Plant Foods for Human Nutrition **55:** 97–110, 2000.

Effect of soaking, cooking and germination on the oligosaccharide content of selected Nigerian legume seeds

H.A. OBOH, M. MUZQUIZ*, C. BURBANO, C. CUADRADO, M.M. PEDROSA, G. AYET and A.U. OSAGIE[1]
Area de Tecnología de Alimentos, SGIT-INIA, Apdo. 8111, 28080 Madrid, Spain;
[1]*Department of Biochemistry, University of Benin, P.M.B. 1154, Benin City, Nigeria* (**author for correspondence*)

Received 6 April 1999; accepted in revised form 15 January 2000

Abstract. The identity and quantity of and effect of processing on raffinose oligosaccharides in raw, mature seeds of lima beans (*Phaseolus lunatus*), pigeon peas (*Cajanus cajan*), African yam beans (*Sphenostylis sternocarpa*) and jackbeans (*Canavalia ensiformis*) were investigated. Sucrose, raffinose, stachyose and verbascose were identified by HPLC in all the legume seeds. The total α-galactoside contents of the seeds in decreasing order were African yam beans 3.84 mg/100 mg; white lima beans 3.62 mg/100 mg; cream pigeon peas 3.51 mg/100 mg; red lima beans 3.37 mg/100 mg; jackbeans 2.83 mg/100 mg and brown pigeon peas 2.34 mg/100 mg. The predominant oligosaccharide was verbascose in pigeon peas and stachyose in the other three legumes. Cooking unsoaked seeds brought about a greater reduction in the total α-galactoside content than soaking for nine hours. The removal of oligosaccharides was higher in legumes cooked in alkaline solution than in water. Germination quantitatively reduced raffinose, stachyose and verbascose while sucrose was increased in all seeds except red lima beans and jackbeans.

Key words: Legumes, Oligosaccharides, Processed, Raffinose, Stachyose, Verbacose

Introduction

Grain legumes are a major source of cheap protein for humans in West Africa. Animal protein is expensive, therefore, supplementing the diet with legume seeds helps to alleviate protein deficiency in human nutrition [1].

Lima beans, pigeon peas, African yam beans and jackbeans are underutilized legumes in Nigeria [2]. The chemical compositions of these grain legumes were evaluated and shown to contain high quantities of proteins, amino acids and minerals [3]. Despite the rich composition of nutrients, these legumes are not normally selected as food sources because of their hard seed coats which lead to long cooking periods and the use of expensive, scarce fuels. Furthermore, the antinutritional factors in legume seeds reduce protein digestibility [4] and the nutritive value [5].

Raffinose oligosaccharides (e.g., stachyose, raffinose and verbascose), are present in legumes [6]. They produce flatulence in man and animals due to the absence of the enzyme α-galactosidase which is needed for hydrolysis of the α-1,6 galactosidic linkage of these oligosaccharides in the lower intestine. These sugars then undergo anaerobic fermentation by bacteria producing carbon dioxide, hydrogen and small amounts of methane gas that cause flatulence which is characterized by abdominal rumblings, cramps, diarrhea and nausea [7].

Plant breeding could be one approach to the reduction of flatulence; but Ryan & Farmer [8] have suggested that oligosaccharides may play a role in seed viability. Eliminating oligosaccharides from the plant itself could, therefore, adversely affect the growth and yield of the legumes. In Nigeria, legume seeds are processed and consumed in a variety of forms. The most common domestic methods include soaking, cooking, cooking in alkali solution and dehulling. The effects of soaking, cooking and dehulling on the oligosaccharide content of cowpeas have been reported [9]. The effects of domestic processing on the oligosaccharide content of faba beans, lentils, common beans and cowpeas have also been investigated [10]. Little information is available, however, on oligosaccharide contents in legumes such as lima beans and African yam beans. The reduction of flatulence is necessary for the utilization of these legumes as more acceptable sources of inexpensive protein.

The present investigation was aimed at identifiying and quantifiying the oligosaccharide content after soaking in water, cooking of unsoaked and soaked seeds, cooking of unsoaked seeds in 0.1% alkaline solution and germination of lima beans, pigeon peas, African yam beans and jackbeans and to describe a method suitable for reducing flatus-producing factors in legumes.

