



## The Producing Technology of Resistant Starch from Buckwheat Using Hydrochloric Acid (HCl) Hydrolysis Treatment

Lijun Wang<sup>1\*</sup> and Xue Bai<sup>2</sup>

<sup>1</sup>The College of Life Science, Yangtze University, Jingzhou, Hubei, China

<sup>2</sup>The First People's Hospital of Jingzhou, Jingzhou, Hubei, China

### ABSTRACT

Resistant starch (RS) has various functions in controlling the glycemic index (GI), lowering concentration of cholesterol and triglycerides, inhibiting fat accumulation, preventing colonic cancer, reducing gall stone formation, maintaining intestinal tract healthy and enhancing the absorption of minerals. Elevated RS in food is an important and effective approach for public health. RS is also an important material for industries. In this paper, the producing technologies of resistant starch from buckwheat were investigated. The results showed that the optimum parameters for producing technology of resistant starch from buckwheat using hydrochloric acid (HCl) hydrolysis treatment are heating temperature after HCl hydrolysis at 120 °C, HCl concentration 3%, solid-to-liquid ratio after HCl hydrolysis 1:5, heating time after HCl hydrolysis 0.5h.

**Keywords:** Buckwheat; Resistant starch; Orthogonal design

### INTRODUCTION

RS is also called enzyme resistant starch, defined as the starch and starch degradation products which cannot be digested and absorbed in the healthy small intestine of human [1]. RS provides functional properties in controlling GI [2], lowering concentration of cholesterol and triglycerides [3,4], inhibiting fat accumulation [5], preventing colonic cancer [6], reducing gall stone formation [7], maintaining intestinal tract healthy [8,9] and enhancing the absorption of minerals [10]. Resistant starch (RS), a novel insulin receptor sensitizer, is benefited to diabetes, which can enhance insulin function and regulate blood glucose [11]. Elevated RS in food is an important and effective approach for public health. RS is also an important material for industries. Buckwheat (*Fagopyrum esculentum*) belonging to plants of the genus Polygonaceae Buckwheat, is edible biologic medicine with relative high starch content, with various values of nutritional therapy health care [12]. The mechanism of RS formation is largely unknown. There are several factors affect the RS formation. It's reported that RS content is positive related to AC [13,14]. Starch granule size and structure are related the RS content. Starch granule in potato is larger than that in cereals, the potato starch digested more slowly than that of cereals [15]. Starch Crystalline structure can be classified into A type, B type and C type, according X-ray scattering pattern. The digestibility of the starch with B type less than A type, C type in the middle [16,17]. The chain length of amylose and amylopectin is another major factor affect the RS formation. RS increase according degree of polymerization (DP) of amylose (from 10 DP to 610 DP) by hydrothermal treatment with retention [18]. The effect of the chain length amylopectin on RS formation is unclear in detail. It reported that amylopectin starch debranched by pullulanase followed by heat-processing can increase RS content [19]. It's due to long unbranched chains of amylopectin involve into RS formation [20]. Other components in cell such as protein, lipid, cellulose etc. can also effect RS content [21,22]. Among them, lipids is most important effect on RS formation. Lipids can decrease RS content significantly [20]. Food additives and food processing technologies are another factors can affect RS content [23,24]. We analyzed the effects of the preparing

conditions to buckwheat RS content and got the optimal preparing conditions for buckwheat RS content. The results of this work will lay the foundation of theory and application for the further study of buckwheat RS.

## EXPERIMENTAL SECTION

### Preparation of Buckwheat Flour

Buckwheat was purchased from Jilin City. Buckwheat was grinded into flour using flour mill, then filtered using 200 mesh sieve.

