ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 08, Issue, 06, pp.21109-21114, June, 2018

ORIGINAL RESEARCH ARTICLE

OPEN ACCESS

NUTRITIONAL QUALITY OF COMMON BEAN FORTIFIED WITH AMINO ACIDS

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ARTICLE INFO

Article History:
Received 12th March, 2018
Received in revised form
17th April, 2018
Accepted 20th May, 2018
Published online 30th June, 2018

Key Words:

Biological value, Edible coating,
Grain, Leguminous,
Polymer.

ABSTRACT

This work aimed to cover common bean grains, Carioca variety, with edible biopolymers added of amino acids methionine and cysteine in the amount recommended by Food and Agricultural Organization/World Health Organization, for the daily ingestion of students in the age group from 2 to 5 years and to quantify them through chromatographic analysis in high performance liquid chromatography. The tests were conducted in theLaboratory of Packaging, Department of Food Technology, Federal University of Viçosa, Minas Gerais, Brazil. For the formulation of treatments, 1% cassava starch and 4% carnauba wax used, individually and in association 1:1 w/w, added of amino acids.Treatments containing cassava starch in the coating presented higher concentration of added amino acids both in the bean soup as in cooked bean grains. The addition of sulphur amino acids limiting in the common bean in edible coatings can be a viable alternative for the daily ingestion of these amino acids in diet.

Citation: Sousa Solange de, Soares Nilda de F. Ferreira and Faroni, Lêda R. D´Antonino, 2018. “Nutritional quality of common bean fortified with amino acids”, *International Journal of Development Research*, 8, (06), 21109-21114.

INTRODUCTION

Nowadays, constant search realized for foods that enable the supply of nutritional needs, with quality, safety and low cost, in addition to the presence of functional bioactive compounds. Due to their high cost, foods of animal origin that are sources of proteins of high biological value and bioavailable minerals (Molina *et al*., 2001) become inaccessible to a large portion of the population. Therefore, vegetables are of great importance in the diet and, according to Slupski(2010), bean can compete with meat, for being a healthy and nutritious food. Among legumes, bean occupies an important place in human nutrition, especially among low-income populations in developing countries (Siddiq *et al*., 2010), being well established in the cookery of developed countries (Tang *et al*., 2009). Despite the presence of anti-nutritional factors that reduce the biological activity of various chemical compounds (nutrients) or metabolites, in addition to affecting the bioavailability of these nutrients to consumers (Wang *et al*., 2010).

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According to Boye *et al*. (2010), there is estimation that 149.6 million young children undernourished worldwide below the age of five in terms of weight and age. Protein deficiency during the development of individuals, especially in early childhood may be related to disorders, often irreversible, which can produce disorders in physical and intellectual development. For Mubarak (2005), the structure and conformation of proteins and their interaction with other components of seed or grain can form complexes, also assigned to the low availability of proteins. Moreover, the low methionine content in common bean has worsened the nutritional value of bean proteins (Montoya *et al*., 2010). For Ribeiro *et al*. (2007), the levels of methionine and cysteine in bean vary with the commercial group, cultivar and growing environment. However, cysteine is an essential amino acid partly in certain clinical conditions, such as premature infants (Ribeiro *et al*., 2009). Methionine lacks of adequate metabolism to meet the body needs. Legumes usually cooked before consumed, as this improves the quality of the protein by promoting the destruction or inactivation of thermosensitive antinutritional factors. However, according to Lisiewska *et al*. (2008) and Wang *et al*. (2009), cooking causes considerable changes in the composition of many chemicals, including amino acids, vitamins and minerals, depending on temperature and heat treatment time. Edible coatings can be used to increase the shelf life of many products, especially those of vegetable origin, preventing weight loss while maintaining color and when added of antioxidant, antimicrobial or enzyme inactivating substances, they may control microbial growth, prevent enzymatic browning, etc. Bioactive edible coatings used in the coating of fruits and other vegetables, mostly those minimally processed. An alternative to balance limiting amino acids in beans is the coating of grains with emulsions incorporated with these amino acids. Studies related to the coating of grains with edible characteristics and added of nutrients are very scarce, as well as behavioral studies of their physiology. Thus, the use of amino acids in the insertion of edible coatings for application in bean grains, as well as its quantification was the aim of this work.

MATERIALS AND METHODS

Beans purchased at a farm in the municipality of Venda Nova, state of Espirito Santo, Brazil, without having undergone chemical treatment with fumigant. The analyses conducted at the Packaging Laboratories of Food Technology Department, Grain Laboratory of Agricultural Engineering Department of Federal University of Viçosa – MG, Brazil.