Materials and methods

Red lima beans (*Phaseolus lunatus*), white lima beans (*Phaseolus lunatus*), brown pigeon peas (*Cajanus cajan*), cream pigeon peas (*Cajanus cajan*), black African yam beans (*Sphenostylis sternocarpa*) and jackbeans (*Canavalia ensiformis*) were bought from the market in the Uromi and Igueben areas, Edo State, Nigeria. All bean samples were cleaned by hand, dried in an air oven (Gallenkamp, UK) at 70 °C for five hours and milled to pass through a 100-mesh sieve (Cyclotec 1093, Tecator AB, Sweden). All flour samples were stored in air-tight plastic containers at –10 °C until analyzed.

Preparation of processed samples

Cooking (W/C): One hundred grams (100 g) of bean seeds were added to distilled water (1:5 w/v) at 100 °C and cooked in a glass beaker on an electric hot plate (Ikatherm HCT, Ika, Staufen, Germany) for 3 h.

Cooking in 0.1% alkaline solution (W/C/K): 'Kanwa' rock salt was purchased from New Benin market, Benin City, Nigeria. The rock salt was ground into fine powder in an analytical grinder (A10, Ika, Staufen, Germany) and dried at 70 °C for 16 h in an oven (Gallenkamp, UK). The dried powder was cooled in a desiccator for 24 h and stored in a stoppered glass bottle at 24 °C. One hundred grams of bean seeds were added to boiling 0.1% Kanwa solution (1:5 w/v) and cooked in a glass beaker on an electric hot plate (Ikatherm HCT, Ika, Staufen, Germany) for 2.5 h. The pH of the Kanwa solution was 11.0.

Soaking and cooking (S12h/C): One hundred grams (100 g) of bean seeds were soaked in a glass beaker for twelve hours in distilled water (1:5 w/v) at 24 °C. The soak water was discarded and the bean seeds were cooked in a glass beaker on an electric hot plate (Ikatherm HCT, Ika, Staufen, Germany) in fresh distilled water at 100 °C for 2.5 h. In all cooking processes, the level of cooking water was kept constant by the addition of boiling distilled water or 0.1% alkaline solution. The cooked seeds were drained and dried at 70 °C in an oven (Gallenkamp, UK) for 16 h. The dried seeds were cooled in a dessicator, milled to pass trough a 100-mesh sieve (Cyclotec 1093, Tecator AB, Sweden). Bean flours were stored in an air-tight container at –10 °C until analyzed (1 month aproximately).

Germination (S9h/G0-S9/G96): One hundred grams (100 g) of bean seeds were surface sterilized for 30 min in a 1% sodium hypochlorite solution. The bean seeds were rinsed five times with distilled water (1:3 w/v) and soaked for 9 h in a glass beaker in tap water (1:3 w/v). The presoaked seeds were allowed to sprout on sterile germinating trays lined with filter paper which was kept moist by layers of damp cotton wool. Germination was carried out at 25 °C with 8 h of daylight per day. Samples were collected at 0 h (control), 12 h, 24 h, 48 h, 72 h and 96 h. The germinating seeds were dried and milled into flour, as indicated above for cooked samples.

Extraction and evaluation of α-galactosides from bean flour

Oligosaccharides were extracted and analyzed as described by Muzquiz et al. [11]. The sample (0.5 g) was homogenized in aqueous ethanol (80%, 5 ml) for 1 min at 24 °C using an Ultraturrax homogenizer (T25, Ika, Staufen, Germany). The mixture was centrifuged (Econospin, Sorvall Instrument, USA) for 5 min at 500 g. The supernatant was decanted and the procedure repeated twice. The combined supernatants were evaporated to dryness in a rotary evaporator (Rotavapor R-3000, Büchi, Switzerland) connected with a vacuum controller (Vacobox B-171, Büchi, Switzerland) at 35 °C and redissolved in 1 ml double deionized water. The extracts were passed through Dowex 50 WX8 (200–400 mesh, Serva, Germany) and QMA minicolumns (Waters, USA) with a Supelco vacuum system (Bellefonte, PA, USA). Water (3 × 1 ml portions) was added to flush the columns and the combined extracts and washings were collected and injected (20 μl) into the HPLC system.

