JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Free Cyclitol, Soluble Carbohydrate and Protein Contents in *Vigna unguiculata* and *Phaseolus vulgaris* Bean Sprouts

Elane da Silva Ribeiro,[†] Danilo da Cruz Centeno,[‡] Rita de Cássia Figueiredo-Ribeiro,[‡] Kátia Valevski Sales Fernandes,[†] José Xavier-Filho,[†] and Antônia Elenir Amancio Oliveira*[,†]

[†]Laboratório de Química e Função de Proteínas e Peptídeos-LQFPP, Centro de Biociências e Biotecnologia-CBB, Universidade Estadual do Norte Fluminense Darcy Ribeiro-UENF, Campos dos Goytacazes, RJ, Brazil

[‡]Instituto de Botânica, Núcleo de Fisiologia e Bioquímica de Plantas, Universidade de São Paulo, SP, Brazil

ABSTRACT: Seeds sprouts have been used as a good source of basic nutrients and nutraceutical compounds. The high nutritional value of seeds derives from the deposition of compounds during development. However some of these molecules are used in metabolic processes like germination, which leads to a considerable variation in their concentrations once these events are completed. In this work, we investigate the levels of inositols (*myo*-inositol, D-pinitol and ononitol), soluble carbohydrates and proteins in cotyledons of *Phaseolus vulgaris* and *Vigna unguiculata* sprouts. Sprouting increased *myo*-inositol and glucose content and reduction of raffinose and ononitol was observed. The protein levels increased in *P. vulgaris* and decreased in *V. unguiculata* sprouting. The level of sucrose was maintained in both sprouts. D-Pinitol was detected only in quiescent seeds. Our results suggested that bean sprout is an important source of proteins, sucrose, glucose and *myo*-inositol. Additionally, bean sprouts have low levels of raffinose, an antinutritional compound.

KEYWORDS: raffinose, sucrose, *myo*-inositol, D-pinitol, germination, seedling development

INTRODUCTION

Some works have reported bioactive components in plant foods which show nutritive and nutraceutical properties related to the maintenance of health. Among these substances are proteins, carbohydrates, antioxidant molecules, polyunsaturated fatty acids and inositols derivates.[1] Legume seeds contain two major classes of soluble α-galactosyl: raffinose family oligosaccharides RFOs (e.g., raffinose, stachyose, and verbascose) and galactosyl cyclitols.[2] RFOs accumulate during seed development and disappear rapidly during germination. Seeds often contain homologous series of soluble α-galactosides based on cyclitol compounds.[3] There are four major families of galactosyl cyclitols: *myo*-inositols (galactinol and digalactosyl-*myo*-inositol), D-ononitol (galactosylononitol), D-pinitol (galactopinitol) and D-*chiro*-inositol (fagopyritols).[4,5] *myo*-Inositol (MI) is a component of all eukaryotic cells, and it is the most abundant inositol in plants. MI is the most well-known nutritionally active form of inositol, and it has been used for hyperlipidemia treatment,[6] as agent of antiepileptic therapy[7] and as chemopreventive agent against cancer.[8] MI is derived from glucose 6-phosphate, and it plays a number of physiological roles in growth and normal cell function. Production of other cyclitols, cyclitol galactosides and RFOs involves metabolic processing of *myo*-inositol (MI).[9] In plants, these MI-derived compounds play important physiological roles in phloem transport, seed development, seed desiccation and stress-related responses.[10] RFOs accumulate during seed development in the range of 2−10% per dry mass and are mobilized early during germination. They play protective physiological functions in plants and serve as transport carbohydrates in the phloem.[11] They also function as cryoprotectants in frost-hardy plant organs and act as protective agents during seed desiccation and storage.[12] In contrast to their important function to plant development, RFOs represent antinutritional compounds for monogastric animals.[13] Previous studies have suggested bean sprouts as a healthy food, rich in nutrients and nutraceutic phytochemicals. Germination has often been proposed as a simple processing method by which the nutrient composition and certain functional properties of cowpea seeds might be improved.[14] Diaz-Batalla et al.[15] showed that some *Phaseolus vulgaris* seed compounds were affected by germination, as a result of a de novo synthesis of flavonols, phytoestrogens and phenolic acids. Based on that, the authors suggested that germinated bean seeds or bean sprouts of *P. vulgaris* (wild and cultivated Mexican varieties) might be good source of antioxidants and phytoestrogens.