### Determination of RS Content

RS content was measured according to AOAC method (2002.02) with a slight modification [25].  $100 \pm 1$  mg milled maize flour (only endosperm) were accurately weighed and placed directly into screw-cap tubes ( $16 \times 125$  mm). 500  $\mu$ L water was added into each tube, then boiled in electric cooker for 20 min and at warm keeping status at 50°C for 10 min. Tubes were taken out and cooled to room temperature. KCl-HCl buffer (pH=1.5) containing 6 IU/mg pepsin was added into each tube and the rice floury was ground and dispersed by a stirring rod, mimicking the chewing in mouth and warmed at 37°C for 1 h. Other procedures were carried out as described in the method AOAC (2002.02).

### The Optimization of the Preparation Process of Buckwheat RS

To optimize the preparation process of buckwheat RS, the major factors and their levels were determined according the effects of various factors (such as heating temperature after HCl hydrolysis, HCl concentration, Heating time after HCl hydrolysis, Solid-to-liquid ratio after HCl hydrolysis (S/L) on RS content using HCl hydrolysis treatments. The optimum preparation conditions of buckwheat RS were further determined using orthogonal test.

## RESULTS AND DISCUSSION

### The Effects of the Concentration of HCl on Buckwheat RS Content

The buckwheat starch neutralization with NaOH after hydrolysis using different concentration HCl for 1 h with 1:2 solid-to -liquid ratio, was autoclaved at 100°C for 0.5 h, then after storage at 4°C for 24 h, dried at 50°C for 18 h. The RS content of the dried buckwheat was analyzed. The optimum concentration of HCl is 4% (Figure 1).

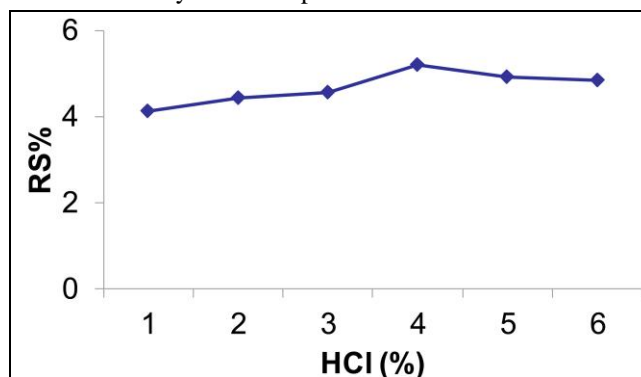


Figure 1: The effects of the concentration of HCl on buckwheat RS content

### The Effects of HCl Hydrolysis Time on Buckwheat RS Content

The buckwheat starch neutralization with NaOH after hydrolysis using 2% HCl for different time with 1:2 solid-to -liquid ratio, was autoclaved at 100°C for 0.5 h, then after storage at 4°C for 24 h, dried at 50°C for 18 h. The RS content of the dried buckwheat was analyzed. The optimum HCl hydrolysis time is 1 h (Figure 2).

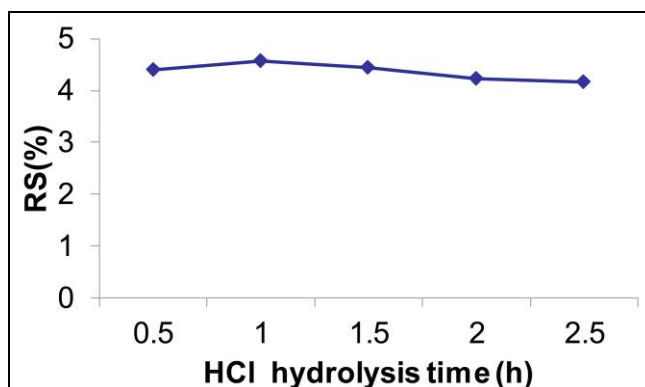


Figure 2: The effects of HCl hydrolysis time on buckwheat RS content

#### The Effects of Heating Temperature After HCl Hydrolysis on Buckwheat RS Content

The buckwheat starch neutralization with NaOH after hydrolysis using 3% HCl for 1h with 1:2 solid-to-liquid ratio, was autoclaved at different temperature for different times, then after storage at 4°C for 24 h, dried at 50°C for 18 h. The RS content of the dried buckwheat was analyzed. The optimum heating temperature is 120°C (Figure 3).