Samples were analyzed using a Beckman System Gold Instrument (USA) composed of a pump (Programmable Solvent M 126), a refractive index detector (M 156), an Analog Interface (M 406), an injection valve (Altex) and the System Gold Software for integration. A Spherisorb-5-NH_2 column (250 x 4.6 mm i.d., Waters, USA) was employed with acetonitrile/water (65:35, v/v) at 1 ml/min as the mobile phase. Individual sugars were quantified by comparison with external standards of pure sucrose, raffinose and stachyose (Sigma, St. Louis, MO, USA); verbascose was supplied and purified by Prof K Gulewicz. Calibration curves were constructed for all four sugars and a linear response was evident for the range of 0–5 mg/ml with a correlation coefficient of 0.99. Two extractions were made for each flour sample and one injection for each extract.

Statistical analysis. The data were analyzed for variance using a BMDP–7D ANOVA program (WJ Dixon, BMDP Statistical Software Release, 1988). The mean values were compared using Duncan's multiple range test. Significance was accepted at $p \leqslant 0.05$ level.

Results and discussion

The total galactoside contents and the different types of raffinose oligosaccharides present in the raw bean seeds are shown in Table 1. HPLC revealed the presence of sucrose, raffinose, stachyose and verbascose.

Stachyose was found to be the predominant sugar in lima beans, jackbeans and African yam beans while verbascose was the predominant oligosaccharide in pigeon peas. Similar results were reported by Revilleza et al. [12] in lima beans and jackbeans. The results revealed significant ($p < 0.05$) differences between species and varieties of the raw bean seeds. The lowest oligosaccharides content was found in brown pigeon peas and jackbeans (2.35 mg/100 mg and 2.83 mg/100 mg, respectively). Brown pigeon peas and cream pigeon peas had an oligosaccharides content that was significantly ($p < 0.05$) different, while red and white lima beans were not significant

Table 1. Composition of α-galactosides of raw of lima beans, pigeon peas, African yam beans and jackbeans (mg/100 mg)[1]

Sample	Sucrose	Raffinose	Stachyose	Verbascose	Total galactosides
Lima beans (Red)	0.806 ± 0.021	0.297 ± 0.016	2.829 ± 0.077	0.246 ± 0.063	3.372 ± 0.002 [a]
Lima beans (White)	0.770 ± 0.018	0.277 ± 0.016	3.157 ± 0.112	0.194 ± 0.011	3.628 ± 0.085 [a,b]
Pigeon Peas (Brown)	1.186 ± 0.149	0.423 ± 0.057	0.857 ± 0.065	1.067 ± 0.110	2.346 ± 0.232
Pigeon peas (Cream)	1.666 ± 0.012	0.620 ± 0.003		1.562 ± 0.070	3.517 ± 0.079 [a,b]
African Yam beans	1.090 ± 0.003	0.664 ± 0.002	2.863 ± 0.036	0.317 ± 0.027	3.844 ± 0.011 [b]
Jackbeans	1.443 ± 0.260	0.284 ± 0.012	2.298 ± 0.038	0.248 ± 0.016	2.830 ± 0.034

[1] Values are means ± standard error of two determinations. Means followed by the same superscripts are not signicantly different at 5% level by Duncan's multiple range test.

($p > 0.05$) different. These results agree with those reported by Burbano et al. [13] who established that oligosaccharide content was influenced by both variety and environment.

Effects of processing on legume oligosaccharides

The effects of processing on the sugars and total α-galactoside contents of the legumes are shown in Tables 2–7. Cooking in water (W/C) resulted in a loss of total α-galactosides ranging from 21% in brown pigeon peas (Table 4) to a 67% in white limas (Table 3) compared to the corresponding raw samples. Cooking in 0.1% alkaline solution produced a significantly ($p < 0.05$) higher reduction of total α-galactosides, in relation to the content of raw seeds, in all seeds except white lima beans. Both processes involved cooking; therefore, these reductions may have been due to heat induced hydrolysis of the oligosaccharides to simple disaccharides and monosaccharides [14]. The current findings are in agreement with those reported for cowpeas [15]. The greatest losses in total oligosaccharide content of alkaline-cooked samples compared to water-cooked samples may have been due to the fact that the alkaline medium aids leaching and solubility of sugars during cooking.

