Sprouted bean flour as a novel functional ingredient for the formulation of bakery products

Sara M. Borgonovi, S. Iametti, A. Marti, A. Sergiacomo, M. Di Nunzio
Dept. Of Food, Enviromental and Nutritional Science (DeFENS), University of Milan, Italy

1. Introduction and aim

Due to their balance nutritional profile legumes are widely consumed as human food. However, legumes as a staple food is hampered by the presence of some anti-nutritional components and of low digestibility di- and oligosaccharides. Many biotechnological approaches have been tested to overcome one or more of these restrictions. Among them, sprouting has been used to alter the macromolecular composition of certain grains and legumes, to decrease the content in anti-nutritional elements. Among legumes, cowpea (*Vigna unguiculata*) is a versatile crop, and represents a good candidate for developing novel products. This study aims to investigate the impact of short time sprouting (48 and 72 hours) on the biomolecular features and on the antioxidant and antinutritional factors in cowpea seeds.

2. Methods

Sprouting was carried out on previously soaked seeds for 24, 48 and 72 hours. SDS-PAGE and the OPA test for free amino groups were used to analyze the protein profile and hydrolysis in the aqueous extract of the resultant flours after drying and grinding. Trypsin inhibitors were evaluated in accordance with the EN ISO 14902 standard, whereas residual phytate and di/oligosaccharides were evaluated using a commercial kit. Folin assay was used to assess the release of water soluble phenolics.

3. Results: anti-nutritional components

Sprouting for 48 and 72 hours consistently decreased the phytic acid content (Fig. 1) and the trypsin inhibitory activity (TIA) (Fig. 2). Di- and oligosaccharides in the raffinose series, also decrease upon sprouting (Fig. 3).

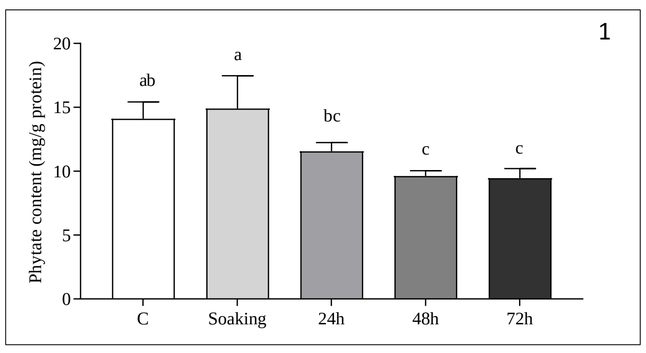


Figure 1. *Phytate content in unsprouted (C), soaked (S), and sprouted cowpea (24h, 48h, and 72h). Data are expressed as mg/g protein and are the mean ± SD of two different extractions. Statistical analysis was by one-way ANOVA ($p < 0.05$) with Tukey's post-hoc test (different letters indicate significant differences).*

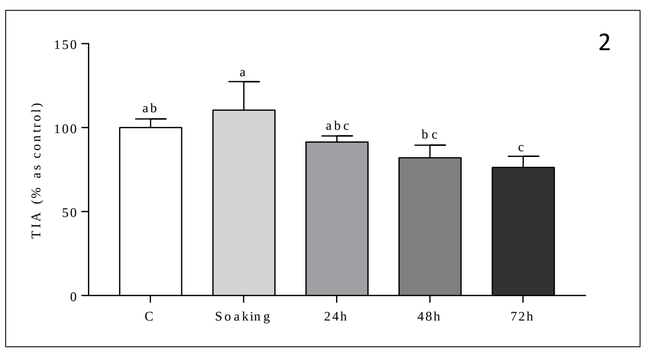


Figure 2. *Trypsin inhibitory activity (TIA) in unsprouted (C), soaked (S), and sprouted cowpea (24h, 48h, and 72h). Data are expressed as the percent of levels in unsprouted cowpea and are the mean ± SD of two different extractions. Statistical analysis was by one-way ANOVA ($p < 0.05$) with Tukey's post-hoc test (different letters indicate significant differences).*

