Effect of different processing methods on resistant starch content and \textit{in vitro} starch digestibility of some common Indian pulses

D M Kasote, S S Nilegaonkar* and V V Agte
Microbial Sciences Division, MACS-Agharkar Research Institute, Pune-411004, MS, India

Received 16 May 2013; revised 29 January 2014; accepted 22 April 2014

In the present study, the effect of various processing methods like soaking, autoclaving, storage and pullulanase treatment on resistant starch content and \textit{in vitro} starch digestibility of \textit{dhals} (split pulses) of pulses pigeon pea, green gram and black gram was assessed. Results showed that these processing methods including pullulanase treatment significantly decreased the resistant starch (RS) content in all the samples. Further, results of \textit{in vitro} starch digestibility demonstrated that autoclaving significantly increased the predicted glycemic index (pGI) and slowly digested starch (SDS). However, decrease in rapidly digested starch (RDS) and starch digestive index (SDI) was observed after autoclaving. Results suggested that RS and RDS are getting converted into SDS after processing treatments. Moreover, present findings suggest that, the processed \textit{dhals} of pigeon pea, green gram and black gram could have added health promoting potential due to their high content of SDS.

**Keywords**: Pulses; autoclaving; \textit{in vitro} starch digestibility; resistant starch; rapidly digestible starch

**Introduction**

Pulses are one of the main components of Indian diets. India has been growing 12 different pulse crops\(^1\). Indian pulses mainly include chickpea, pigeon pea, mung bean, black gram, lentil, field pea etc. They are the excellent source of proteins, carbohydrates and minerals. Pulses also act as the main source of protein in a diet of vegetarian Indian population, which supply almost all essential amino acids. Legumes are nutritionally important but their use in diet is recommended to be limited owing to presence of antinutritional factors. Starch is the major carbohydrate in pulse seeds, which accounts for 22–45% of the dry matter\(^2\). Legumes consumption is generally recommended in diabetic condition, as they have low glycemic index (GI)\(^3\). The GI of the food is usually obtained by comparing the postprandial blood glucose response with reference food\(^4\). Starch is generally classified as digestible and non-digestible starch. Digestible starch further comprises of rapidly digestible starch (RDS) and slowly digestible starch (SDS). Non-digestible starch is popularly known as resistant starch (RS). These days, RS is receiving nutritional importance due to its various health benefits, generally mediated through production of short chain fatty acids after fermentation in large intestine\(^5\). RS is further categorized as RS1, RS2, RS3 and RS4\(^6\). RS1 represents physically entrapped starch within whole or partly milled grains or native seeds. RS2 is ungelatinized starch, which is unavailable to amylotic enzymes due to its compact and anhydrated structure. Retrograded starch is of RS3 category, occurs in heat treated or cooked foods. RS4 represent chemically modified starch\(^6, 7, & 8\). RS is a functionally important type of starch, found to be increased in cereals by moist heat processes especially autoclaving, autoclaving-cooling cycles\(^9, 10\). In addition, soaking and pullulanase debranching treatments were also found to be useful in increasing RS content\(^9, 11\). Therefore, the present study was undertaken to explore the effect of various processing methods like soaking, autoclaving, storage and pullulanase treatment on resistant starch content and \textit{in vitro} starch digestibility of some pulses of Indian geographical origin such as pigeon pea, green gram and black gram.

**Materials and methods**

**Materials**

Polished \textit{dhals} (split pulses) of pigeon pea (\textit{Cajanus cajan}), green gram (\textit{Vigna radiata}) and black gram (\textit{Vigna mungo}) were purchased from local markets of Pune. Flours of \textit{dhals} were prepared by using blender. Enzymes such as α-amylase, pepsin,
amylglucosidase and pullulanase were procured from Sigma-Aldrich. All chemicals used were of analytical grade.

Isolation of starch

Starch from dhal flours of pigeon pea, green gram and black gram was isolated according to method described by Aċkar et al.\textsuperscript{12} with some modifications. Briefly, 50 g flour of each dhal was suspended in 0.15% NaOH (1:3 w/v), stirred for 1 h at room temperature. Suspension was centrifuged at 3000 rpm for 5 min and upper greyish layer was discarded. Washing was repeated until grey layer disappeared. Washed starch was neutralized with 1 M HCl, centrifuged once again and oven air-dried at 50°C. Sediment containing starch was washed with distilled water, again centrifuged and the supernatant was discarded. Washing was repeated until grey layer disappeared. Washed starch was neutralized with 1 M HCl, centrifuged once again and oven air-dried at 50°C. Isolated starches were powdered in blender and used for further studies.