In this work, we investigate the levels of free cyclitols (*myo*-inositol, D-pinitol and ononitol), soluble carbohydrates (raffinose, glucose and sucrose) and proteins in cotyledons of *Phaseolus vulgaris* and *Vigna unguiculata* sprouts. The information is relevant both for the understanding of metabolic changes in seed composition during germination events and for the characterization of adequacy of the bean sprout usage as food and phytoterapic source.

MATERIALS AND METHODS

Seeds. *Phaseolus vulgaris* and *Vigna unguiculata* seeds were commercially obtained from local markets in Campos dos Goytacazes, RJ, Brazil. The seeds were stored at −20 °C.

Received: December 28, 2010
Accepted: March 17, 2011
Revised: March 11, 2011
Published: March 17, 2011

dx.doi.org/10.1021/jf104979m | *J. Agric. Food Chem.* 2011, 59, 4273–4278

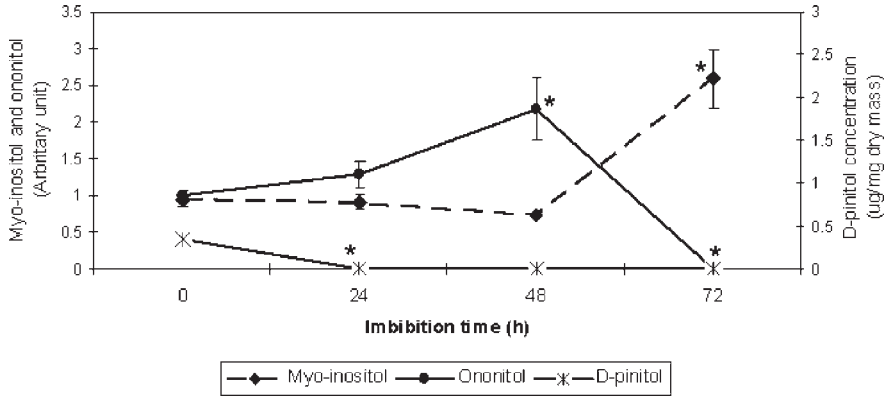

Figure 1. Changes in *myo*-inositol, ononitol and D-pinitol levels in cotyledons from *Vigna unguiculata* sprouts. Experiments were run in triplicate, and the results shown are averages. *Significantly different from the quiescent cotyledons (0 h) according to a *t* test ($p < 0.05$).

Standards and Reagents. The D-pinitol standard used in this work was isolated from *Bougainvillea spectabilis* leaves and supplied by Dr. Selwyn Yorke (New Zealand Pharmaceuticals LTD). Reagents for Bradford assay (Coomassie brilliant blue G, phosphoric acid, ethanol), methanol, chloroform, sodium phosphate, adonitol, phosphopentoxide (phosphorus pentoxide) and BSTFA (*N*,*O*-bis[trimethylsilyl] trifluoroacetamide) were commercially obtained from Sigma-Aldrich.

Seed Germination and Seedling Development. Seeds of *P. vulgaris* and *V. unguiculata* (cowpea) were germinated in Petri dishes containing cotton soaked with sterile distilled water. Seeds were germinated in a controlled environment cabinet (28 °C and 60% relative humidity), and seedlings were allowed to develop for 72 h (light/dark cycle of 12/12 h). After each imbibition time (0−72 h), the cotyledons were separated, manually grounded to a flour in the presence of liquid nitrogen and submitted for analysis. Experiments were run in triplicate with 20 seeds/dish (a total of 60 seeds per treatment), and the data shown are the average of these replicates. The results were analyzed using a *t* test for dependent samples,[16] and differences were considered significant at the level of $P < 0.05$. The cotyledons obtained from each germination time were freeze-dried and ground to a fine powder (flour).

