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Comparison of Six Different Techniques for Producing Resistant Starch Type III from High Amylose Rice

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Abstract. This research aimed to compare six different techniques for producing retrograde resistant starch (type III). High amylose (26.73 %) rice flour was dissolved with water, 95% ethanol, lactic acid (10 mmol/L), lactic acid (20 mmol/L), lactic acid (10 mmol/L) and ethanol, lactic acid (20 mmol/L) and ethanol (95%) prior to heating in autoclave at 121°C and incubation for four different period of times, including 0, 24, 48, and 72 h at 60 ℃. Overall, the results indicated that the rice flour heated with water and applied no incubation process yielded the highest RS content (13.91%), 6 times higher than untreated rice flour. The rice flour heated with ethanol and 10 mmol lactic acid, with no incubation after heating, had the moderate RS content (8.11-9.34%), whereas the flour heated with 20 mmol lactic acid gave lower level of RS content, regardless the incubation times.

Keywords: Resistant starch III, High amylose rice, Retrograde starch, Rice flour

1. Introduction

Resistant starch (RS) is the sum of starch and starch degradation products that are unable to be absorbed in the human small intestine; because they are resistant to the enzyme digestion [1]. RS appeared to have various physiological effects such as a reduction of plasma cholesterol, alteration in microbial populations, and increase in large intestinal short-chain fatty acid production. Therefore, RS has gained a lot of interesting from consumers at present, as one of the most bioactive constitutions in food. Rice is an important cereal as a staple food of over half the world's population and it is harvested with the large quantity per year. Rice, basically, is a primary dietary source of carbohydrate, but the freshly rice contains a lower percentage of RS (below 3%). Therefore, it is attractive source to produce RS. Several methods have been studied to anneal RS formation from varieties of flour. Heat moist treatment is one of the most popular techniques studied as it was safe and inexpensive. Cooling and storing of cooked rice is known to entail starch retrogradation, thus increasing the level of resistant starch type III (RS III) through recrystallization [2]. Therefore, the objective of the study was to investigate the resistant starch contents of different treatments of high amylose rice flour.

2. Materials and Methods

2.1. Sample Preparation

Broken rice (*Oryza sativa* cultivar Leuang 11) was obtained from Kalasin province, Thailand. The milled rice was ground into powder using a stainless hammer mill and passed through a sieve (100 mm mesh size). The powdered samples were packed in sealed plastic and stored at 4 ℃ until use.

2.2. Formation of RS III

Rice flour was suspended (25 g in 100 mL) in six different solvents, including water, ethanol (95%), 10 and 20 mmol/L lactic acid, 10 mmol/L lactic acid in ethanol, 20 mmol/L lactic acid in ethanol. Subsequently,

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the flours were heated in autoclave at 121 ℃ for 1 h [3,4]. To determine the optimum incubation time, the experiment was set-up as a completely randomised design by incubating heated flours at four different levels (0, 24, 48 or 72 h) at 60 ℃. The powdered samples were tray-dried at 45 ℃ for 12 h. The milled flours were ground and stored at 4 ℃ in sealed plastic containers until use.

2.3. Determination of Chemical Compositions

Proximate compositions of rice flour samples were analyzed; moisture content was done by drying in an oven at 105±2 ℃ until constant weight, ash content was determined using a muffle furnace temperature at 550 °C, crude fat was determined by Soxhlet extraction method, and protein content was evaluated by the Kjeldahl method, using 5.95 as the conversion factor, carbohydrate content was calculated from 100–(%protein +%fat +%moisture +%ash +%fiber) [5]. Amylose content was determined using spectrophotometric method. fiber was determined by crude fiber method. All determinations were carried out in triplicates, following the official methods and average values were reported.

2.4. Determination of Digestible Starch, Resistant Starch and Total Starch

The enzymatic method described by [6] was used of quantitative determination of digestible starch (DS), resistant starch (RS), and total starch (TS) using the Megazyme resistant starch assay kit (Megazyme International Ireland Ltd, Bray, Ireland).

