Effect of popping and fermentation on starch fractions, starch digestibility, rheological property of *Amaranthus caudatus* grain.

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Introduction

- Amaranth is an underutilized plant with high biodiversity, distribution and productivity.

- It has high tolerance to arid and semi arid conditions and poor soils.

- Has resistance to drought, pests and ability to adapt to environments which are not conducive to conventional cereals.

- It has excellent nutritional qualities compared to commonly utilized cereals.
Cont’d….

- Starch is the commonest storage carbohydrate in plants and also the largest source of carbohydrates in human food.

- The degree of starch hydrolysis determines the postprandial blood glucose level.

- Highly processed, low fiber and energy-dense carbohydrate food products can lead to over consumption and obesity related diseases.
Cont’d…

- Starch could be classified as **rapidly digestible (RDS)**, **slowly digestible (SDS)** and **resistant starch (RS)** based on in vitro digestion by simulating stomach and intestinal conditions and measuring glucose release at different time interval (Englyst & Cummings, 1992).

- RDS: hydrolyzed in 20 minutes.
- SDS: hydrolyzed between 20 min and 120 min.
- RS: undigested part after 120 min.
The duration and extent of starch digestion could be influenced by several intrinsic and extrinsic factors.

- Starch granule morphology
- Amylose to amylopectin ratio
- Molecular structure
- Degree of branching in terms of steric hindrance
- Interaction of starch or its components with fiber, antinutrients (e.g., phytate), protein and lipid in the food,
- The physical form of the starch or the effect of processing (e.g., raw or cooked, ground or whole).
Cont’d…..

- Starch is also the main component in amaranth grain and plays an important role for food applications.

  - food thickeners,
  - for soups,
  - fat replacers, and
  - in breakfast cereals, muffins, cookies, snacks, pastas and health foods.

- However, there are limited studies conducted on starch digestibility of raw amaranth and effect of common processing methods.
Objectives

General Objective

- Evaluate the effect of popping and fermentation on starch fractions, starch digestibility, energy density and rheological property of porridge prepared from *Amaranthus caudatus* grain.

Specific objectives

- Determine the free glucose content and starch fractions in raw and processed amaranth.
- To determine the starch digestibility of raw and processed amaranth.
- To evaluate the effect of processing on rheological properties and energy density of amaranth porridge.
Methodology

- Sample collection and preparation

✓ The three different types of *Amaranthus caudatus* grains were collected from Bench Majji Zone, SNNPR, Ethiopia.

✓ Preparation of raw amaranth flour
  The collected grains were sorted, cleaned and washed to remove immature seeds, sand and soil. The washed seeds were sun dried, milled, and sieved using 0.425 mm sieve.

✓ Popping was done as described in Amare et al. (2015)

✓ Natural fermentation was done as described in Ibrahim et al. (2005) with modifications.
Cont’d…

➢ Preparation of porridge

i. By cooking: followed the method of Mouquet and Treche (2001)

  o Briefly, the flour was mixed with cold demineralized water into slurry and cooked with continuous stirring over a hot plate for 5 min once the mixture starts to boil.

ii. With instant procedure: followed the method of Mouquet et al. (2003).

  o Briefly, popped amaranth flour was mixed with hot water at 70 °C and the viscosity was measured at the same dry matter content as that of porridge prepared using the cooking procedure described above.

➢ Viscosity Measurement was done based on the method of Mouquet and Treche (2001)

  o Porridge prepared in both cooking methods were allowed to cool to 45 °C and viscosity was measured using Haake VT550 viscometer.

Cont’d

- **Total starch**
  - A Megazyme total starch assay kit was used.

- **Free Sugars**
  - A modified method of Englyst et al. (1999) was used.