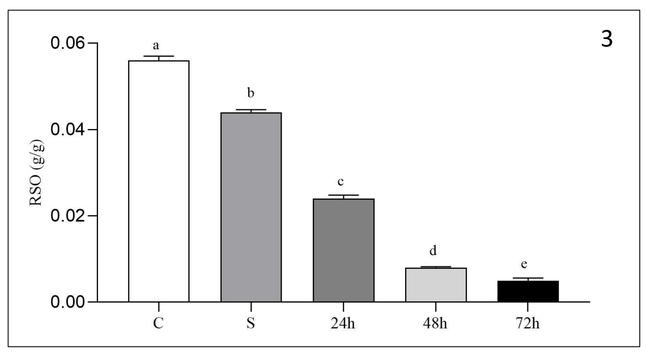


Figure 3. *RSO (Raffinose Series Oligosaccharides) in unsprouted (C), soaked (S), and sprouted cowpea (24h, 48h, and 72h). Data are expressed as g/g flour and are the mean ± SD of two different extractions. Statistical analysis was by one-way ANOVA ($p < 0.05$) with Tukey's post-hoc test (different letters indicate significant differences).*

4. Results: Polyphenols content

Sprouting leads to an increase in the phenolics content (Fig. 4), with appreciable antioxidant activity already present after 48 hours of germination.

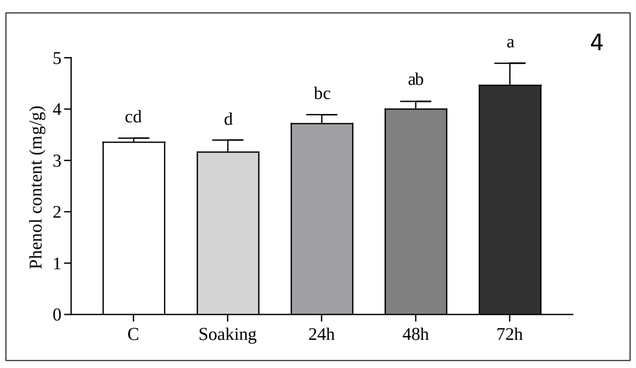
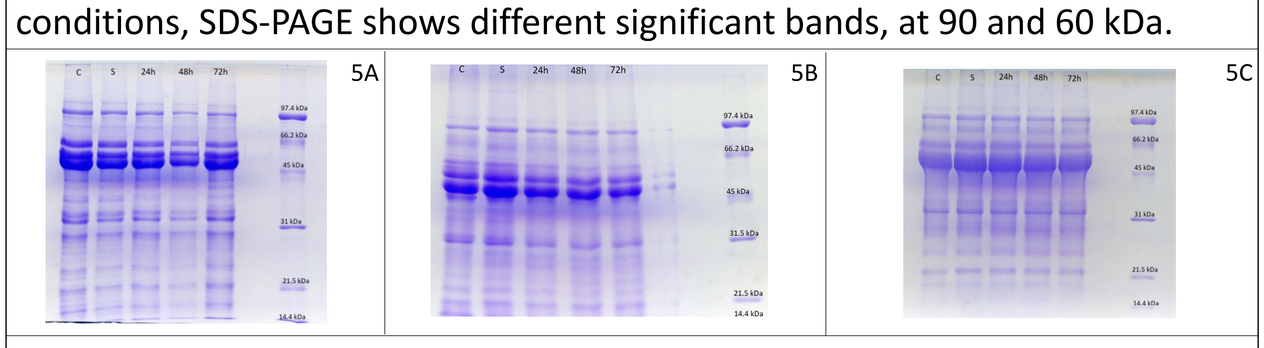