Processing methods

Dhals of pulses pigeon pea, green gram and black gram were soaked overnight in distilled water at room temperature. Soaking water was then drained and dhals were dried at 50°C in an oven for 24 h. The dried dhals of pulses pigeon pea, green gram and black gram were ground into fine powder and used for RS content analysis. Isolated Starch samples were dispersed in water (1:5, w/v) and autoclaved at 120°C for 1 h. Cooled starch materials was dried in oven at 50°C for 2 days. Additionally, a batch of autoclaved samples was also stored at 4°C for 24 h and then in oven at 50°C for 2 days.

Pullulanase treatment

Pullulanase debranching was carried out according to method described by Gonza’lez-Soto et al.\textsuperscript{13} with some modifications. Briefly, 25 ml of acetate buffer (0.1 M, pH 5.2) was added to 5 g of starch samples. The mixture was gelatinized for 10 min in a boiling water bath (with stirring), then autoclaved at 121°C for 30 min. After this, the gel was re-dissolved with 125 ml of acetate buffer. The gel (10% w/v) was cooled to 50°C and 10.6 U/g of pullulanase was added. The mixture was incubated with constant stirring for 24 h at 40°C. Subsequent to this mixture was kept in boiling water bath for 10 min, and then again stored at 4°C for 24 h. Finally, the reaction mixture was dried in oven at 50°C for 2 days.

Determination of resistant starch (RS)

RS content was estimated according to the modified A.O.A.C official method described by Tribess et al.\textsuperscript{14}. Briefly, 100 mg samples were washed twice with 8 ml ethanol (8%, v/v), centrifuged at 1500 X g for 10 min. The residues were treated with 4 mL tris maleate/NaOH buffer (0.1M, pH 6) containing amylglucosidase (4 U/ml), α-amylase (300 U/ml) and pepsin (500 U/ml). Then, the tubes were tightly capped, mixed and incubated at 37°C for 16 h with continuous shaking. Caps were removed and contents were treated with 8 ml ethanol, mixed and centrifuged at 1500 X g for 10 min and residue separated from supernatant. This suspension and centrifugation step was repeated two times. The tubes were placed in an ice water bath with shaking. Three ml of 2 M KOH was added to each tube and the slurry was kept on shaker for 20 min. Then, each tube was treated with 10 ml 1.2 M sodium acetate buffer (pH 3.8). Immediately, 0.1 ml amylglucosidase was added (3200 U/ml, sodium acetate buffer, pH 4.75), with vigorous mixing. Tubes were capped and incubated in shaker bath at 50°C for 30 min. After incubation, the tube volume was adjusted to 20 ml with distilled water and centrifuged at 1500 X g for 10 min. Finally, glucose was quantified in the supernatants with GOD/POD reagent assay kit (ACCUREX, Biomedical Pvt., Ltd., India). RS was calculated as ΔE x F/W x 9.27. Where, ΔE = absorbance of reaction against blank, F = conversion from absorbance to mg, w = weight of sample.

In vitro starch digestion

In vitro starch digestion studies were carried out according to method described by Goñi et al.\textsuperscript{15} with some modifications. Fifty mg dried sample was incubated with 10 ml HCl–KCl buffer (pH 1.5) and 0.2 ml 10% pepsin at 40°C for 1 h with constant shaking. The pH was raised by adjusting volume to 25 ml with tris-maleate buffer (pH 6.9). 5 ml of pancreatic α-amylase solution (1 mg/5 ml in tris-maleate buffer) was then added to the reaction mixture and incubated at 37°C with constant shaking for 30 min. Aliquots of 1 ml was taken at 30, 90 and 120 min from the reaction mixture and immediately placed into the boiling water for 5 min to inactivate the enzyme reaction. Afterwards, 1 ml of 0.4 M sodium acetate buffer (pH 4.75) was added to each 1 ml aliquot and treated with 60 μl of amylglucosidase (3,300 U/ml). The reaction mixture was then incubated at 60°C for 45 min with constant shaking. The glucose released was quantified using (GOD/POD) reagent assay kit. The starch was calculated as glucose (mg %) x 0.9\textsuperscript{16}. The rate of
starch digestion was expressed as a percentage of the total starch hydrolyzed at different time intervals. The 30 and 120 min hydrolysis represented the RDS and SDS respectively. Total starch (TS) was calculated by formula TS = RS + (RDS + SDS). According to Goñi et al., 90 min hydrolysis was used to calculate predicted glycemic index (pGI), \[ pGI= 39.21 + 0.803(H90) \]. The starch digestion Index (SDI) was calculated by formula SDI = RDS/TS X100.

**Statistical analysis**

Results were expressed as means ± SD. Means were compared by one way analysis of variance (ANOVA), followed by the Bonferroni’s multiple comparison test with use of GraphPad Prism Software.