2.5. Statistical Analysis

The results of individual samples are reported as the mean ± standard deviation. Data was analyzed by analysis of variance (ANOVA) and Duncan's multiple-range using SPSS statistical software. The significance differences were set at 95% confidence level.

3. Results and Discussion

3.1. Chemical Compositions of Flour

In this study, rice flour (cv. Leuang 11) was prepared by grinding and sieving of cleaned, milled broken rice. This variety of rice is native to Thailand and has widely been used for rice noodle production. The results pertaining to its chemical compositions are presented in Figure 1. Carbohydrate was the predominant chemical components in rice flour, accounting for 80.53% on wet weight basis while the mean values of the moisture, protein, ash, fat and crude fiber contents were 11.88%, 5.81%, 0.47%, 0.93% and 0.38%, respectively. These results are in agreement with the previous studies reported by [7]. Resistant starch and amylose contents of rice flour were found to be 2.67% and 26.73%, respectively. As the rice flour had amylose content of more than 25%, this rice flour could be classified into high amylose rice flour.

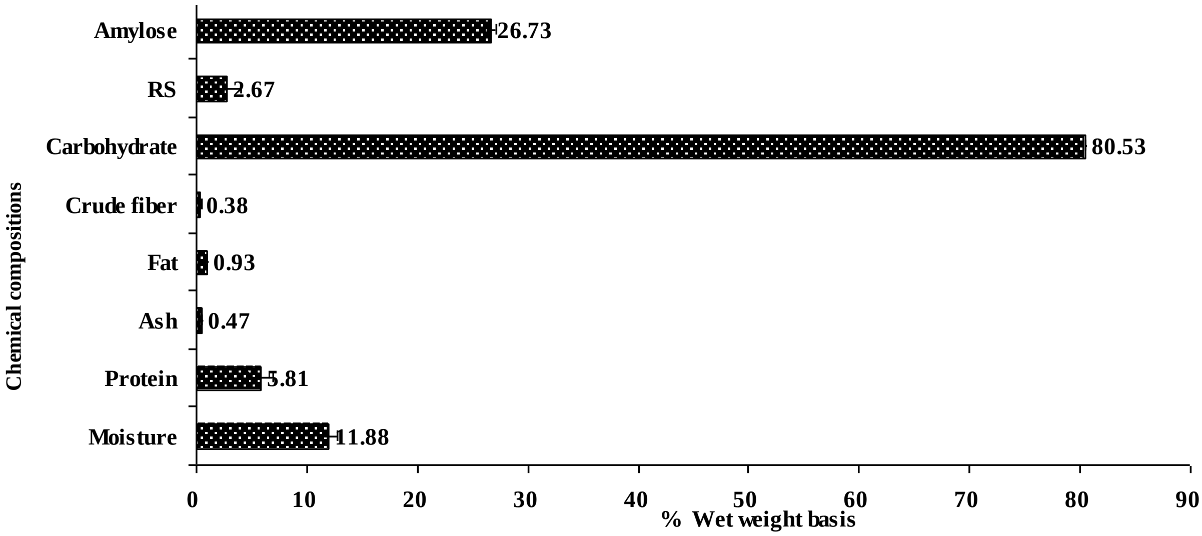

Fig. 1: Chemical compositions of rice flour (cv. Leuang 11)

3.2. Effect of Solvent Types and Incubation Times on Formation of RS III

The RS III contents of rice flours treated with six different solvents and incubated for four different periods of times are showed in Table I. Compared to the amount of RS in the native rice starch (2.67%), most rice flours autoclaved with different solvents had a significant higher content of RS III ($p \leq 0.05$). However, the change of RS content was not pronounced in sample autoclaved with 10 mmol/L lactic acid in ethanol and incubated for 72 h, showing RS level of 2.33%. The highest content of RS was observed in sample treated with water and no incubation (13.91%), followed by sample treated with 10 mmol/L lactic acid in ethanol and incubated for 24 h (9.66%).