Formulation and application of coatings

The coatings formulated using polymeric bases as carbohydrate (1% cassava starch) and lipids (carnauba wax emulsion at concentration of 4%), individually or forming a 50% film solution of each compound. Each coating was added of essential amino acids methionine + cysteine at concentrations recommended by Food and Agriculture Organization/World Health Organization (FAO / WHO) (2007), which is 25 mg kg body weight $^{-1}$ day^{-1} for school children aged two to five years. Coating made from cassava starch obtained upon addition of 1 g starch in 100 ml distilled water and submitted to heating to 70°C when gelation of its granules occurs. The solution left to cool for further use. Beans were immersed in solutions (cassava starch, carnauba wax and a mixture of two polymers) remaining for one minute, and then they were drained in a sieve, following by drying step carried out in oven at 25°C for 12 hours. The treatments were control (without coating and without amino acids), cassava starch + amino acids (Faa), carnauba wax + amino acids (Caa) and cassava starch + carnauba wax + amino acids (FCaa), for room and cooling temperatures, totaling eight treatments. The storage period of bean grains with about 13 % moisture, packed in low-density polyethylene bags with thickness of 0.09 mm, measuring 25.5 cm in width by 35 cm in length, was 60 days, kept under refrigerated conditions (domestic refrigerator at 4 ± 2 C, 85 % RH) and room temperature (16.9°C, 77.6 % RH).

Determination of amino acids added to treatments

Sample preparation: Samples of 100 g of beans per treatment (control, Faa, Caa and FCaa) at storage times zero, 30 and 60 days were cooked in 700 ml distilled water in a domestic pressure cooker for 25 minutes without being submitted to maceration before cooking. Bean soup and grains separately placed in polypropylene containers and stored at refrigeration temperature (4 ± 2ºC, 85 % RH). Over a period of 12 hours after cooking and subsequent cooling, the samples dried in oven at 40°C and airflow. Then, samples ground in a Wiley mill, conditioned in low-density polyethylene bags and frozen in ultra-freezer at -80°C until further analyses. After thawing in domestic refrigerator, bean soup samples submitted to two filtrations: one on filter paper and another in Millipore ® vacuum filter using 0.45 µm filter (Millipore ®). For crushed bean grain samples, 200 ml of high purity water were added, mixed and submitted to vacuum filtration using 0.45 µm filter (Millipore ®). Then, the samples were stored under refrigeration (4 ± 2ºC, 85 % RH) for subsequent analyses.

Derivatization

Derivatization solution was prepared according to Gomis *et al*. (1990), Pripis-Nicolau *et al*. (2001), Paramás *et al*. (2006). The methodology modified because the extraction period occurred only after 24 hours of contact of sample with the solution. For cysteine analysis, a step added, in which an aliquot of 0.1 ml of iodoacetic acid (IDA) added to 0.2 ml of samples and submitted to derivatization. The IDA solution was prepared as recommended by Pripis-Nicolau *et al*. (2001) (modified methodology as mentioned above).

Quantification of amino acids

The quantification of amino acids present in crushed cooked bean grains and bean soup performed by high performance liquid chromatography (HPLC) with fluorescence detection. The HPLC system consists of two high-pressure pumps (model LC - 10 AD), with a fluorescence detector RF 10 AxL interface SCL - 10Avp and the Class - Shimadzu VP software version 6.12 used for calculations of areas. The chromatographic separation performed on C_{18} column (150 x 4.6 mm), flow rate of 1.0 ml min $^{-1}$ and wavelengths were 340 nm and 440 nm for excitation and emission, respectively. The injection volume was 50 µl. For methionine determination, solvents used were methanol (solvent B), and sodium acetate buffer 4.2 x 10^{-2}mol l^{-1} pH 6.80 (solvent A). Cysteine quantification used the mobile phase containing phosphate buffer pH 7.4 - acetonitrile 89:11 v / v. Detection of cysteine isoindole derivatives accomplished with emission at 425 nm. The standard curves of methionine (L- methionine, purchased from SIGMA- ALDRICH™, Japan) and cysteine (L- cysteine, SIGMA - ALDRICH™, Japan) were constructed by plotting a graph of peak areas obtained by the injection of 50 µl of the prepared amino acid solutions. The method repeatability verified by the injection of amino acid solutions in duplicate. The equations and coefficients of determination of the standard curve for methionine and cysteine were $y = 124064x – 1415.7$, $R^2 = 0.9935$ and $y = 1E+07x + 3E+06$, $R^2 = 0.9922$, respectively.