The combined effects of soaking and cooking (S12h/C) led to increased sugar losses in most of the legumes studied, which is in agreement with data reported by Uzogara et al. [16]. The total α-galactosides content of soaked and cooked seeds was significantly ($p < 0.05$) lower than water-cooked seeds of brown pigeon peas (Table 4), cream pigeon peas (Table 5) African yam beans (Table 6) and jackbeans (Table 7). In contrast, Rao and Belavady [17] reported an increase ranging from 30% to 100% in oligosaccharide content after cooking. The difference could be explained by the fact that the cooking water was not discarded in the study by Rao & Belavady [17] whereas in the current study and that of Iyer et al. [18], the soak and cooking waters were eliminated as is commonly practiced.

Soaking for nine hours (S9h/G0) reduced the total α-galactoside contents of the seeds to different extents. Losses of 2% in jackbeans (Table 7) and 58% in cream peas (Table 5) were observed when compared with raw seeds. These losses were significant ($p < 0.05$) for all seeds except red lima (Table 2) and jackbeans (Table 7). This could be a result of the relative hardness of the seed coat which limits the uptake of water and may prevent the leaching of the α-galactosides into the soak water. Significant reductions in the α-galactoside contents of soaked common beans, including cowpeas, lentils and faba beans, were reported by Abdel-Gawad [10]; the extent of the losses was enhanced as the soaking time increased.

Tables 2–7 data also show the changes in total and individual α-galactosides due to germination. Stachyose declined progressively until 96 h of

Table 2. The composition of α-galactosides (mg/100 mg)[1] of raw, processed and germinated red lima beans (*Phaseolus lunatus*)

Treatments	Sucrose	Raffinose	Stachyose	Verbascose	Total galactosides
Raw	0.806 ± 0.021	0.297 ± 0.016	2.829 ± 0.077	0.246 ± 0.063	3.372 ± 0.002 ^{g}
W/C	0.232 ± 0.011	0.123 ± 0.013	1.614 ± 0.005	0.100 ± 0.010	1.837 ± 0.008 ^{c,d,e}
W/C/K	0.274 ± 0.021	0.087 ± 0.010	1.043 ± 0.054	n.d.[2]	1.130 ± 0.064 ^{a,b}
S12h/C	0.392 ± 0.024	0.182 ± 0.017	1.447 ± 0.027	0.106 ± 0.006	1.735 ± 0.050 ^{c,d}
Germination					
S9h/G0	0.818 ± 0.042	0.305 ± 0.013	2.830 ± 0.027	0.124 ± 0.009	3.259 ± 0.049 ^{g}
S9h/G12	0.467 ± 0.007	0.166 ± 0.011	1.950 ± 0.060	0.116 ± 0.005	2.232 ± 0.076 ^{e,f}
S9h/G24	0.403 ± 0.012	0.143 ± 0.003	1.658 ± 0.065	0.120 ± 0.009	1.921 ± 0.072 ^{d,e}
S9h/G48	0.400 ± 0.005	0.136 ± 0.002	1.680 ± 0.008	0.113 ± 0.000	1.929 ± 0.009 ^{d,e}
S9h/G72	0.344 ± 0.005	0.124 ± 0.001	1.334 ± 0.023	n.d.	1.458 ± 0.024 ^{b,c}
S9h/G96	0.231 ± 0.003	0.103 ± 0.006	0.830 ± 0.039	0.137 ± 0.009	1.070 ± 0.024 ^{a,b}

[1] Values are mean ± standard deviation of duplicate determinations.
[2] n.d.: not detected.
W/C: cooking, W/C/K: cooking in 0.1% alkaline solution, S12/C: soaking and cooking, S9h/G0-S9h/G96: germination. Means followed by the same superscripts are not significantly different at 5% level by Duncan's multiple range test.