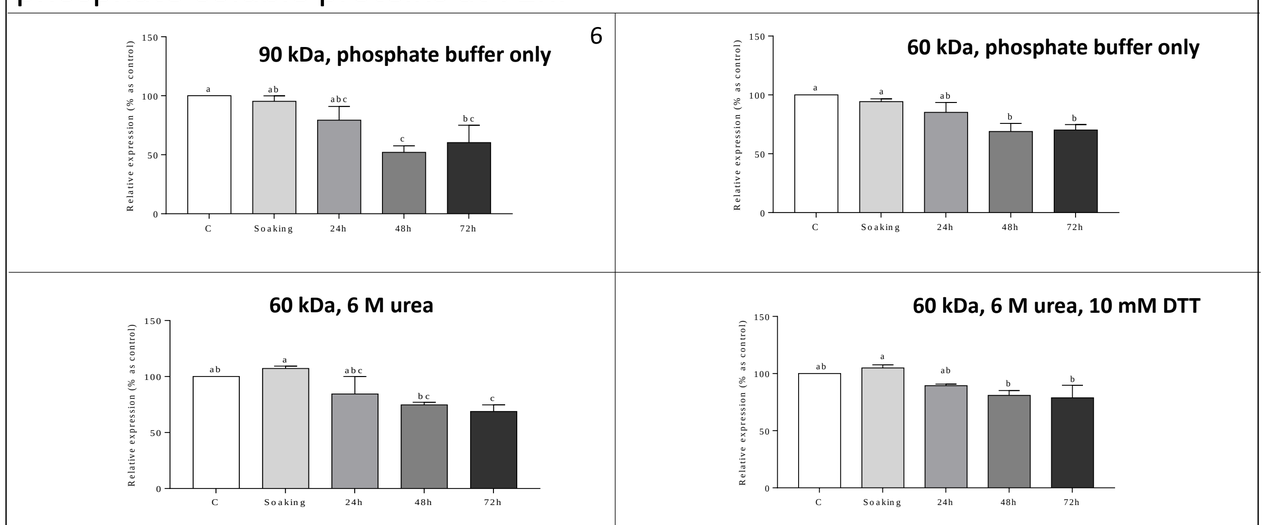
Figure 4. *Polyphenols content in unsprouted (C), soaked (S), and sprouted cowpea (24h, 48h, and 72h). Data are expressed as mg/g dw and are the mean ± SD of two different extractions. Statistical analysis was by one-way ANOVA ($p < 0.05$) with Tukey's post-hoc test (different letters indicate significant differences).*

5. Results: protein profile and hydrolysis

Short sprouting modifies the proteins pattern. SDS-PAGE analysis was performed on protein extracted under conditions suited to break down different types of protein-protein interactions (phosphate buffer, +/- 6M urea, +/- 10 mM DTT). The different protein profiles are compared in Figure 5: phosphate buffer (A), + 6M urea (B); +6M urea and 10 mM DTT (C). In all conditions, SDS-PAGE shows different significant bands, at 90 and 60 kDa.



Densitometric analysis (Fig. 6) shows that the overall protein pattern was insensitive to the presence of chaotropes and reductants, suggesting that the same proteins were involved in various types of covalent and non-covalent interactions. Proteins not linked through disulfide bonds were the easiest target for sprouting-related degradation, that apparently addressed regions relevant to interprotein interactions, as suggested by the increase in phosphate-soluble proteins at 72h.



Sprouting-dependent hydrolysis was confirmed by the OPA assay, that measures the release of small peptides and amino acids.

The compactness of the protein aggregates was studied by assessing the accessibility of SH groups (Fig. 7). Data indicated no significant difference in the number of SH group exposed between the germinated and untreated flour samples, regardless of the presence/absence of chaotropes, suggesting no major sprouting-related modification in the compactness of individual proteins and its insensitivity to 6M urea.

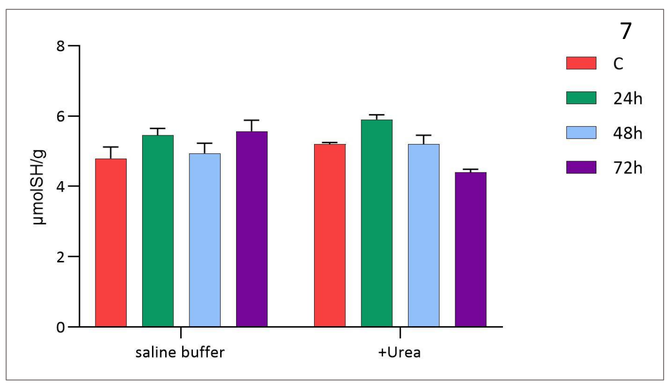


Figure 7. *Content of SH group in unsprouted (C), soaked (S), and sprouted cowpea (24h, 48h, and 72h). Data are expressed as µmol SH/g flour and are the mean ± SD of two different extractions. Statistical analysis was by one-way ANOVA ($p < 0.05$) with Tukey's post-hoc test.*

6. Conclusions

Short sprouting modifies the protein pattern, with a possible improvement in the features required for formation of a protein network. Sprouting also leads to a decrease of anti-nutritional factors and of low digestibility oligosaccharides while improving the content in phenolics with potential antioxidant activity. Although the maximum effects on phytate and oligosaccharides content (and protein hydrolysis) are observed after 48h, the highest decrease of trypsin inhibitor activity was observed after 72h.

These findings suggest that sprouted cowpea flour may be a food ingredient with enhanced nutritional and technological value. A characterization of wheat bread containing 25% of 72h-sprouted cowpea flour is currently undergoing to support this assumption.

7. Acknowledgment

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