It is known that the formation of RS III involves recrystallisation of amylose, subsequent to gelatinization, into enzyme-resistant double helices. There are three-stages of recrystallisation process, including nucleation (formation of critical nuclei), propagation (crystal growth from the nuclei formed) and maturation (continued crystal growth and perfection). The nucleation and propagation rates determine the overall recrystallisation rate while the maturation rate is more temperature dependent [8]. Nucleation generally proceeds rapidly when the incubation temperature is close to the glass transition temperature of starch, at about 5°C [9].

Table 1. Effects of solvent types and incubation times on the formation of retrograde RS from rice flour

Solvents	Incubation time (h)	Resistant starch (%)	% Relative change compared to native flour
Water	0	13.91±0.16[a]	420.97
	24	7.07±1.23[cdef]	164.79
	48	9.53±1.26[b]	256.93
	72	8.46±1.96[bcd]	216.85
Ethanol	0	8.79±0.94[bc]	229.21
	24	6.08±1.73[ef]	127.72
	48	7.03±0.25[cdef]	163.30
	72	9.32±1.09[b]	249.06
10 mmol/L Lactic acid	0	8.11±0.78[bcde]	203.75
	24	9.34±0.26[b]	249.81
	48	6.51±0.19[def]	143.82
	72	6.91±0.82[cdef]	158.80
10 mmol/L Lactic acid in ethanol	0	5.39±0.81[f]	101.87
	24	9.66±1.85[b]	261.80
	48	6.05±0.94[ef]	126.59
	72	2.33±0.49[g]	-12.73
20 mmol/L Lactic acid	0	8.13±1.08[bcde]	204.49
	24	6.28±0.05[def]	135.20
	48	6.14±0.17[ef]	129.96
	72	5.95±0.27[ef]	122.85
20 mmol/L Lactic acid in ethanol	0	6.47±0.37[def]	142.32
	24	6.18±0.22[ef]	131.46
	48	6.18±0.22[ef]	131.46
	72	5.87±1.02[ef]	119.85

[a-f] Means with different superscripts are significantly different ($p \leq 0.05$)

Irrespective of the incubation time, water was found to the most favorable for the formation of RS, followed by ethanol and lactic acid. Rice flours autoclaved with 10 and 20 mmol/L lactic acid, without incubation after heating, had statistically similar level of RS (8.11 and 8.13%, respectively). These results however differed from what was observed by [3]. This could be caused by an excessive acid hydrolysis of glycosidic bond that may limit amylose recrystallization during subsequent incubation. The combination of lactic acid and ethanol also seemed to lower the formation of RS level of rice flour.

Although the recrystallisation process is probably affected by incubation temperature and time after autoclave heating, the results showed the highest RS formation in autoclaved flours without incubation. Increasing incubation times after autoclaving seemed to affect the RS formation of rice flour differently, depending on types of solvent used. There was a growing increase in RS content from 6.08-7.07% to 8.46-9.32% with increasing time (24 to 72 h) in samples autoclaved with water or ethanol. However, the RS content decreased from 6.18-9.66% to 2.33-6.91% as incubation time increased from 24 to 72 h in samples autoclaved with acid or acid in ethanol.

3.3. Effect of Solvent Types and Incubation Times on Digestible Starch and Total Starch Contents

Figures 2 and 3 showed the digestible starch and total starch contents of rice flours treated with different solvents and incubated for different duration of times. It was noticed that the digestible starch content decreased with the increasing RS III content. The sample treated with 10 mmol/L lactic acid in ethanol, with no incubation after autoclaving, had the highest digestible starch (83.48%) and significantly different ($p \leq 0.05$) from the other samples. The lowest content of digestible starch (72.63%) was observed in sample autoclaved with water, without incubation after heating. However, treatments with different solvents had no significant effect on total starch content of rice flour ($p > 0.05$). All rice flours contained similar amount of total starch, ranging between 83.42 and 87.32% after heat treatment.