**Results and discussion**

Processing methods such as soaking, autoclaving or pressure cooking have been found to be useful in increasing RS content of most of the food materials such as black bean and lima bean, banana, maize, rice, barley, sorghum etc. However, there are controversial reports available in the context of effect the different processing treatments on RS, RDS and SDS contents of pulses and cereals (Table 1). There is consistency about effect of Conventional cooking, high pressure steaming, microwave cooking, autoclaving, splitting, soaking and boiling which seem to decrease the RS content. However, effect of retrogradation is controversial with some reports showing decrease in RS and some stating increase of RS. It has been found that autoclaving processing treatment decreases RS content in Indian legumes such as moth bean and horse gram. However, legumes like black bean and lima bean increases in RS content after steam cooking.

Table 2 shows the effect of soaking, autoclaving, storage and pullulanse treatment on RS content of pigeon pea, green gram and black gram. Results showed that autoclaving-cooling treatments were found to be affecting RS content of all the studied materials.

---

**Table 1—Effect of different processing treatments on resistant starch (RS), rapidly digested starch (RDS) and slowly digested starch (SDS) contents of pulses and cereals**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Pulses/cereal materials</th>
<th>Process</th>
<th>RS, RDS and SDS Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Raw black, red, and lima beans</td>
<td>Conventionally cooking and high-pressure steaming</td>
<td>Resistant starch was 3-5 times higher than in the raw pulses</td>
<td>20</td>
</tr>
<tr>
<td>2.</td>
<td>Cowpeas, black beans</td>
<td>Conventional cooking and microwave reheating</td>
<td>No major difference in RS formation was observed</td>
<td>31</td>
</tr>
<tr>
<td>3.</td>
<td>Moth bean, horse gram black gram</td>
<td>Cooking and storage</td>
<td>RS decreased after cooking and storage</td>
<td>23</td>
</tr>
<tr>
<td>4.</td>
<td>Lentils, chickpea, beans</td>
<td>Retrogradation</td>
<td>RS decreased after retrogradation</td>
<td>32</td>
</tr>
<tr>
<td>5.</td>
<td>Raw chickpeas, beans</td>
<td>Conventional cooking and microwave cooking</td>
<td>RS decreased However, RDS increased.</td>
<td>33</td>
</tr>
<tr>
<td>6.</td>
<td>White and black varieties of Mucuna pruriens var. utilis</td>
<td>Cooking and autoclaving</td>
<td>Significant improvement in the digestibility of starch</td>
<td>34</td>
</tr>
<tr>
<td>7.</td>
<td>Common Beans (Phaseolus vulgaris L.)</td>
<td>Pressure (autoclaving) and traditional cooking</td>
<td>Retrograded resistant starch content was higher in beans cooked with the traditional process than in autoclaved beans</td>
<td>35</td>
</tr>
<tr>
<td>8.</td>
<td>Whole chick peas</td>
<td>Precooked/vacuum-packaged; and canned and domestically boiled products</td>
<td>The commercially precooked/vacuum-packaged whole had higher RDS than the commercially canned and domestically boiled products</td>
<td>36</td>
</tr>
<tr>
<td>9.</td>
<td>Corn staches</td>
<td>Chemically modification</td>
<td>Interconversion of RS to RDS</td>
<td>28</td>
</tr>
<tr>
<td>10.</td>
<td>Chickpea, lentil</td>
<td>Cooking</td>
<td>RS decreased</td>
<td>37</td>
</tr>
<tr>
<td>11.</td>
<td>Legumes, cereals</td>
<td>Cooking</td>
<td>RS increased</td>
<td>38</td>
</tr>
<tr>
<td>12.</td>
<td>Red kidney bean, Yellow and Green peas</td>
<td>Splitting, soaking, boiling and pressure-cooking</td>
<td>Decrease in RS and increase in RDS</td>
<td>39</td>
</tr>
<tr>
<td>13.</td>
<td>Maize</td>
<td>Pullulanase debranching treatment</td>
<td>RS increased</td>
<td>11</td>
</tr>
<tr>
<td>14.</td>
<td>Wheat, rice, barley, Bengal gram, pea kidney bean, lentils, potato, sweet potato</td>
<td>Boiling and pressure cooking</td>
<td>Decreased RS after heat treatment</td>
<td>6</td>
</tr>
<tr>
<td>15.</td>
<td>Bean, pea, lentil seeds</td>
<td>Cooking</td>
<td>Significantly decreased RS and SDS</td>
<td>26</td>
</tr>
</tbody>
</table>
samples. RS content of pigeon pea, green gram and black gram were significantly decreased after various autoclaving-cooling treatments (p<0.05). In addition, soaking and pullulanase debranching treatments were also found to be significantly decreasing the RS content of all the pulses (p<0.05). The observed high RS content in untreated starch samples of all dhal flours could be the cause for low bioavailability of starch, high amylose content, high soluble dietary fiber content and presence of amylase inhibitors. Nevertheless, gelatinization of starch may have decreased antinutritional factors including amylase inhibitors which could be responsible for the observed decrease in RS content in all studied samples after autoclaving.