Experimental design and statistical analysis

A completely randomized design (CRD) used, with treatments arranged in a 4 × 2 × 5 factorial, four coatings, two storage temperatures (room and refrigeration), five storage periods and three replicates. The experimental units represented by treatments packed in low-density polyethylene bags, stored in laboratory without control of lighting conditions and under refrigeration. The analyses were performed using the Statistical Analysis System software (SAS ®, 2009), version 9.1, licensed by the Federal University of Viçosa – MG, Brazil.

RESULTS AND DISCUSSION

Amino Acids: The results of the chromatographic analysis for the quantification of cysteine and methionine added to Faa, Caa and FCaa treatments and control treatment (without coating and without amino acids) for bean soup and cooked bean grains at time zero, 30 and 60 days of storage showed differences in relation to the added and quantified contents.

Methionine content in bean soup and bean grains: All treatments showed differences in relation to the methionine concentration in bean soup. The storage times also differed ($p < 0.05$), where time zero was different from the other times (30 and 60), while the latter did not differ from one another. An interaction between treatment and storage time observed. In the Figure 1 we can see that no content of methionine quantified in the soup bean grains of control treatment (without coating). The average methionine values in bean soup was higher in Faa treatment (12.02 mg ml $^{-1}$) (Figure 2), followed by Caa (4.63 mg ml $^{-1}$) (Figure 3) and FCaa treatments (1.31 mg ml $^{-1}$) (Figure 4), for the period of 60 days of storage.

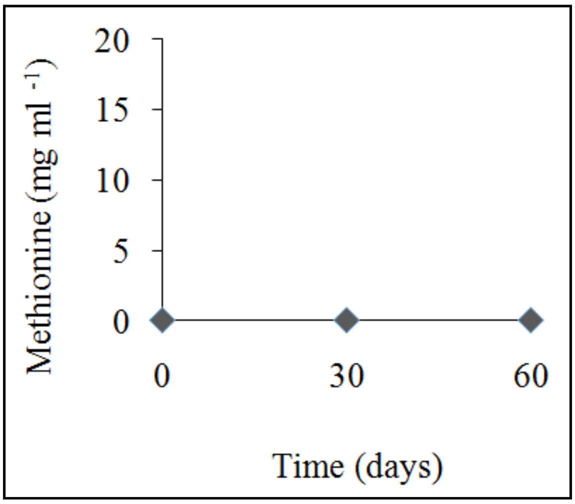

Figure 1. Averages of methionine concentration in the cooked bean soup for the control treatment

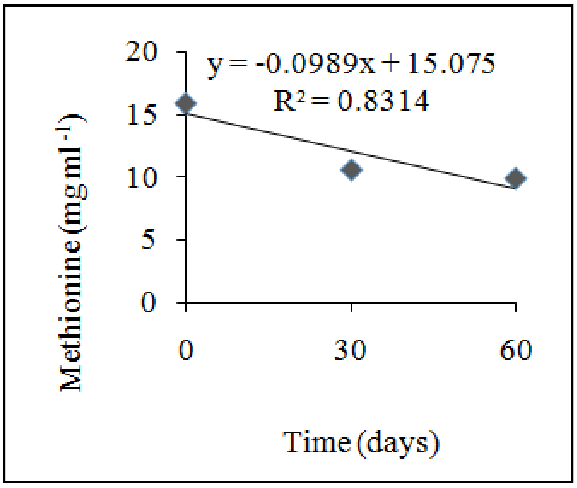

Figure 2. Averages of methionine concentration in the cooked bean soup for the cassava starch + amino acids - Faa treatment

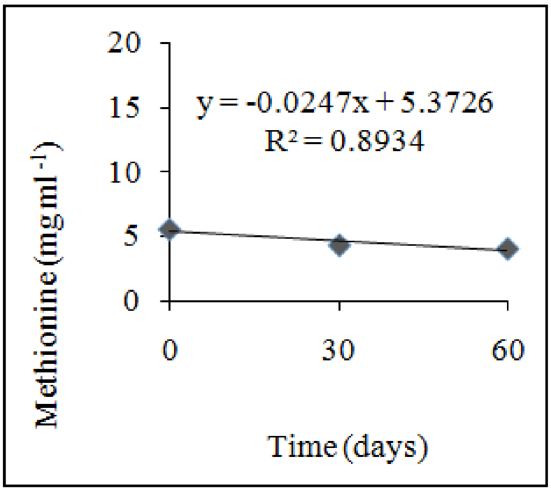

Figure 3. Averages of methionine concentration in the cooked bean soup for the carnauba wax + amino acids - Caa treatment