Table 3. The composition of α-galactosides (mg/100 mg)[1] of raw, processed and germinated white lima beans (*Phaseolus lunatus*)

Treatments	Sucrose	Raffinose	Stachyose	Verbascose	Total galactosides
Raw	0.770 ± 0.018	0.277 ± 0.016	3.157 ± 0.112	0.194 ± 0.011	3.628 ± 0.085
W/C	0.294 ± 0.004	0.162 ± 0.007	1.013 ± 0.014	n.d.[2]	1.176 ± 0.007
W/C/K	0.526 ± 0.012	0.173 ± 0.003	1.383 ± 0.007	0.100 ± 0.001	1.656 ± 0.009 [c]
S12h/C	0.340 ± 0.011	0.179 ± 0.000	1.078 ± 0.004	0.085 ± 0.007	1.342 ± 0.010
Germination					
S9h/G0	0.349 ± 0.015	0.309 ± 0.014	1.927 ± 0.011	0.180 ± 0.007	2.416 ± 0.032
S9h/G12	1.090 ± 0.022	0.239 ± 0.003	1.155 ± 0.006	0.137 ± 0.037	1.531 ± 0.027 [c]
S9h/G24	1.255 ± 0.002	0.230 ± 0.025	1.678 ± 0.055	0.128 ± 0.018	2.036 ± 0.098
S9h/G48	2.120 ± 0.239	0.077 ± 0.006	0.570 ± 0.031	n.d.	0.647 ± 0.025 [b]
S9h/G72	2.952 ± 0.037	0.056 ± 0.000	0.514 ± 0.013	n.d.	0.570 ± 0.013 [b]
S9h/G96	3.107 ± 0.047	n.d.	0.402 ± 0.071	n.d.	0.402 ± 0.071 [a]

[1] Values are mean ± standard deviation of duplicate determinations.
[2] n.d.: not detected.
W/C: cooking, W/C/K: cooking in 0.1% alkaline solution, S12/C: soaking and cooking, S9h/G0-S9h/G96: germination. Means followed by the same superscripts are not significantly different at 5% level by Duncan's multiple range test.

Table 4. The composition of α-galactosides (mg/100 mg)[1] of raw, processed and germinated brown pigeon peas (*Cajanus cajan*)

Treatments	Sucrose	Raffinose	Stachyose	Verbascose	Total galactosides
Raw	1.186 ± 0.149	0.423 ± 0.057	0.857 ± 0.065	1.067 ± 0.110	2.346 ± 0.232
W/C	1.770 ± 0.024	0.454 ± 0.007	0.755 ± 0.005	0.648 ± 0.023	1.858 ± 0.036 [d]
W/C/K	1.445 ± 0.054	0.377 ± 0.036	0.603 ± 0.002	0.457 ± 0.004	1.436 ± 0.039 [c]
S12h/C	1.439 ± 0.009	0.358 ± 0.003	0.508 ± 0.000	0.379 ± 0.001	1.245 ± 0.005 [c]
Germination					
S9h/G0	1.155 ± 0.046	0.268 ± 0.008	0.670 ± 0.008	0.821 ± 0.010	1.759 ± 0.007 [d]
S9h/G12	1.201 ± 0.016	0.152 ± 0.003	0.544 ± 0.017	0.652 ± 0.009	1.348 ± 0.011 [c]
S9h/G24	2.254 ± 0.036	n.d.[2]	0.436 ± 0.016	0.178 ± 0.004	0.614 ± 0.020 [b]
S9h/G48	2.306 ± 0.030	0.060 ± 0.009	0.350 ± 0.016	0.181 ± 0.018	0.591 ± 0.008 [b]
S9h/G72	3.079 ± 0.153	n.d.	0.485 ± 0.049	0.120 ± 0.005	0.605 ± 0.054 [b]
S9h/G96	1.913 ± 0.110	n.d.	0.473 ± 0.023	n.d.	0.473 ± 0.023 [a,b]

[1] Values are mean ± standard deviation of duplicate determinations.
[2] n.d.: not detected.
W/C: cooking, W/C/K: cooking in 0.1% alkaline solution, S12/C: soaking and cooking, S9h/G0-S9h/G96: germination. Means followed by the same superscripts are not significantly different at 5% level by Duncan's multiple range test.

