation from triplicate determinations.
Different letters are significantly different along the rows ($p < 0.05$).
ww = wet weight, dw = dry weight

Rice flour and Rice starch are interactions between enzyme concentrations and digestion times. The influence of time factors in digestion affects the quantities of glucose and maltose isomaltose (DP2). Based on the statistical analysis (f-test) demonstrating the relationship as depicted in Table 2,3, the influence of enzyme quantities significantly impacts the production of oligosaccharides, maltotriose, isomaltotriose, and panose (DP3). It can be inferred that the longer digestion times of 12 and 24 hours result in increased glucose quantities in proportion to the increased time. Conversely, the use of enzymes in the range of 0.7-1.4% based on the total dry substance (TDS), with the highest concentration, leads to the most significant impact on the production of oligosaccharides with a high DP3 range. In the experiment, the study aimed to investigate modified starches with low glucose content and a high proportion of DP2 and DP3, in high quantities to slow down the digestion process of this type of starch and provide energy over a more extended period. Consequently, based on the study of modified rice starch, the optimal conditions for producing modified starch using this combined digestion process are a concentration of 1.4 % per tds and a digestion time of 12 hours.The modified rice flour is present 13.19 % of glucose (DP1), 17.66 % of isomaltose and maltose (DP2), and 14.35 % of isomaltotriose maltotriose and panose (DP3) which high value of isomalto-oligasaccharide than he modified rice starch that is present 9.96 % of glucose (DP1), 12.23 % of isomaltose and maltose (DP2), and 10.12 % of isomaltotriose maltotriose and panose (DP3).

TABLE II

F-ratio from ANOVA test (factorial design) of modification rice flour by TGD L Amano for Glucose, DP2, and DP3.

Rice flour: TGD L Amano				
Dependent Variable	df	Glucose	DP2	DP3
dose	2	55.13***	638.19***	1001.73***
time	1	3.580	118.71***	1416.91***
time *dose	2	16.11***	514.10***	1657.46***
R^2		0.924	0.995	0.998
Adjusted R^2		0.892	0.993	0.997

*, **, *** indicate significant p <0.05, 0.01, 0.01 respectively. N=6

TABLE III

F-ratio from ANOVA test (factorial design) of modification rice starch by TGD L Amano for Glucose, DP2, and DP3.

Rice starch: TGD L Amano				
Dependent Variable	df	Glucose	DP2	DP3
dose	2	230.07***	3940.88***	288.65***
time	1	1519.63***	9475.21***	47.36***
time* dose	2	72.95***	5468.37***	294.28***
R2		0.0994	0.9990	0.9900
Adjusted R2		0.9920	0.9990	0.9860

*, **, *** indicate significant p <0.05, 0.01, 0.01 respectively. N=6

C. The effect of transglucosidase concentrations and digestion times on the physical properties of the modified rice flour and starch

The modified rice flour and starch are evaluated for color using the L*, a*, b* system, water activity (Aw), water solubility index (WSI), and water absorption index (WAI) to study their characteristics and compare them with commercially produced modified starch or sugar. Rice flour shows an interaction between enzyme concentrations and digestion times in Table 4, except for the water absorption index (WAI) value, white rice starch shows the interaction between enzyme concentrations and digestion times in Table 5, except for the water solubility index (WSI) value.

1) color (L, a*, b* system)*

The modified rice flour and starch present a light- or light-yellow appearance with L* values ranging from 93 to 95, a* values ranging from -0.36 to -0.56, and b* values ranging from 2.12 to 3.22, while the commercial product has L*, a*, b* values of 96.62, -0.57, and 1.06, respectively. Rice flour and starch (1.4% TDD, 12-hour condition) exhibit b* values of 2.63 and 2.33, respectively, impacting the yellow hue of the product. However, these values remain within the acceptable range for Isomalto-oligosaccharides (IMOs) products. Moreover, prolonged digestion time contributes to the yellow color alteration due to the gradual progression of the Maillard reaction between sugar and amino acids [20]. With higher protein content, rice flour reacts more with sugar and amino acids, leading to an increased b* value.

2) water activity (Aw)

Modified rice flour and starch exhibit lower water activity, ranging from 0.12 to 0.21, compared to commercial products, which have a value of 0.32. However, these values satisfy the standards for a dry product. The results indicate the presence of microbial activity in the product, suggesting the potential for a longer shelf life.

3) water solubility index (WSI) and water absorption index (WAI)

The commercial product has a high solubility in water, exceeding 99.5%, and a water absorption index (WAI) of less than 2%. This indicates that the product possesses characteristics like sugar. However, the modified rice flour and starch undergo an incomplete purification process. Consequently, their solubility (more than 90%) is lower than that of commercial products, and they exhibit a higher water absorption index (WAI). This is because these products retain. There is some residual starch, albeit in the form of large molecules that dissolve in water to a lesser extent. Nevertheless, the nutritional value of the small amount of remaining starch in the product may contribute to enhancing the viscosity, good mouthfeel, and low sweetness taste of certain foods.