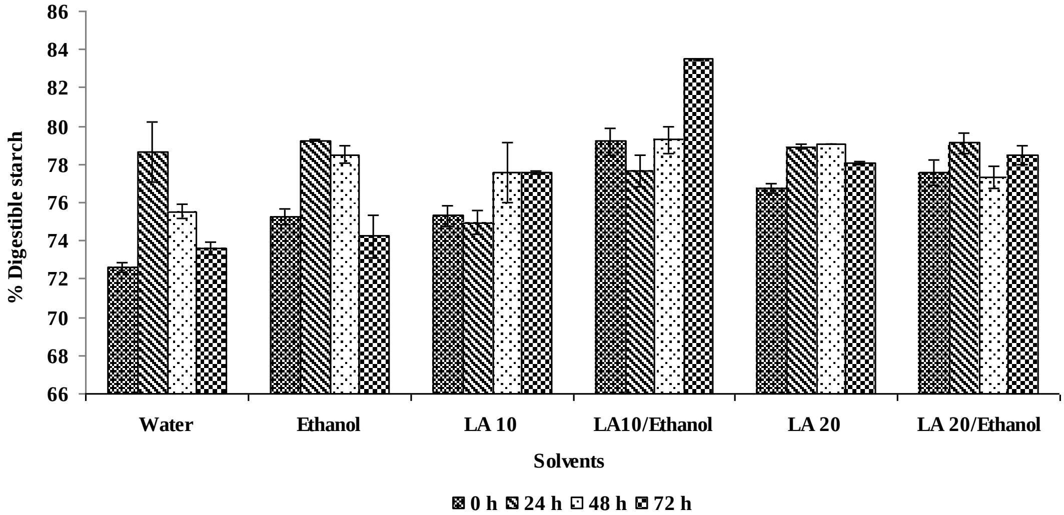

Fig. 2: Percentage of digestible starch of rice flours autoclaved with six different solvents and incubated for four different time periods (LA10 = lactic acid 10 mmol; LA 20 = lactic acid 20 mmol)

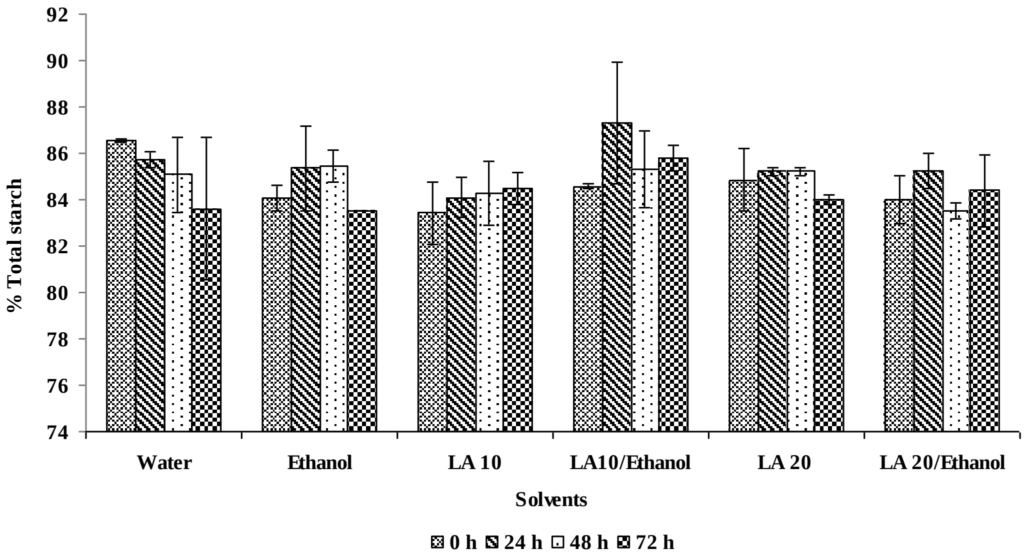

Fig. 3: Percentage of total starch of rice flours autoclaved with six different solvents and incubated for four different time periods (LA10 = lactic acid 10 mmol; LA 20 = lactic acid 20 mmol)

4. Conclusions

The findings suggest that autoclave heat treatment (121 ℃ for 1 h) in the presence of water, with no incubation after heating, significantly increased the level of RS III of rice flour (cv. Leuang 11). This process could be adapted to improve RS content of rice flour and to add value to the rice products.

5. Acknowledgements

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