- **In vitro starch digestibility**
  
  The method of Englyst et al. (1999) was followed
  
- \[
  \text{RDS} = (G_{20} - FG) \times 0.9
  \]
  
- \[
  \text{SDS} = (G_{120} - G_{20}) \times 0.9
  \]
  
- \[
  \text{RS} = (TG - G_{120}) \times 0.9
  \]
  
- \[
  \text{SD(\%)} = 100 - (RS \times 100 / TS)
  \]
  
- \[
  \text{SDRI} = \text{RDS} \times 100 / TS
  \]
Fig 1: Schematic diagram used to analyze starch fractions

Sample + Guar gum
Add acetate buffer
10 min at 37 °C
Add enzyme mixture
(amyloglucosidase, invertase and pancreatin)
Incubate with shaking at 37 °C
After 20 min remove portion
After 100 min remove portion
Mix, 30 min at 100 °C
Cool to 0 °C, add KOH, mix
30 min at 0 °C with shaking
Take portion in to acetic acid
Add Amyloglucosidase
30 min at 70 °C
10 min at 90 °C
Cool, dilute and centrifuge
Measure Total Glucose (TG)
Measure glucose released after 120 min (G_{120})
Measure glucose released after 20 min (RAG)

Place in ethanol
Place in to ethanol

Centrifuge
Centrifuge
Statistical Analysis

• Data were submitted to Statgraphics plus 5.1 (Statpoint, Warrenton, USA) software and analyzed using analysis of variance (ANOVA) to determine significant differences among the processing methods.

• Duncan multiple range test was used to compare the means and differences were considered significant when $p < 0.05$. 
Results and Discussion

Free sugars, total starch and starch fractions of raw A. Caudatus grain porridge and the effect of popping and fermentation.

Table 1 Total starch and its fractions in raw and processed Amaranthus caudatus grain porridge (g/100g DM)*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>White</th>
<th>Red</th>
<th>Brown</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Free sugars</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>0.67</td>
<td>0.60</td>
<td>0.64</td>
<td>0.64 ± 0.01c</td>
</tr>
<tr>
<td>Popped</td>
<td>0.63</td>
<td>0.59</td>
<td>0.53</td>
<td>0.60 ± 0.03c</td>
</tr>
<tr>
<td>Fermented</td>
<td>1.47</td>
<td>1.13</td>
<td>1.15</td>
<td>1.25 ± 0.05b</td>
</tr>
<tr>
<td>Corn flakes</td>
<td></td>
<td></td>
<td></td>
<td>3.30 ± 0.04a</td>
</tr>
<tr>
<td><strong>Total starch</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>56.02</td>
<td>54.12</td>
<td>47.73</td>
<td>52.62 ± 0.22c</td>
</tr>
<tr>
<td>Popped</td>
<td>58.15</td>
<td>57.14</td>
<td>50.81</td>
<td>55.37 ± 0.15b</td>
</tr>
<tr>
<td>Fermented</td>
<td>54.95</td>
<td>53.36</td>
<td>46.97</td>
<td>51.76 ± 0.27d</td>
</tr>
<tr>
<td>Corn flakes</td>
<td></td>
<td></td>
<td></td>
<td>76.82 ± 0.39a</td>
</tr>
<tr>
<td><strong>Rapidly digestible starch</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>33.18</td>
<td>32.61</td>
<td>27.41</td>
<td>31.06 ± 0.24d</td>
</tr>
<tr>
<td>Popped</td>
<td>35.63</td>
<td>35.11</td>
<td>30.48</td>
<td>33.74 ± 0.11c</td>
</tr>
<tr>
<td>Fermented</td>
<td>36.58</td>
<td>36.00</td>
<td>31.13</td>
<td>34.56 ± 0.18b</td>
</tr>
<tr>
<td>Corn flakes</td>
<td></td>
<td></td>
<td></td>
<td>50.89 ± 0.34a</td>
</tr>
<tr>
<td><strong>Slowly digestible starch</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>13.89</td>
<td>13.03</td>
<td>10.89</td>
<td>12.61 ± 0.28d</td>
</tr>
<tr>
<td>Popped</td>
<td>14.65</td>
<td>14.21</td>
<td>12.07</td>
<td>13.64 ± 0.31c</td>
</tr>
<tr>
<td>Fermented</td>
<td>16.33</td>
<td>15.33</td>
<td>13.12</td>
<td>14.93 ± 0.16b</td>
</tr>
<tr>
<td>Corn flakes</td>
<td></td>
<td></td>
<td></td>
<td>24.51 ± 0.50a</td>
</tr>
<tr>
<td><strong>Resistant starch</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>8.95</td>
<td>8.48</td>
<td>9.43</td>
<td>8.96 ± 0.37a</td>
</tr>
<tr>
<td>Popped</td>
<td>7.87</td>
<td>7.82</td>
<td>8.26</td>
<td>7.98 ± 0.37a</td>
</tr>
<tr>
<td>Fermented</td>
<td>2.04</td>
<td>2.03</td>
<td>2.72</td>
<td>2.28 ± 0.37b</td>
</tr>
<tr>
<td>Corn flakes</td>
<td></td>
<td></td>
<td></td>
<td>1.42 ± 0.53b</td>
</tr>
</tbody>
</table>

*Mean values with different letters in column are significantly different at p < 0.05.
Free sugars in raw amaranth porridge: 0.60-0.67 g/100g DM.