Extraction and Analysis of Cyclitols and Carbohydrates. The compounds were extracted and analyzed according to the method of Richter et al.[17] Fifty milligrams of *P. vulgaris* and *V. unguiculata* powdered cotyledons (quiescent and germinated for 24, 48, and 72 h) were extracted in 500 μL of methanol:chloroform:water (12:5:1, by vol.) containing 10 μg of adonitol as an internal standard. The solutions were heated at 60 °C for 30 min (tubes were shaken every 10 min in vortex) and centrifuged at 10000*g* for 2 min at 25 °C. Into a 1.5 mL tube, 350 μL of supernatant was transferred followed by the addition of 350 μL of water; the solution was vortexed, and the mixture was placed on the bench for 5 min. The suspension was centrifuged at 10000*g* for 5 min, and the supernatant (100 μL) was transferred to 1.5 mL tube, dried in a speed-vac for at least 2 h and placed in a desiccator with phosphopentoxide for several hours to remove traces of water. The samples were derivatized with 200 μL of pyridine and 50 μL of BSTFA (*N*,*O*-bis[trimethylsilyl] trifluoroacetamide), shaken carefully and placed on a heating block at 75 °C for 1 h (tubes were shaken every 10 min in vortex). The samples were then transferred to a crimp-cap GC vial and submitted for analysis by GC−MS (gas chromatography/mass spectrometer). The gas chromatograph (Agilent GC 6890, Agilent Technologies, USA) was equipped with an HP-5 capillary column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies, USA) and coupled to a quadrupole mass spectrometer (Agilent MSD 5973N, Agilent Technologies, USA). Helium was used as a carrier gas with a flux of 1 mL min^{-1}. The analysis was performed using the following temperature protocol: 5 min of isothermal heating at 70 °C, a 5 °C min^{-1} oven temperature ramp to 310 °C, a temperature hold for 1 min at 310 °C for metabolic profile analysis.[18] For cyclitol analysis we used the temperature protocol of 2 min of isothermal heating at 130 °C, an 8 °C min^{-1} oven temperature ramp to 320 °C, and a temperature hold for 2 min at 320 °C.[19] The injector port was operated in the splitless mode at 230 °C, and the mass spectrometer was held at 150 °C. Mass spectra were recorded at 2 scan s^{-1} with a scanning range of 50 to 600 *m*/*z*. The chromatograms and spectra were evaluated using the ChemStation software (Agilent Technologies, USA). The levels of D-pinitol were measured based on the standard curve of D-pinitol purified from *Bougainvillea spectabilis* leaves. Minor compounds present at the spectra were not evaluated. These experiments were run in triplicate, and the results shown are averages.

Protein Determination. *P. vulgaris* and *V. unguiculata* cotyledonary flours (quiescent and germinated for 24, 48, and 72 h) were extracted (2 mg/mL) with 50 mM sodium phosphate buffer (pH 7.6) for 30 min at 4 °C and centrifuged (10000*g*, 10 min). The resulting supernatant was assayed by the Bradford method.[20] These experiments were run in triplicate, and the results shown are averages.

Statistical Analysis. Data were analyzed using a *t* test for dependent samples,[16] and differences were considered significant at the level of $P < 0.05$. Data are presented as means ± SEM.

RESULTS AND DISCUSSION

Change in *myo*-Inositol, D-Pinitol and Ononitol Levels during Sprouting. Bean sprouts have been used in healthy food diets as a good source of nutrients and phytochemicals related to disease prevention. Reports have shown that nutrient transformation could be maximized during sprouting.[15] In this work a time course of *myo*-inositol, D-pinitol and ononitol levels was followed in *V. unguiculata* and *P. vulgaris* cotyledons during sprouting. Our results showed the presence of *myo*-inositol in all germinated seed cotyledons. The level of MI was constant during 48 h of imbibition and increased at 72 h (Figures 1 and 2). *V. unguiculata* cotyledonary sprouts showed the highest variation (2.5 times) in *myo*-inositol levels, at 72 h, when compared to quiescent seeds (Figure 1). *P. vulgaris* cotyledons showed a 1.5 times increase in *myo*-inositol level at 72 h of imbibition (Figure 2).

myo-Inositol is derived from glucose-6-phosphate, and the metabolic process of MI produces other forms of inositol. MI can be further methylated to sequoyitol or ononitol, which are epimerized to D-pinitol.[1] Research has shown that MI, D-pinitol and other inositols are present in high concentrations in legume seeds.[10,21] Jaindl and Popp[22] suggested that accumulation of