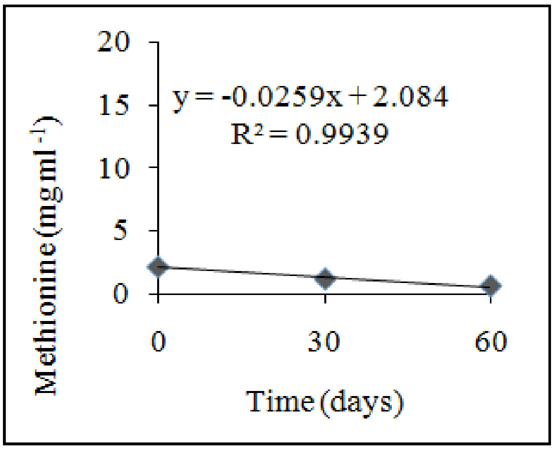

Figure 4. Averages of methionine concentration in the cooked bean soup for the cassava starch + carnauba wax + amino acids – Fcaatreatment

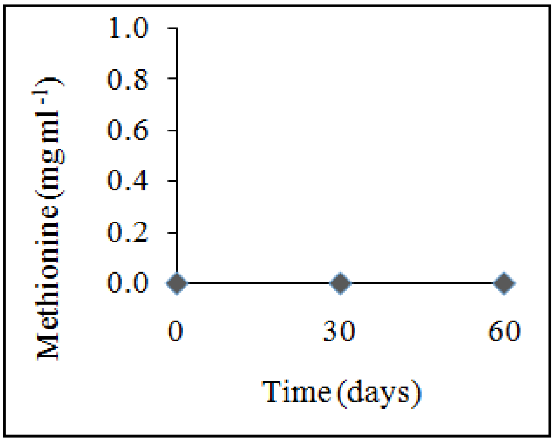

Figure 5. Averages of methionine concentration in the cooked bean grains for the control treatment

With respect to the storage time, the higher mean methionine value in the bean soup observed at time zero (5.87 mg ml $^{-1}$), followed by time 30 days (3.97 mg ml $^{-1}$) and 60 days (3.63 mg ml $^{-1}$). In Figures 3 and 4 shows the content of methionine in the cooked bean soup of Caa treatment. A probable complexation of amino acids with coatings containing carnauba wax may have occurred during the storage period, thus explaining the decrease in the methionine content observed in this experiment.

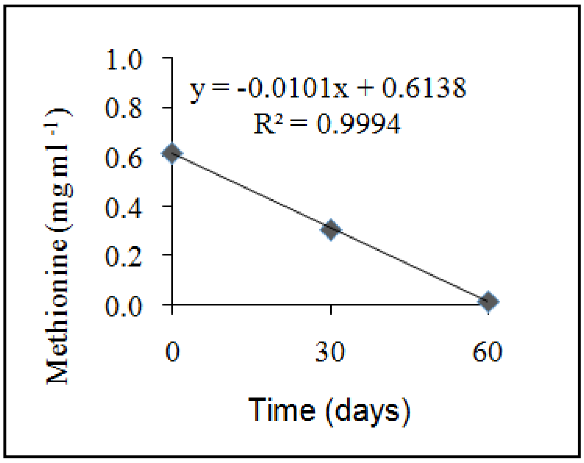

Figure 6. Averages of methionine concentration in the cooked bean grains for the cassava starch + amino acids - Faatreatment

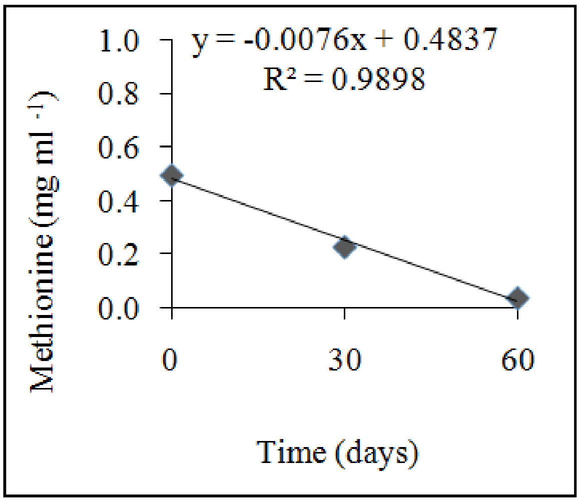

Figure 7. Averages of methionine concentration in the cooked bean grains for the carnauba wax + amino acids - Caatreatment