Total starch content in raw *A. caudatus* was in the range of 48-56 g/100g DM.

The amount is comparable to that found in other *A. spp* (*A. cruentus, A. hybridus and A. hypochondriacus*) (Capriles et al., 2008; Cai et al., 2004)

Compared to other cereals, the starch content is less than that of rice, sorghum and teff but higher than barley.
Cont’d…

- Popping significantly increased \((p < 0.05)\) the amount of RDS, SDS and TS contents by a percentage of 8.6, 8.2 and 5.2 %, respectively.

- Degradation of phytic acid and denaturation of protein allows the starch accessible to enzymatic attack.

- Fermentation significantly increased \((p < 0.05)\) free sugars, RDS and SDS but significantly decreased TS and RS.

  - Similar results were reported in finger millet and sorghum (Antony & Chandra, 1998; Pranoto et al., 2013).
In vitro starch digestibility in raw and processed amaranth

Table 2 Rapidly available glucose, Bioavailable starch and, starch digestibility and starch digestibility rate index of porridge from raw and processed Amaranthus caudatus grain*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>White</th>
<th>Red</th>
<th>Brown</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RAG (g/100g DM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>33.85</td>
<td>33.21</td>
<td>28.05</td>
<td>31.70 ± 0.23d</td>
</tr>
<tr>
<td>Popped</td>
<td>36.26</td>
<td>35.70</td>
<td>31.01</td>
<td>34.34 ± 0.28c</td>
</tr>
<tr>
<td>Fermented</td>
<td>38.05</td>
<td>37.13</td>
<td>32.28</td>
<td>35.81 ± 0.17b</td>
</tr>
<tr>
<td>Corn flakes</td>
<td></td>
<td></td>
<td></td>
<td>54.19 ± 0.39a</td>
</tr>
<tr>
<td><strong>BAS (g/100g DM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>47.07</td>
<td>45.64</td>
<td>38.30</td>
<td>43.67 ± 0.31d</td>
</tr>
<tr>
<td>Popped</td>
<td>50.28</td>
<td>49.31</td>
<td>42.55</td>
<td>47.38 ± 0.23c</td>
</tr>
<tr>
<td>Fermented</td>
<td>52.90</td>
<td>51.30</td>
<td>44.24</td>
<td>49.48 ± 0.18b</td>
</tr>
<tr>
<td>Corn flakes</td>
<td></td>
<td></td>
<td></td>
<td>75.40 ± 1.16a</td>
</tr>
<tr>
<td><strong>SD[%]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>84.03</td>
<td>84.34</td>
<td>80.24</td>
<td>82.87 ± 0.55d</td>
</tr>
<tr>
<td>Popped</td>
<td>86.47</td>
<td>86.32</td>
<td>83.76</td>
<td>85.52 ± 0.32c</td>
</tr>
<tr>
<td>Fermented</td>
<td>96.27</td>
<td>96.15</td>
<td>94.21</td>
<td>95.54 ± 0.29a</td>
</tr>
<tr>
<td>Corn flakes</td>
<td></td>
<td></td>
<td></td>
<td>98.16 ± 0.51a</td>
</tr>
<tr>
<td><strong>SDRI (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>59.22</td>
<td>60.28</td>
<td>57.43</td>
<td>58.98 ± 0.68b</td>
</tr>
<tr>
<td>Popped</td>
<td>61.27</td>
<td>61.45</td>
<td>60.00</td>
<td>60.91 ± 0.51b</td>
</tr>
<tr>
<td>Fermented</td>
<td>66.57</td>
<td>67.43</td>
<td>66.28</td>
<td>66.76 ± 0.76a</td>
</tr>
<tr>
<td>Corn flakes</td>
<td></td>
<td></td>
<td></td>
<td>68.00 ± 1.18a</td>
</tr>
</tbody>
</table>

*Mean values with different letters in column are significantly different at p < 0.05
Cont’d...