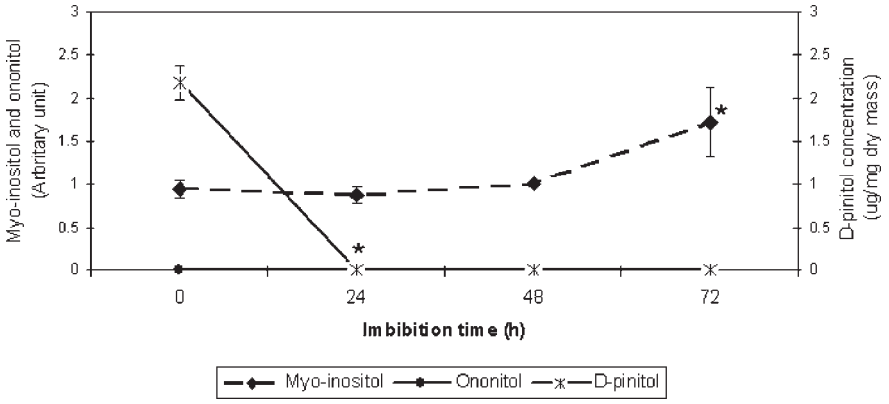

Figure 2. Changes in *myo*-inositol, ononitol and D-pinitol levels in cotyledons from *Phaseolus vulgaris* sprouts. Experiments were run in triplicate, and the results shown are averages. *Significantly different from the quiescent cotyledons (0 h) according to a *t* test ($p < 0.05$).

cyclitols in plants, including seeds, is a widespread response that provides protection against various environmental stresses. MI has been considered an important phytochemical related to disease prevention.[6] In parallel, MI has demonstrated therapeutic efficacy in obsessive−compulsive disorder (OCD), panic and depression,[6,23] as well as an antiepileptic activity.[7,24] Nozadze et al.[25] showed evidence of anticonvulsant action of MI in rats. MI treatment significantly decreased the severity and duration of seizures and delayed the onset of seizures. Solomonia et al.[24] showed that an aqueous extract of the plant *Aquilegia vulgaris* contains compounds that have antiepileptic activity. In this extract the authors have identified two constituents, MI and oleamide. These data suggest a strong potential of MI as an antiepileptic agent. Dietary *myo*-inositol significantly decreased hepatic concentrations of total lipids, triglyceride and cholesterol in rats.[26] *myo*-Inositol has been also used as a substitute for conventional treatment for hyperlipidemia[6] and as a chemopreventive agent in animal pulmonary, lung and liver cancer.[8] Hecht et al.[8] showed that inhibition of lung tumor was obtained with *myo*-inositol diet doses of 1 and 0.5%.

Our results showed that D-pinitol was present only in *P. vulgaris* and *V. unguiculata* quiescent seeds and disappeared during sprouting. *V. unguiculata* quiescent cotyledons had a D-pinitol concentration of 0.35 μg/g of dry mass (Figure 1). *P. vulgaris* cotyledons showed a concentration of 2.17 μg/g of D-pinitol per dry mass (Figure 2). Ononitol was detected in *V. unguiculata* cotyledons from quiescent seeds, in accordance with previous reports from Yasui et al.[21] The concentration increased at 48 h of imbibition, and the ononitol disappeared from the cotyledons after 72 h (Figure 1). Ononitol was not detected in *P. vulgaris* cotyledons (Figure 2). Previous findings have shown in vitro synthesis of *myo*-inositol-β-glucoside and diglucosyl-*myo*-inositol when incubating a particulate enzyme fraction prepared from mung-bean (*Phaseolus aureus*) seedling hypocotyls with a low concentration of UDP-glucose.[27] However the coumpounds themselves have not been detected in the crude seedling extracts.

D-Pinitol (3-*O*-methyl-chiroinositol) is claimed to exert insulin-like effects in mammals.[28] Sivakumar et al.[28] demonstrated the renoprotective nature of D-pinitol by attenuating the hyperglycemia-mediated proinflammatory cytokines and antioxidant competence in kidney tissues of diabetic rats. Oral administration of D-pinitol to a diabetic group of rats showed a significant increase in the level of total protein and significant decrease in the levels of blood urea, serum uric acid and creatinine.[28] Oral intake of D-pinitol and *myo*-inositol stimulated translocation of glucose transporter 4 in skeletal muscle of mice.[29]

Our results showed that *V. unguiculata* and *P. vulgaris* bean sprouts contain high levels of *myo*-inositol, when compared to quiescent seeds. We suggest that the consumption of these bean sprouts enriches the diet with *myo*-inositol and this can be beneficial for health promotion and prevention of disease.