In order to understand the mechanism of decrease in RS content, and role of processing methods on GI, RDS and SDS content, further in vitro starch digestion studies were carried out. Figure 1 shows the in vitro starch digestibility of native and autoclaved pigeon pea, green gram and black gram starch samples. Autoclaved samples showed significantly high content of SDS than native one. The SDS content of autoclaved starch samples were in the order pigeon pea>green gram>black gram. SDS is generally considered as healthy food component and found to be useful in stabilization of glucose metabolism, diabetes management, mental performance, and satiety. Chung et al. reported that RS is converted into RDS by retrogradation process. The increased SDS and decreased RS contents in autoclaved starch sample of pigeon pea, green gram and black gram dhals could be due to the fact that RS might be converted into SDS by chemical modifications and/or by gelatinization of starches.

Results further demonstrated that autoclaving significantly increased the predicted glycemic index (pGI) of pigeon pea, green gram and black gram starch samples. The observed pGI of autoclaved starches of all studied samples were far less than 70%, confirmed their moderate glycemic index. Autoclaved pigeon pea starch samples, the amount of RDS was significantly decreased than the native one. Non-significant

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Treatment</th>
<th>Pigeon pea</th>
<th>Green gram</th>
<th>Black gram</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Native starch sample</td>
<td>16.86 ±0.26</td>
<td>11.60 ± 2.42</td>
<td>11.35 ± 0.77</td>
</tr>
<tr>
<td>2.</td>
<td>Soaked for 12 h</td>
<td>9.09 ± 1.21</td>
<td>5.01 ± 0.75</td>
<td>5.27 ± 0.40</td>
</tr>
<tr>
<td>3.</td>
<td>Retrograded, cooled at room temperature</td>
<td>4.81 ± 0.95</td>
<td>3.03 ± 0.50</td>
<td>2.93 ± 0.43</td>
</tr>
<tr>
<td>4.</td>
<td>Retrograded, stored at 4°C for 24 h</td>
<td>3.96 ± 0.15</td>
<td>3.16 ± 0.16</td>
<td>4.07 ± 0.15</td>
</tr>
<tr>
<td>5.</td>
<td>Pullulanase debranching</td>
<td>9.97 ± 0.16</td>
<td>8.44 ± 0.32*</td>
<td>6.01 ± 0.24*</td>
</tr>
</tbody>
</table>

All values are expressed as Mean ± SD.
*Statistically significant at P<0.05, **statistically significant at P<0.0001.

![Fig. 1—In vitro starch digestibility of common Indian pulses: A) Pigeon pea B) Green gram and C) Black gram](image-url)

Foot note
RDS= rapidly digested starch; SDS= slowly digested starch; pGI= predicted glycemic index; SDI= starch digestive index. All values are expressed as Mean ± SD. ns = statistically non-significant, **Significant at P<0.001, *** Significant at P<0.0001
decrease in RDS amount was observed in autoclaved starch samples of black gram and green gram than the native one. Thus in studied pulses, RDS could be converted into RS after autoclaving.

Conclusion
The processing methods including pullulanase debranching showed significantly decreased RS content of dhals of pulses pigeon pea, green gram and black gram. Results of in vitro starch digestibility study further showed that RS and RDS were converted to SDS during autoclaving processing. Augmented starch bioavailability, decrease in RS content, and conversion of RS to SDS after processing treatments in pigeon pea, green gram and black gram, are the major findings of our current study. Findings of this study further suggest, processed dhals of pigeon pea, green gram and black gram could have added health promoting potential due to their high content of SDS, especially in treatment of diabetes.

Acknowledgements
We are grateful to Department of Biotechnology (DBT), India for providing financial assistance. Authors wish to thank the Director, MACS-Agharkar Research Institute, Pune for providing research facility.

References
3 Jenkins D J A, Wolever T M S, Jenkins A L, Thorne M J, Kalmusky J, Reichert R. & Wong G, The glycemic index of starch samples of black gram and green gram than the native one. Thus in studied pulses, RDS could be converted into RS after autoclaving.
9 Niba L L & Hoffman J, Resistant starch and β-glucan levels in grain sorghum (Sorghum bicolor M.) are influenced by soaking and autoclaving, Food Chem, 81 (2003) 113–118.
24 Martián-cabrejas M A, Sanfiz B, Vidal A, Mollaí E, Esteban R. & Loí pe-Andreà FJ, Effects of fermentation and