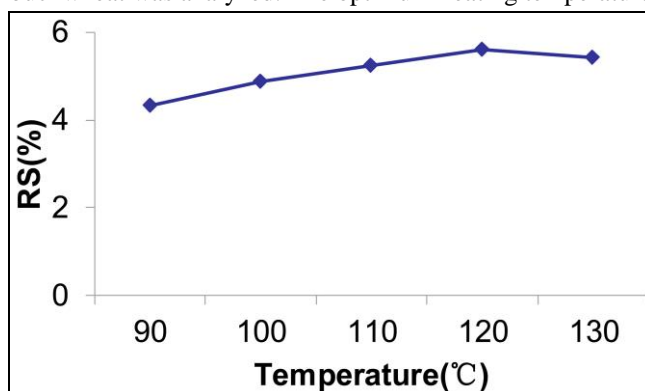


Figure 3: The effects of heating temperature after HCl hydrolysis on buckwheat RS content

#### The Effects of Heating Time After HCl Hydrolysis on Buckwheat RS Content

The buckwheat starch neutralization with NaOH after hydrolysis using 3% HCl for 1h with 1:2 solid-to-liquid ratio, was autoclaved at 100°C for different times, then after storage at 4°C for 24 h, dried at 50°C for 18 h. The RS content of the dried buckwheat was analyzed. The optimum heating time is 0.5 h (Figure 4).

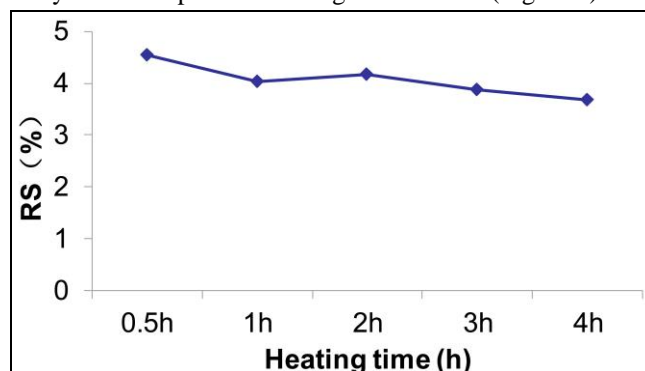


Figure 4: The effects of heating time after HCl hydrolysis on buckwheat RS content

#### The Effects of Solid-To-Liquid Ratio After HCl Hydrolysis on Buckwheat RS Content

The buckwheat starch neutralization with NaOH after hydrolysis using 3% HCl for 1 h with different solid-to-liquid ratio, was autoclaved at 100°C for 0.5 h, then after storage at 4°C for 24 h, dried at 50°C for 18 h. The RS content of the dried buckwheat was analyzed. The optimum solid-to-liquid ratio is 1:5 (Figure 5).

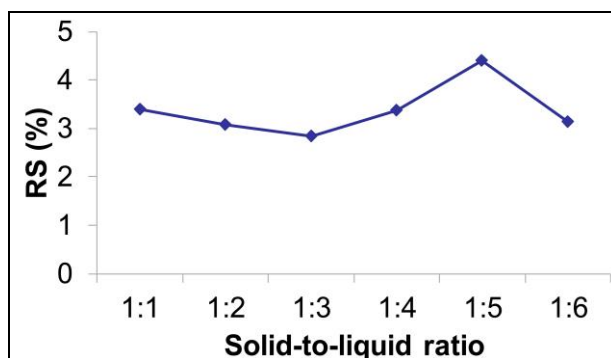


Figure 5: The effects of solid-to-liquid ratio after HCl hydrolysis on buckwheat RS content

### RS Processing Orthogonal Experiment

According to the effects of individual factors on the RS contents, orthogonal experiments were conducted using HCl concentration, heating temperature after HCl hydrolysis, heating time after HCl hydrolysis and solid-to-liquid ratio after HCl hydrolysis as factors and RS content as index (Tables 1 and 2).