Changes in Raffinose, Glucose and Sucrose Levels during Sprouting. Sucrose, raffinose and glucose were detected in *V. unguiculata* (Figure 3) and *P. vulgaris* (Figure 4) seed cotyledons during germination and seedling development. Sucrose content remained relatively constant in both species during 72 h of imbibition, indicating that sucrose was not mobilized as an energy source for germination and seedling development of *V. unguiculata* and *P. vulgaris*. Raffinose was present in quiescent seeds, and the concentration decreased during imbibition for both seed types (Figures 3 and 4). After 72 h, raffinose levels were 6 times lower in *V. unguiculata* cotyledons (Figure 3) and raffinose disappeared in *P. vulgaris* cotyledons (Figure 4). Unlike raffinose, glucose was not detected in quiescent seeds. However, glucose content increased during imbibition at 24 and 48 h in both seeds (Figures 3 and 4) and disappeared in *V. unguiculata* cotyledons after 72 h (Figure 3).

Although plant foods are considered good sources of important compounds to animal nutrition, depending on the types of these compounds, their biological effects can also be toxic or antinutritional. Legume seeds contain a number of antinutritional compounds, and these molecules reduce the nutritive value of these seeds.[13] In both cowpea and common bean dry seeds a number of antinutritional chemicals have been described.[13,15] Among them raffinose represents an antinutritional compound for animals, including humans, in contrast to their important functions to plant development. This sugar is fermented by bacteria producing carbon dioxide, hydrogen and methane gas that cause flatulence, abdominal rumblings, cramps, diarrhea and nausea.[13] RFOs are present in seeds in the range of 2−10% per dry mass and are the first stored energy source to be degraded during seed germination in some legume seeds.[11] Consequently their levels are low in germinated seeds. In some seed species, the breakdown of RFOs occurs in the endosperm and embryo, indicating that RFOs are important reserves in both tissues.[30,31] Blöchl et al.[30] showed that the RFO levels rapidly decreased to 50% within the first 40 h after imbibition. Oboh et al.[31] showed that germination reduced raffinose in *Phaseolus*

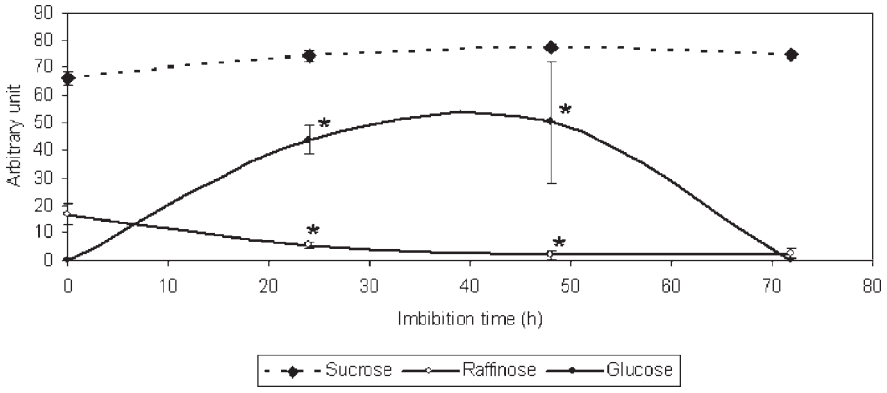

Figure 3. Changes in sucrose, raffinose and glucose levels in cotyledons from *Vigna unguiculata* sprouts. Experiments were run in triplicate, and the results shown are averages. *Significantly different from the quiescent cotyledons (0 h) according to a *t* test ($p < 0.05$).

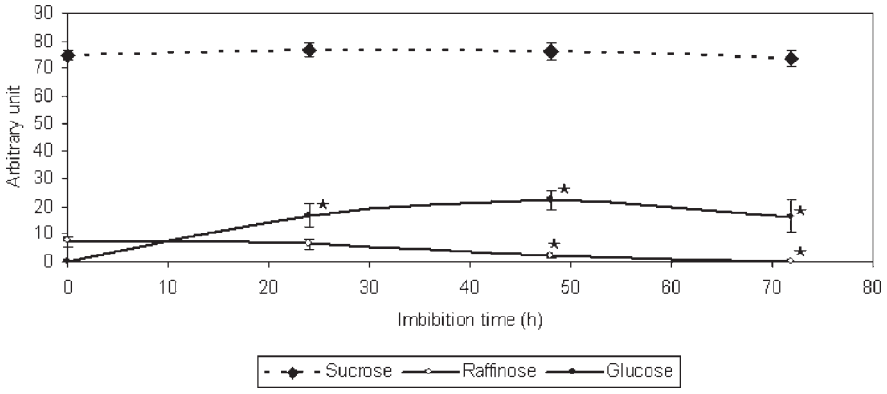

Figure 4. Changes in sucrose, raffinose and glucose levels in cotyledons from *Phaseolus vulgaris* sprouts. Experiments were run in triplicate, and the results shown are averages. *Significantly different from the quiescent cotyledons (0 h) according to a *t* test ($p < 0.05$).