Table 5. The composition of α-galactosides (mg/100 mg)[1] of raw, processed and germinated cream pigeon peas (*Cajanus cajan*)

Treatments	Sucrose	Raffinose	Stachyose	Verbascose	Total galactosides
Raw	1.666 ± 0.012	0.620 ± 0.003	1.335 ± 0.006	1.562 ± 0.070	3.517 ± 0.079
W/C	1.796 ± 0.276	0.423 ± 0.069	0.596 ± 0.000	0.629 ± 0.108	1.350 ± 0.121 [c]
W/C/K	n.d.[2]	n.d.	0.367 ± 0.044	0.135 ± 0.000	0.434 ± 0.023 [a,b]
S12h/C	0.876 ± 0.019	0.039 ± 0.003	0.390 ± 0.014	0.417 ± 0.011	0.846 ± 0.027
Germination					
S9h/G0	1.362 ± 0.013	0.033 ± 0.004	0.632 ± 0.032	0.824 ± 0.040	1.489 ± 0.076 [c]
S9h/G12	1.773 ± 0.099	0.034 ± 0.006	0.684 ± 0.039	0.742 ± 0.059	1.460 ± 0.092 [c]
S9h/G24	2.603 ± 0.049	n.d.	0.159 ± 0.006	0.224 ± 0.012	0.383 ± 0.018 [a]
S9h/G48	2.879 ± 0.045	n.d.	0.150 ± 0.002	0.172 ± 0.002	0.321 ± 0.004 [a]
S9h/G72	3.199 ± 0.189	n.d.	0.371 ± 0.002	n.d.	0.371 ± 0.002 [a]
S9h/G96	2.176 ± 0.085	n.d.	0.597 ± 0.015	n.d.	0.597 ± 0.015 [b]

[1] Values are mean ± standard deviation of duplicate determinations.
[2] n.d.: not detected.
W/C: cooking, W/C/K: cooking in 0.1% alkaline solution, S12/C: soaking and cooking, S9h/G0-9h/G96: germination. Means followed by the same superscripts are not significantly different at 5% level by Duncan's multiple range test.

Table 6. The composition of α-galactosides (mg/100 mg)[1] of raw, processed and germinated African yam beans (*Sphenostylis stenocarpa*)

Treatments	Sucrose	Raffinose	Stachyose	Verbascose	Total galactosides
Raw	1.090 ± 0.003	0.664 ± 0.002	2.863 ± 0.036	0.317 ± 0.027	3.844 ± 0.011
W/C	1.124 ± 0.105	0.669 ± 0.076	1.746 ± 0.104	0.142 ± 0.038	2.558 ± 0.218 ^{e}
W/C/K	0.705 ± 0.021	0.183 ± 0.025	0.757 ± 0.033	0.081 ± 0.025	1.021 ± 0.033 ^{b,c}
S12h/C	0.603 ± 0.008	0.102 ± 0.011	0.560 ± 0.041	n.d.[2]	0.663 ± 0.030 ^{a}
Germination					
S9h/G0	1.086 ± 0.058	0.423 ± 0.018	2.403 ± 0.099	0.214 ± 0.022	3.040 ± 0.095 ^{f}
S9h/G12	1.170 ± 0.062	0.403 ± 0.038	1.962 ± 0.062	0.223 ± 0.000	2.588 ± 0.100 ^{e}
S9h/G24	0.948 ± 0.029	0.433 ± 0.008	2.583 ± 0.009	0.220 ± 0.017	3.235 ± 0.000 ^{f}
S9h/G48	1.191 ± 0.015	0.341 ± 0.009	1.708 ± 0.012	0.190 ± 0.015	2.238 ± 0.037
S9h/G72	1.752 ± 0.207	0.166 ± 0.023	1.085 ± 0.002	0.163 ± 0.021	1.414 ± 0.046 ^{d}
S9h/G96	1.394 ± 0.144	0.145 ± 0.004	0.801 ± 0.027	0.138 ± 0.002	1.085 ± 0.029 ^{c}

[1] Values are mean ± standard deviation of duplicate determinations.
[2] n.d.: not detected.
W/C: cooking, W/C/K: cooking in 0.1% alkaline solution, S12/C: soaking and cooking, S9h/G0-S9h/G96: germination. Means followed by the same superscripts are not significantly different at 5% level by Duncan's multiple range test.