Table 1: Factor level table

Level	A (HCl/%)	B (heating temperature/°C)	C (heating time/h)	D (solid-liquid ratio)
1	2	110	0.5	01:04
2	3	120	1	01:05
3	4	130	2	01:06

Table 2:  $L_9(3^4)$  RS processing orthogonal experiment design and results

No.	A (HCl/%)	B (heating temperature/°C)	C (heating time/h)	D (solid-liquid ratio)	RS (%)
1	1(2)	1(110)	1(0.5)	1(1:4)	5.74
2	1(2)	2(120)	2(1)	2(1:5)	6.78
3	1(2)	3(130)	3(2)	3(1:6)	5.54
4	2(3)	1(110)	2(1)	3(1:6)	5.97
5	2(3)	2(120)	3(2)	1(1:4)	7.37
6	2(3)	3(130)	1(0.5)	2(1:5)	7.18
7	3(4)	1(110)	3(2)	2(1:5)	6.05
8	3(4)	2(120)	1(0.5)	3(1:6)	6.23
9	3(4)	3(130)	2(1)	1(1:4)	5.96
K1	18.06	17.76	19.15	19.07	
K2	20.52	20.38	18.71	20.01	
K3	18.24	18.68	18.96	17.74	
R	2.46	2.62	0.44	2.27	

As the results shown in the Table 2, heating temperature after HCl hydrolysis had the largest effect on RS content. HCl concentration had the second largest effect on RS content. Solid-to-liquid ratio after HCl hydrolysis had the third largest effect on RS content. Heating time after HCl hydrolysis had the fourth largest effect on RS content. The optimum parameters for producing technology of resistant starch from buckwheat using HCl hydrolysis treatment are  $A_2B_2C_1D_2$ , that is heating temperature after HCl hydrolysis at 120°C, HCl concentration 3%, Heating time after HCl hydrolysis 0.5 h, Solid-to-liquid ratio after HCl hydrolysis 1:5. The sequence of effects on RS content:  $B > A > D > C$ .

## CONCLUSION

The major effects on RS content using HCl hydrolysis treatment are heating temperature after HCl hydrolysis, HCl concentration, Solid-to-liquid ratio after HCl hydrolysis, Heating time after HCl hydrolysis. The optimum parameters for producing technology of resistant starch from buckwheat using HCl hydrolysis treatment are heating temperature after HCl hydrolysis at 120°C, HCl concentration 3%, solid-to-liquid ratio after HCl hydrolysis 1:5, heating time after HCl hydrolysis 0.5 h.

## ACKNOWLEDGEMENTS

The work was supported by Dr. Scientific Research Fund (801100010121) from Yangtze University, China.

**Data availability statement:** All relevant data are within the paper and its Supporting Information files.

**Funding:** This research was supported by Dr. Scientific Research Fund(801100010121). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