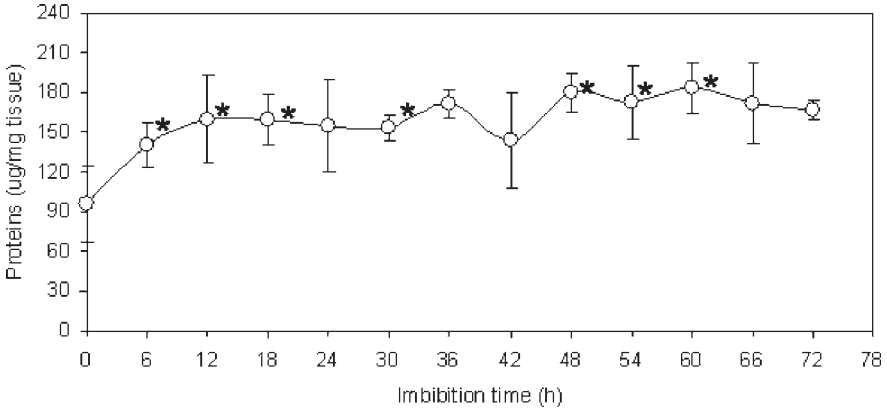

Figure 5. Changes in soluble proteins in cotyledons from *Phaseolus vulgaris* sprouts. Experiments were run in triplicate, and the results shown are averages. *Significantly different from the quiescent cotyledons (0 h) according to a *t* test ($p < 0.05$).

lunatus (red lima beans and white lima beans), African yam beans and jack beans (*Canavalia ensiformis*). The sugar was completely eliminated after 96 h of germination in these species.[31]

Change in Protein Levels during Sprouting. Our results showed an increase in the soluble protein levels in *P. vulgaris* cotyledons during sprouting; these levels reached increases of over 85% (Figure 5). In *V. unguiculata* cotyledonary sprouts we observed increases in protein levels at 12 h and 30 h of imbibition followed by a decrease (Figure 6). Legume seeds accumulate large amounts of proteins during development. Part of the energy for germination and seedling development is provided by the mobilization of these stored proteins.[32] Proteins in legume seeds represent up to 20−40% and are an important source of amino acids for animal nutrition.[14,32] Previous reports have stated that germination is a simple processing event by which the nutrient composition and certain functional properties of *V. unguiculata* seeds might be improved.[14] Germination increased the crude protein content, total phosphorus content, and nitrogen

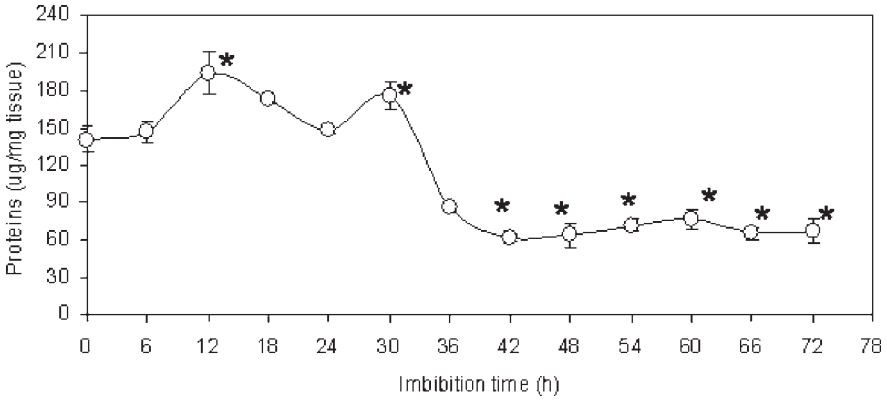

Figure 6. Changes in soluble proteins in cotyledons from *Vigna unguiculata* sprouts. Experiments were run in triplicate, and the results shown are averages. *Significantly different from the quiescent cotyledons (0 h) according to a *t* test ($p < 0.05$).

solubility of germinated Nigerian lines of *V. unguiculata*.[14] At this work, such increase was observed only at the early sprouting stages for *V. unguiculata*. As a concluding remark, the consumption of the cowpea and common bean young sprouts (72 h after imbibition) may be considered a valuable way to enrich the diet with sucrose and soluble proteins, and especially with *myo*-inositol which can be beneficial for health promotion and prevention of diseases.