Table 7. The composition of α-galactosides (mg/100 mg)[1] of raw, processed and germinated jackbeans (*Canvalia ensiformis*)

Treatments	Sucrose	Raffinose	Stachyose	Verbascose	Total galactosides
Raw	1.443 ± 0.260	0.284 ± 0.012	2.298 ± 0.038	0.248 ± 0.016	2.830 ± 0.034 [d]
W/C	1.068 ± 0.009	0.070 ± 0.007	1.073 ± 0.017	0.152 ± 0.001	1.295 ± 0.025
W/C/K	0.838 ± 0.038	0.059 ± 0.012	0.965 ± 0.012	0.107 ± 0.006	1.131 ± 0.018
S12h/C	0.523 ± 0.050	0.030 ± 0.000	0.638 ± 0.002	0.082 ± 0.025	0.750 ± 0.023 [b,c]
Germination					
S9h/G0	1.531 ± 0.006	0.369 ± 0.021	2.207 ± 0.035	0.208 ± 0.014	2.784 ± 0.042 [d]
S9h/G12	0.748 ± 0.003	0.152 ± 0.005	1.158 ± 0.043	0.131 ± 0.005	1.441 ± 0.043
S9h/G24	0.981 ± 0.051	0.052 ± 0.001	0.778 ± 0.008	0.033 ± 0.033	0.863 ± 0.024 [c]
S9h/G48	0.999 ± 0.028	0.030 ± 0.005	0.617 ± 0.087	n.d.[2]	0.647 ± 0.091 [b]
S9h/G72	0.812 ± 0.020	n.d.	0.516 ± 0.004	n.d.	0.516 ± 0.004 [a]
S9h/G96	0.385 ± 0.001	n.d.	0.485 ± 0.043	n.d.	0.485 ± 0.043 [a]

[1] Values are mean ± standard deviation of duplicate determinations.
[2] n.d.: not detected.
W/C: cooking, W/C/K: cooking in 0.1% alkaline solution, S12/C: soaking and cooking, S9h/G0-S9h/G96: germination. Means followed by the same superscripts are not significantly different at 5% level by Duncan's multiple range test.

germination in lima beans, African yam beans and jackbeans achieving reductions higher than 70% relative to the raw seeds. In brown and cream pigeon peas, verbascose was completely eliminated at 96 h of germination while stachyose was reduced around a 50% (Tables 4 and 5). Raffinose and verbascose exhibited a similar trend during the germination of red lima beans (Table 2), white lima beans (Table 3), African yam beans (Table 6) and jackbeans (Table 7), being completely eliminated at 96 hours in white lima beans and jackbeans. Sucrose levels increased during germination of white lima beans, brown pigeon peas, cream pigeon peas and African yam beans (Tables 3–6). Conversely, red lima beans and jackbean seeds (Tables 2 and 7, respectively) had decreased sucrose levels as the period of germination increased as has been reported for germinating soybeans and cottonseeds [19].

The changes in total α-galactoside content, due to germination, indicated that there were significant ($p < 0.05$) reductions in all the germinating seeds. In general, the greatest losses of total α-galactosides were obtained at 96 h of germination. Similar findings have been reported for related legumes such as black gram and mung beans [20]. Oligosaccharides of the raffinose family are degraded by α-galactosidase which selectively cleaves galactose from raffinose, stachyose and verbascose leaving behind sucrose. Different legume seeds have variable levels of α-galactosidase activity [7]. The fact that sucrose decreased during germination of red lima beans and jackbeans indicated that sucrose, in addition to α-galactosides, can be degraded to release energy for the germinating embryo.

It may be concluded that all the local methods of processing substantially reduced raffinose family oligosaccharides in the grain legumes studied. Germination and cooking in alkaline medium caused the greatest losses of total α-galactosides.

