

Optimization of the Sustainable Production of Resistant Starch in Rice Bran and Evaluation of Its Physicochemical and Technological Properties

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SUPPLEMENTARY MATERIALS

S.1. Chemical analysis

Raw material was tested for protein, fibre, fat, and ash contents according to the AACC Official Methods [1]. The crude protein content was determined by the Kjeldahl nitrogen (method 920.152), the percentage of protein was estimated by multiplying the total nitrogen content by a factor of 5.95. Ash was determined by combustion of the sample in a muffle oven at 550 °C (method 942.05). The fat content was determined by Soxhlet extraction method (method 996.01). Total dietary fibre content was determined according to the method 985.29.

S.2. Determination of technological properties

S.2.1. Water and oil absorption capacity

For water absorption capacity (WAC) and oil absorption capacity (OAC) determination Ahmad et al. [2], 0.5 g of sample was placed in a pre-weighted centrifuge tube and mixed with 6 mL of distilled water or rapeseed oil. After 1 h shaking in a 37°C water bath, the liquid fraction was carefully removed by centrifugation (2,000×g, 15 min). The wet residue was weighed (wet weight), and the water/oil holding capacity was expressed as grams of water/oil absorbed by the gram of starch (g/g).

S.2.2. Water solubility and swelling power

The measurement of solubility and swelling power of starch was performed according to Li et al. [3] with slight modifications. The sample (0.5 g) of starch (W) was mixed with distilled water (5 mL), and was incubated at 30 °C with continuously shaking for 30 min in a water bath. After, it was cooled to room temperature and centrifuged (3,500×g, 20 min) (W_d). The supernatant was transferred to a glass tube and dried in an oven at 105°C to constant weight as water-soluble starch (S). The solubility and swelling power were calculated according to the following equations:

$$\text{Solubility (\%)} = S \times 100 / W \quad (1)$$

$$\text{Swelling power (\%)} = (W_d \times 100) / [W (100 - S)], \quad (2)$$

where S is the weight of dried supernatant (g), W_d – is the weight of wet sediment (g), and W is the sample weight (g). All samples were analysed in triplicate.

S.3. Experimental section

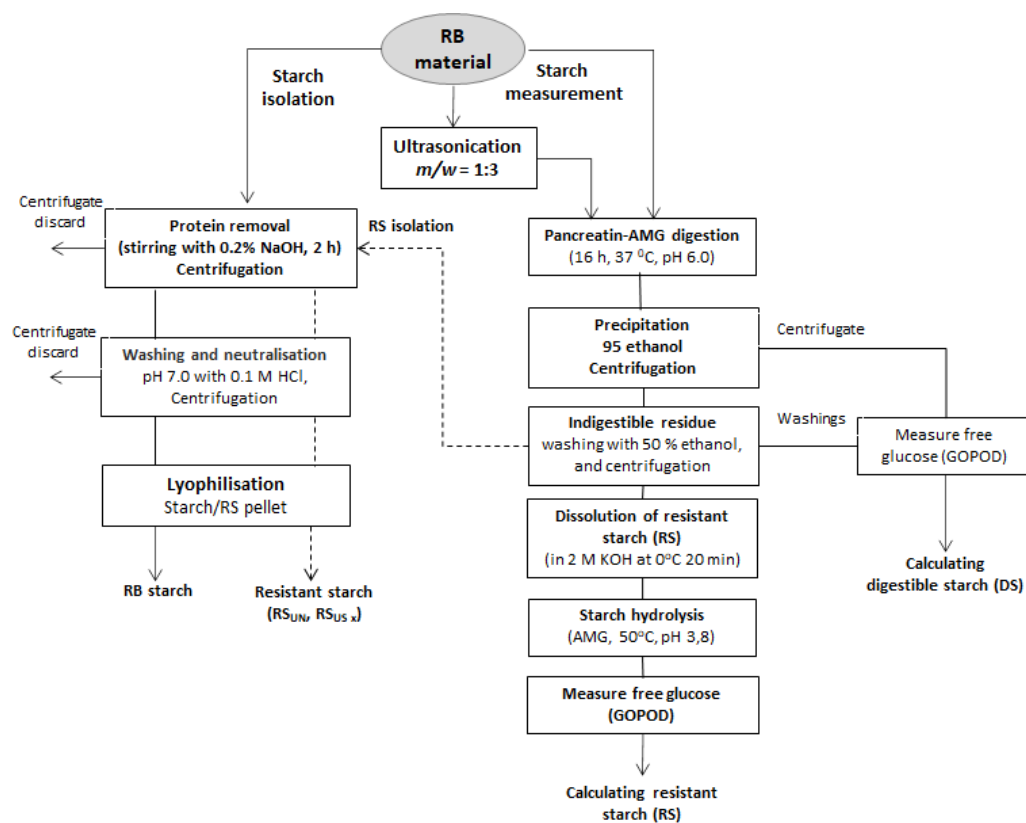


Figure S1. Rice bran starch and resistant starch isolation and measurement scheme.