AUTHOR INFORMATION

Corresponding Author

*Tel: 55 (22) 2739 7132. Fax: 55 (22) 2739 7178. E-mail: elenir@uenf.br.

Funding Sources

This work was supported by grants from the Brazilian agencies FAPERJ, CNPq, and TECNORTE and from the Universidade Estadual do Norte Fluminense Darcy Ribeiro.

ACKNOWLEDGMENT

We would like to thank Dr. Selwyn Yorke (New Zealand Pharmaceuticals LTD) for kindly providing D-pinitol.

REFERENCES

(1) Lampe, J. W. Health effects of vegetables and fruits: assessing mechanisms of action in human experimental studies. *Am. J. Clin. Nutr.* **1999**, *70*, 174–490.

(2) Karner, U.; Peterbauer, T.; Raboy, V.; Jones, D. A.; Hedley, C. L.; Andreas Richter, A. Myo-Inositol and sucrose concentrations affect the accumulation of raffinose family oligosaccharides in seeds. *J. Exp. Bot.* **2004**, *55*, 1981–1987.

(3) Peterbauer, T.; Richter, A. Biochemistry and physiology of raffinose family oligosaccharides and galactosyl cyclitols in seeds. *Seed Sci. Res.* **2001**, *11*, 185–197.

(4) Richter, A.; Peterbauer, T.; Brereton, I. Structure of galactosylononitol. *J. Nat. Prod.* **1997**, *60*, 749–751.

(5) Gomes, C. I.; Obendorf, R. L.; Horbowicz, M. *Myo*-inositol, D-*chiro*-inositol, and D-pinitol synthesis, transport, and galactoside formation in soybean explants. *Crop Sci.* **2005**, *45*, 1312–1319.

(6) Harvey, B. H.; Brink, C. B.; Seedat, S.; Stein, D. J. Defining the neuromolecular action of myo-inositol: Application to obsessive–compulsive disorder. *Prog. Neuro-Psychopharmacol.* **2002**, *26*, 21–32.

(7) Solomonia, R.; Mikautadze, E.; Nozadze, M.; Kuchiashvili, N.; Lepsveridze, E.; Kiguradze, T. Myo-inositol treatment prevents biochemical changes triggered by kainite-induced status epilepticus. *Neurosci. Lett.* **2010**, *468*, 277–281.

(8) Hecht, S. S.; Kenney, P. M. J.; Wang, M.; Upadhyaya, P. Dose-response study of myo-inositol as an inhibitor of lung tumorigenesis induced in A/J mice by benzo[a]pyrene and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. *Cancer Lett.* **2001**, *167*, 1–6.

(9) Loewus, F. A.; Murthy, P. P. N. *Myo*-inositol metabolism in plants. *Plant Sci.* **2000**, *150*, 1–9.

(10) Obendorf, R. L. Oligosaccharides and galactosyl cyclitols in seed desiccation tolerance. *Seed Sci. Res.* **1997**, *7*, 63–74.

(11) Peterbauer, T.; Richter, A. Biochemistry and physiology of raffinose family oligosaccharides and galactosyl cyclitols in seeds. *Seed Sci. Res.* **2001**, *11*, 185–197.

(12) Pennycooke, J. C.; Jones, M. L.; Stushnoff, C. Down-regulating α-galactosidase enhances freezing tolerance in transgenic petunia. *Plant Physiol.* **2003**, *133*, 901–909.

(13) Price, K. R.; Lewis, J.; Wyatt, G. M.; Fenwick, R. Review article. Flatulence—Causes, relation to diet and remedies. *Die Nahrung* **1988**, *32*, 609–626.

(14) Giami, S. Y.; Akusu, M. O.; Emelike, J. N. Evaluation of selected food attributes of four advanced lines of ungerminated and germinated Nigerian cowpea (*Vigna unguiculata* (L.) Walp. *Plant Food Hum. Nutr.* **2001**, *56*, 61–73.

(15) Diaz-Batalla, L.; Wildholm, J. M.; Fahey, G. C.; Castano-Tostado, E.; Paredes-Lopez, O. Chemical components with health implications in wild and cultivated mexican common bean seeds (*Phaseolus vulgaris* L.). *J. Agric. Food Chem.* **2006**, *54*, 2045–2052.

(16) Bridge, P. D.; Sawilowsky, S. S. Increasing physician's awareness of the impact of statistic on research outcomes: Comparative power of the T-test and Wilcoxon rank-sum test in small samples applied research. *J. Clin. Epidemiol.* **1999**, *52*, 229–235.