Acknowledgments

The authors are grateful for support from the INIA (SC95-004-C5). One of the authors (H.A.O.) was supported by a grant from AECI of the Spanish Ministry of Asuntos Exteriores. The authors wish to thank the INIA for awarding postdoctoral grants to C.C. and M.M.P.

References

1. Sathe SK, Salunkhe DK (1981) Preparation and utilization of protein concentrates and isolates for nutritional and functional improvement of foods. J Food Qual 4: 145–233.
2. Aletor VA, Aladetimi OO (1989) Compositional evaluation of some cowpea varieties and some underutilised edible legume in Nigeria. Die Nahrung 33(10): 999–1008.

3. Apata DF, Ologhobo AD (1994) Biochemical evaluation of some Nigerian legume seeds. Food Chem 49: 333–338.
4. Jambunathan R, Singh U (1981) Grain quality of pigeon pea. In Proceedings of the International Workshop on Pigeon Pea, Vol 1, ICRISAT, Hyderabad Andhra Pradesh, India, 15–19 December, 1980.
5. Sharma A, Sehgal S (1992) Effect of processing and cooking on the antinutritional factors of faba bean (*Vicia faba*). Food Chem 43: 383–385.
6. Reddy NR, Salunkhe DK, Sharma RP (1980) Flatulence in rats following ingestion of cooked and germinated black gram a a fermented product of black gram and rice blend. J Food Sci 45: 1161–1164.
7. Price KR, Lewis J, Wyatt GM, Fenwick R (1988) Review article. Flatulence – Causes, relation to diet and remedies. Die Nahrung 32(6): 609–626.
8. Ryan CA, Farmer EE (1981) Oligosaccharide signals in plants: A current assessment. Annv Rev Plant Physiol Molec Biol 42: 651–674.
9. Akinyele IO, Akinlosotu A (1991) Effect of soaking, dehulling and fermentation on the oligosaccharides and nutrient content of cowpeas (*Vigna unguiculata*). Food Chem 41: 43–53.
10. Abdel-Gawad AS (1993) Effect of domestic processing on oligosaccharide content of some dry legume seeds. Food Chem 46: 25–31.
11. Muzquiz M, Rey C, Cuadrado C, Fenwick GR (1992) Effect of germination on the oligosaccharide content of lupin species. J Chrom 607: 349–352
12. Revilleza JR, Mendoza EMT, Raymundo LC (1990) Oligosaccharides in several Philippine indigenous food legumes: Determination, localization and removal. Plant Foods Human Nutr 40: 83–93.
13. Burbano C, Muzquiz M, Ayet G, Cuadrado C, Pedrosa MM (1999) Evaluation of antinutritional factors of selected varieties of Phaseolus vulgaris. J Sci Food Agric 79: 1468–1472.
14. Onigbinde AO, Akinyele IO (1983) Oligosaccharide conent of 20 varieties of cowpeas in Nigeria. J Food Sci 48: 1250–1254.
15. Somiari IR, Balogh E (1993) Effect of soaking, cooking and crude α-galactosidase treatment on the oligosaccharide content of cowpea flours. J Sci Food Agric 61: 339–343.
16. Uzogara SG, Morton ID, Daniel JW (1996) Changes in some antinutrients of cowpeas (*Vigna unguiculata*) processed with ‘kanwa’ alkaline salt. Plant Foods Human Nutr 40: 249–258.
17. Rao PU, Belavady B (1978) Oligosaccharide in pulses: Varietal differences and effects of cooking and germination. J Agric Chem 26: 316–319.
18. Iyer V, Salunkhe DK, Shate SK, Rockland LB (1980) Quick-cooking beans (*Phaseolus vulgaris* L). II. Phytates, oligosaccharides and anti-enzymes. Plant Foods Human Nutr 30: 45–52.
19. Kuo TM, Vanmiddlesworth JF, Wolf WJ (1988) Content of raffinose oligosaccharides and sucrose in various plant seeds. J Agric Food Chem 36: 32–36.
20. Kataria ABM, Chauhan BN, Punia D (1990) Effect of domestic processing and cooking methods on the content of carbohydrates of amphidiploids (black gram $\times$ Mung beans). Food Chem 36: 63–72.