- SD in raw amaranth porridge: 80-84 g/100g DM.

  - Small starch granule size and high levels of amylopectin could be the reason for high SD in amaranth.

  - High SD is a desirable attribute for ingredients used in complementary food formulation.

- Popping increased SD by 3.2%. The increase could be attributed to enhanced exposure of seed’s starch matrix and pregelatinization making the starch more susceptible to enzymatic attack (Caprilles et al., 2008).
Fermentation also significantly increased (P < 0.05) SD by 15%.

- Degradation of phytic acid.
- Loosening of protein-starch interaction due to the action of proteases.
Effect of processing on the consistency of amaranth porridge

Fermented amaranth has a lower apparent viscosity compared to raw and popped amaranth.

Hydrolysis of the starch

Figure 1 Viscosity of gruel prepared from three types of *Amaranthus caudatus* grain A) white B) red and C) brown measured at a shear rate of 83 s\(^{-1}\) after 10 minutes shear at a gruel temperature of 45 °C. RWA, RRA, RBA, PWA, PRA, PBA, FWA, FRA and FBA are raw white, raw red, raw brown popped white, popped red, popped brown, fermented white, fermented red and fermented brown amaranth, respectively.
Cont’d….

Evaluation of instant character of popped amaranth

The superimposed viscosity curve shows that nearly all the starch is gelatinized during popping.

Figure 2 Viscosity of three types of popped amaranth prepared using cooking and instant procedure.
Cont’d…

Energy Density of amaranth porridge and the effect of processing

Table 3 Energy density and minimum daily number of meals required to attain the level of energy needed from amaranth based complementary foods of different viscosity for children with average breast milk energy intake according to age group‡

<table>
<thead>
<tr>
<th>Sample</th>
<th>Treatment</th>
<th>DM content</th>
<th>Energy Density (kcal/g gruel)</th>
<th>No. of meals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6-8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 Pa.s</td>
<td>3 Pa.s</td>
<td>1 Pa.s</td>
</tr>
<tr>
<td>White</td>
<td>Raw</td>
<td>11.0</td>
<td>16.8</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>Popped</td>
<td>11.4</td>
<td>17.2</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>Fermented</td>
<td>12.9</td>
<td>20.2</td>
<td>0.54</td>
</tr>
<tr>
<td>Red</td>
<td>Raw</td>
<td>10.5</td>
<td>17.3</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>Popped</td>
<td>12.4</td>
<td>17.8</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>Fermented</td>
<td>12.8</td>
<td>20.0</td>
<td>0.54</td>
</tr>
<tr>
<td>Brown</td>
<td>Raw</td>
<td>12.0</td>
<td>19.0</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>Popped</td>
<td>14.5</td>
<td>20.3</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>Fermented</td>
<td>14.8</td>
<td>22.5</td>
<td>0.59</td>
</tr>
</tbody>
</table>

‡Viscosity of gruel (1-3 Pa.s) was considered based on the recommendation by Mouquet and Treche (2001) and estimated total energy requirement is based on new US longitudinal data averages plus 25% (2SD). Assumed functional gastric capacity (30 g/kg reference body weight) is 249 g/meal at 6-8 months, 285 g/meal at 9-11 months, and 345 g/meal at 12-23 months.

At the same viscosity, the DM content of porridge prepared from popped and fermentation is higher, so is the energy density.

The number of meals required for children is less if the porridge is prepared from fermented amaranth than popped and raw amaranth.
Conclusion and Recommendation

• More than 80% of amaranth starch is digestible when served in the form of porridge.

• Both popping and fermentation improves the SD making amaranth porridge a high glycemic food digesting at faster rate.

• The high SD of amaranth allows the crop to be positioned at the top among the candidates used for complementary food formulation as infants could not accommodate bulky foods.

• Popped amaranth flour could be used as an instant powder to prepare instant drink of better nutritional quality than other commonly utilized cereals.
Acknowledgement

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• IRD
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• Wolaita Sodo University
• EPHI