(17) Richter, A.; Thonke, B.; Popp, M. 1-D-1-*O*-methyl-muco-inositol in *Viscum album* and members of the rhizophoraceae. *Phytochemistry* **1990**, *29*, 1785–1786.

(18) Roessner, U.; Luedemann, A.; Brust, D.; Fiehn, O.; Linke, T.; Willmitzer, L.; Fernie, A. R. Metabolic profiling allows comprehensive phenotyping of genetically or environmentally modified plant systems. *Plant Cell* **2001**, *13*, 11–29.

(19) Reid, J. S. G. Reserve carbohydrate metabolism in germinating seeds of *Trigonella foenum-graecum* L. (Legum). *Planta* **1971**, *100*, 131–142.

(20) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254.

(21) Yasui, T.; Tateishi, Y.; Ohashi, H. Distribution of low molecular weight carbohydrates in the subgenus *Ceratotropis* of the genus *Vigna* (Leguminosae). *J. Plant Res.* **1985**, *98*, 75–87.

(22) Jaindl, M.; Popp, M. Cyclitols protect glutamine synyhase and malate dehydrogenase against heat induced deactivation and thermal denaturation. *Biochem. Biophys. Res. Commun.* **2006**, *345*, 761–765.

(23) Chengappa, K. N. R.; Levine, J.; Gershon, S.; Mallinger, A. G.; Hardan, A.; Vagnucci, A.; Pollock, B.; Luther, J.; Buttenfield, J.; Verfaille, S.; Kup-fer, D. J. Inositol as an add on treatment for bipolar depression. *Bipolar Disord.* **2000**, *2*, 47–55.

(24) Solomonia, R.; Kuchiashvili, N.; Berulava, A.; Pkhakadze, V.; Trapaidze, N.; Zhvania, M.; Abesadze, I.; Kojima, H.; Dalakishvili, N. Purification and identification of compounds of the *Aquilegia vulgaris* extract fraction exhibiting anti-epileptic activity. *J. Biol. Phys. Chem.* **2004**, *4*, 185–192.

(25) Nozadze, M.; Mikautadze, E.; Lepsveridze, E.; Mikeladze, E.; Kuchiashvili, N.; Kiguradze, T.; Kikvidze, M.; Solomonia, R. Anticonvulsant activities of myo-inositol and scyllo-inositol on pentylenetetrazol induced seizures. *Seizure* **2011**, *20* (2), 173–176.

(26) Okazaki, Y.; Katayama, T. Effects of dietary carbohydrate and myo-inositol on metabolic changes in rats fed 1,1,1-trichloro-2,2-bis-(p-chlorophenyl) ethane(DDT). *J. Nutr. Biochem.* **2003**, *14*, 81–89.

(27) Kemp, J.; Loughman, B. C. Cyclitol glucosides and their role in the synthesis of a glucan from uridine diphosphate glucose in *Phaseolus aureus*. Characterization of some cyclitol glucoside and their synthesis. *Biochem. J.* **1974**, *142*, 153–159.

(28) Sivakumar, S.; Palsamy, P.; Subramanian, S. P. Impact of D-pinitol on the attenuation of proinflammatory cytokines, hyperglycemia-mediated oxidative stress and protection of kidney tissue ultrastructure in streptozotocin-induced diabetic rats. *Chem. Biol. Interact.* **2010**, *188*, 237–245.

(29) Dang, N. T.; Mukai, R.; Yoshida, K.; Ashida, H. D-pinitol and myo-inositol stimulate translocation of glucose transporter 4 in skeletal muscle of C57BL/6 mice. *Biosci. Biotechnol. Biochem.* **2010**, *74*, 1062–1067.

(30) Blöchl, A.; Peterbauer, T.; Hofmann, J.; Richter, A. Inhibition of raffinose oligosaccharide breakdown delays germination of pea seeds. *Planta* **2008**, *228*, 99–110.

(31) Oboh, H. A.; Muzquiz, M.; Burbano, C.; Cuadrado, C.; Pedrosa, M. M.; Ayet, G.; Osagie, A. U. Effect of soaking, cooking and germination on the oligosaccharide content of selected Nigerian legume seeds. *Plant Food Hum. Nutr.* **2000**, *55*, 97–110.

(32) Bewley, J. D. Seed germination and dormancy. *Plant Cell* **1997**, *9*, 1055–1066.