## REFERENCES

- [1] A Escarpa; MC Gonzalez; MD Morales; F SauraCalixto. *Food Chem.* **1997**, 60, 527-532.
- [2] J Hasjim; SO Lee; S Hendrich; S Setiawan; YF Ai; JL Jane. *Cereal Chem.* **2010**, 87, 257-262.
- [3] M Fukushima; KH Han; M Iijuka; K Shimada; M Sekikawa; K Kuramochi; K Ohba; L Ruvini; H Chiji. *Brit J Nutr.* **2005**, 94, 902-908.
- [4] JF Perez; D Martinez-Puig; J Mourot; V Ferchaud-Roucher; A Anguita; F Garcia; A Krempf. *Livest Sci.* **2006**, 99, 237-247.
- [5] JA Higgins; MR Jackman; IL Brown; GC Johnson; A Steig; HR Wyatt; JO Hill; PS Maclean. *Nutr Metab.* **2011**, 8, 49.
- [6] J Burn; DT Bishop; PD Chapman; F Elliott; L Bertario; MG Dunlop; D Eccles; A Ellis; DG Evans; R Fodde; ER Maher; G Moslein; HF Vasen; J Coaker; RK Phillips; S Bulow; JC Mathers. *Cancer Prev Res.* **2011**, 4, 655-665.
- [7] AM Birkett; JC Mathers; GP Jones; KZ Walker; MJ Roth; JG Muir. *Br J Nutr.* **2000**, 84, 63-72.
- [8] U Lesmes; EJ Beards; GR Gibson; KM Tuohy; E Shimoni. *J Agr Food Chem.* **2008**, 56, 5415-5421.
- [9] J Phillips; JG Muir; A Birkett; ZX Lu; GP Jones; K O'Dea; GP Young. *Am J Clin Nutr.* **1995**, 62, 121-130.
- [10] L Yonekura; H Suzuki. *Eur J Nutr.* **2005**, 44, 384-391.
- [11] MD Robertson; AS Bickerton; AL Dennis; H Vidal; KN Frayn. *Am J Clin Nutr.* **2005**, 82, 559-567.
- [12] ZL Zhang; ML Zhou; Y Tang; FL Li; YX Tang; JR Shao; WT Xue; YM Wu. *Food Res Int.* **2012**, 49, 389-395.
- [13] BS Yadav; A Sharma; RB Yadav. *Int J Food Sci Nutr.* **2009**, 60, 258-272.
- [14] AM Leeman; ME Karlsson; AC Eliasson; IME Bjorck. *Carbohydr Polym.* **2006**, 65, 306-313.
- [15] SG Ring; JM Gee; M Whittam; P Orford; IT Johnson. *Food Chem.* **1988**, 28, 97-109.
- [16] HN Englyst; J Veenstra; GJ Hudson. *Brit J Nutr.* **1996**, 75, 327-337.
- [17] CG Biliaderis. *Can J Physiol Pharm.* **1991**, 69, 60-78.
- [18] RC Eerlingen; M Crombez; JA Delcour. *Cereal Chem.* **1993**, 70, 339-344.
- [19] CS Berry. *J Cereal Sci.* **1986**, 4, 301-314.
- [20] SL Mangala; K Udayasankar; RN Tharanathan. *Food Chem.* **1999**, 64, 391-396.
- [21] A Escarpa; MC Gonzalez. *Food Sci Technol Int.* **1997**, 3, 149-161.
- [22] M Torre; AR Rodriguez; F Sauracalixto. *J Agr Food Chem.* **1992**, 40, 1762-1766.
- [23] T Gelencser; V Gal; A Salgo. *Food Bioprocess Tech.* **2010**, 3, 491-497.
- [24] N Mulinacci; F Ieri; C Giaccherini; M Innocenti; L Andrenelli; G Canova; M Saracchi; MC Casiraghi. *J Agr Food Chem.* **2008**, 56, 11830-11837.
- [25] BV McCleary; M McNally; P Rossiter; P Aman; T Amrein; S Arnouts; E Arrigoni; L Bauer; C Bavor; K Brunt; R Bryant; S Bureau; ME Camire; M Champ; Q Chen; ML Chin; W Colilla; J Coppin; D Costa; G Crosby; J Dean; JD Berrios; J de Valck; J Doerfer; M Dougherty; K Eybye; G Fahey; A Femenia; P Forssell; J Gelroth; J Geske; H Hidaka; MF Isaksen; B Kettlitz; F Kozlowski; HN Laerke; B Li; Y Lincoln; Q Liu; AC Martensson; S Mattys; F Meuser; J Monroe; L Niba; C Niemann; J Panozzo; P Rossiter; JM Roturier; C Sampson; P Sanders; D Suter; AA Tas; H Themeier; C Tudorica; R Watson; L Weber; M Weinstein; M Wilkinson; J Yen; T Zheng. *J Aoac Int.* **2002**, 85, 1103-1111.