Table S1. Multiple comparisons between means of different US (1.3 W/cm²) temperature groups (significant at $p < 0.05$).

| | Mean | | | | |
|--------|----------------|----|---------|---------|--------|
| | Sum of Squares | df | Square | F | Sig. |
| 15 min | 616.423 | 4 | 154.106 | 749.007 | 0.0001 |
| 20 min | 614.204 | 4 | 153.551 | 585.090 | 0.0001 |
| 25 min | 321.119 | 4 | 79.137 | 540.531 | 0.0001 |
| 30 min | 68.8417 | 4 | 19.359 | 412.118 | 0.0001 |
| 35 min | 59.785 | 4 | 14.946 | 267.359 | 0.0001 |

Table S2. Multiple comparisons between means of different US (1.3 W/cm²) time groups (significant at $p < 0.05$).

| | Sum of Squares | df | Mean Square | F | Sig. |
|-------|----------------|----|-------------|---------|--------|
| 30 °C | 185.611 | 4 | 46.403 | 286.885 | 0.0001 |
| 40 °C | 641.174 | 4 | 160.294 | 560.506 | 0.0001 |
| 50 °C | 372.268 | 4 | 93.067 | 537.090 | 0.0001 |
| 60 °C | 177.368 | 4 | 44.342 | 576.919 | 0.0001 |
| 70 °C | 0.516 | 4 | 0.129 | 21.629 | 0.060 |

Table S3. Significant coefficients of quartic model equation in terms of coded factors.

| Factor | Coefficient Estimate | df | Standard Error | 95% CI Low | 95% CI High | VIF |
|-------------------------------|----------------------|----|----------------|------------|-------------|--------|
| Intercept | 11.65 | 1 | 0.0324 | 11.58 | 11.72 | |
| A-Time | -3.33 | 1 | 0.0463 | -3.43 | -3.23 | 3.00 |
| B-Temp | -0.5519 | 1 | 0.0432 | -0.6496 | -0.4542 | 2.82 |
| C-Power | 0.9644 | 1 | 0.0298 | 0.8971 | 1.03 | 2.62 |
| AB | -0.8837 | 1 | 0.0328 | -0.9578 | -0.8097 | 1.0000 |
| AC | -0.2800 | 1 | 0.0463 | -0.3848 | -0.1752 | 3.00 |
| BC | -0.1419 | 1 | 0.0432 | -0.2396 | -0.0442 | 2.82 |
| A ² | -3.60 | 1 | 0.0565 | -3.73 | -3.47 | 2.41 |
| B ² | -6.27 | 1 | 0.0537 | -6.40 | -6.15 | 2.19 |
| ABC | -0.1387 | 1 | 0.0328 | -0.2128 | -0.0647 | 1.0000 |
| A ² B | 0.8807 | 1 | 0.0542 | 0.7581 | 1.00 | 2.74 |
| A ² C | -0.1399 | 1 | 0.0392 | -0.2287 | -0.0512 | 2.15 |
| AB ² | 1.19 | 1 | 0.0567 | 1.06 | 1.31 | 3.00 |
| B ² C | -0.0930 | 1 | 0.0392 | -0.1817 | -0.0042 | 2.32 |
| A ² B ² | 2.47 | 1 | 0.0781 | 2.30 | 2.65 | 3.94 |
| A ² BC | -0.0643 | 1 | 0.0542 | -0.1869 | 0.0583 | 2.74 |
| AB ² C | 0.3412 | 1 | 0.0567 | 0.2129 | 0.4696 | 3.00 |

Table S4. Analysis of variance of the regression parameters for a quartic model for the response factor.

| Source | Sum of Squares | df | Mean Square | F-value | p-value | |
|-------------------------------|----------------|--------------------------------|-------------|----------|----------|-----------------|
| Model | 389.87 | 16 | 24.37 | 2839.52 | < 0.0001 | significant |
| A-Time | 44.36 | 1 | 44.36 | 5168.84 | < 0.0001 | |
| B-Temp | 1.40 | 1 | 1.40 | 163.30 | < 0.0001 | |
| C-Power | 9.01 | 1 | 9.01 | 1049.64 | < 0.0001 | |
| AB | 6.25 | 1 | 6.25 | 728.10 | < 0.0001 | |
| AC | 0.3136 | 1 | 0.3136 | 36.54 | 0.0002 | |
| BC | 0.0927 | 1 | 0.0927 | 10.80 | 0.0094 | |
| A ² | 34.80 | 1 | 34.80 | 4055.14 | < 0.0001 | |
| B ² | 117.02 | 1 | 117.02 | 13636.80 | < 0.0001 | |
| ABC | 0.1540 | 1 | 0.1540 | 17.95 | 0.0022 | |
| A ² B | 2.27 | 1 | 2.27 | 263.98 | < 0.0001 | |
| A ² C | 0.1092 | 1 | 0.1092 | 12.72 | 0.0061 | |
| AB ² | 3.75 | 1 | 3.75 | 437.29 | < 0.0001 | |
| B ² C | 0,0482 | 1 | 0,0482 | 5,62 | 0,0419 | |
| A ² B ² | 8,59 | 1 | 8,59 | 1001,51 | < 0.0001 | |
| A ² BC | 0,0121 | 1 | 0,0121 | 1,41 | 0,2658 | |
| AB ² C | 0,3105 | 1 | 0,3105 | 36,19 | 0,0002 | |
| Residual | 0,0772 | 9 | 0,0086 | | | |
| Lack of Fit | 0,0228 | 1 | 0,0228 | 3,34 | 0,1049 | not significant |
| Pure Error | 0,0545 | 8 | 0,0068 | | | |
| Std. Dev. | 0,0926 | R² | 0,9998 | | | |
| Mean | 7,78 | Adjusted R² | 0,9994 | | | |
| C.V. % | 1,19 | Predicted R² | 0,9736 | | | |
| | | Adeq Precision | 153,6987 | | | |

References

1. *Approved Methods of the AACC International*, 10th ed.; The Association AACC: Saint Paul, MN, USA, 2000.
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3. Li, A.; Gao, Q.; Ward, R. Physicochemical properties and in vitro digestibility of resistant starch from mung bean (*Phaseolus radiatus*). *Starch/Stärke* **2011**, *63*, 171–178.