D. The effect of transglucosidase concentrations and digestion times on in vitro digestibility of the modified rice flour and starch

The modified rice starch and flour have high oligosaccharides (DP2, DP3). The glycemic index (GI) was compared against glucose over a period of time. As shown in Figure 3, the treatment using the TGD Amano enzyme at a concentration level of 1.4% per TDS and a digestion time of 12 hours for rice flour and starch resulted in a glycemic index value of 81.54 for rice flour. In contrast, rice starch had a glycemic index value of 85.04. However, upon analysis, it was found that sugar digestion remains relatively constant after 90 minutes and steadily decreases from 120 to 180 minutes. This digestion pattern is similar to a commercial sample with 90% pure oligosaccharides, which had a GI value of 84. Both modified starches exhibit digestion patterns that result in slower carbohydrate digestion compared to glucose or Jasmine white rice (GI 89) as reported by Pereira C, Lourenço VM, Menezes R, Brites C. (2021) [3]. Due to the action of enzymes, transglucosidases alter the structure of starch, consistsusually consists of amylose and amylopectin in an alpha-1,4 linkage. However, when digested by enzymes in the transferase group, capable of converting it into an alpha-1,6 structure, molecules such as isomalto isomaltotriose and panose are formed. These molecules are reported to be poorly digested in the human body.

TABLE IV
F-ratio from ANOVA test (factorial design) of The properties of modification rice flour by TGD L Amano

Rice flour: TGD L Amano							
Dependent Variable	df	L*	a*	b*	Aw	WSI index	WAI index
Dose	2	6.270	156.17***	154.94***	5933.09***	20.27***	0.26
Time	1	45.21***	104.17***	78.89***	45323.52***	150.25***	3.93
Time * Dose	2	29.62***	301.17***	646.19***	59406.83***	9.38***	0.56
R^2		0.907	0.988	0.993	1	0.946	0.317
Adjusted R^2		0.868	0.984	0.99	1	0.923	0.32

*, **, *** indicate significant p <0.05, 0.01, 0.01 respectively. N=6

Aw (activity water), WSI (water-soluble index) ,WAI (water absorption index)

TABLE V
F-ratio from ANOVA test (factorial design) of The properties of modification rice starch by TGD L Amano

Rice starch: TGD L Amano							
Dependent Variable	df	L*	a*	b*	Aw	WSI index	WAI index
dose	2	233.65*	206.60***	496.00***	40377.02***	1.161	13.32***
time	1	721.39*	2784.80***	2166.33***	34227.41***	0.935	4.558
time * dose	2	301.25*	140.60***	344.31***	43644.05***	0.144	4.63***
R^2		0.993	0.997	0.997	1	0.228	0.771
Adjusted R^2		0.991	0.995	0.996	1	0.094	0.676

*, **, *** indicate significant p <0.05, 0.01, 0.01 respectively. N=6

Aw (activity water), WSI (water-soluble index) ,WAI (water absorption index)

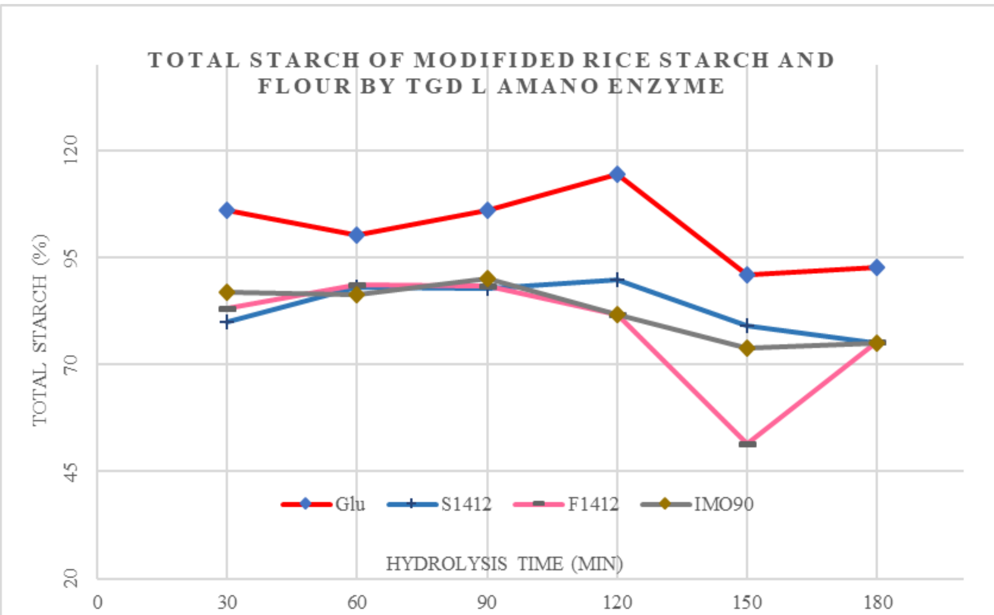

Fig 3 the comparison of total starch of modified rice starch and flour by tgd l amano which treated 1.4 %tds of enzyme for 12 h with glucose and commercial IMO.(s1412=starch 1.4% tds, 12h) ,(f1412= 1.4% tds, 12 h)

Previous reports have shown using transglucosidase enzymes to modify rice starch increases the proportion of resistant starch [21], and reports using the IMOs production process have also found it to slow down the absorption of sugar into the blood.

IV. CONCLUSION

In conclusion, it was found that using different enzyme concentrations, digestion times, and types of carbohydrates influenced the type and quantity of oligosaccharide pattern of product, properties of products and glycemic index (GI). The best condition in this project are rice flour and starch which concentration level 1.4 % per tds and digestion time 12 hour.

ACKNOWLEDGMENT

Research funding for presenting and publishing research is supported by Faculty of Graduate Studies, Mahidol University. This research project was also supported by the Department of Nutrition, Faculty of Public Health, Mahidol University, Thailand.

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