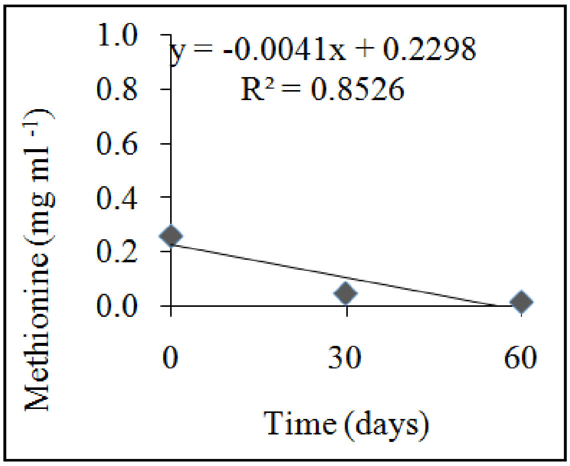

Figure 8. Averages of methionine concentration in the cooked bean grains for the cassava starch + carnauba wax + amino acids - FCaa treatment

The results of the analysis of variance for cooked bean grains showed significant interaction between treatments *versus* time (Figures 5, 6, 7 and 8), for the period of 60 days of storage. There was a significant difference between Faa (Figure 6) and FCaa (Figure 8) treatments, and control and Caa (Figure 8) treatments also differed significantly ($p < 0.05$).

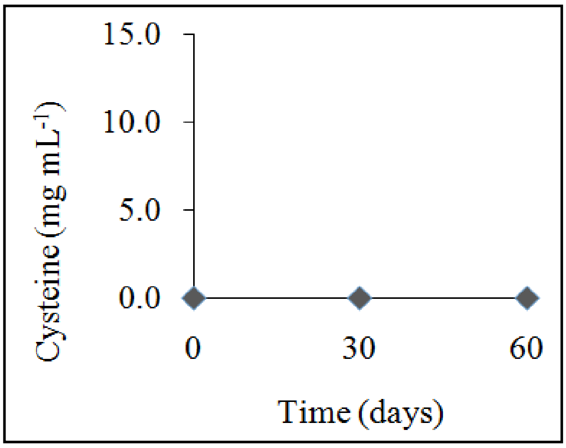

Figure 9. Averages of cysteine concentration in the cooked bean soup for the control treatment

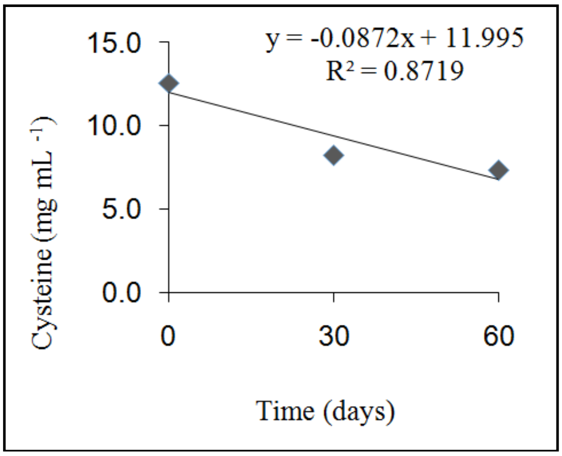

Figure 10. Averages of cysteine concentration in the cooked bean soup for the Faa treatment

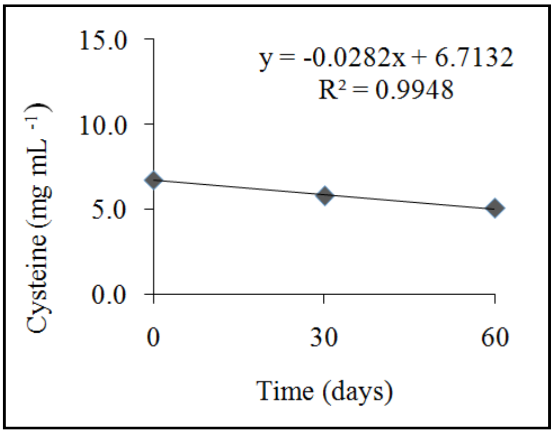

Figure 11. Averages of cysteine concentration in the cooked bean soup for the carnauba wax + amino acids - Caatreatment

The time periods analyzed also showed differences, where time zero showed higher mean methionine concentration for cooked bean grains (0.33 mg g^{-1}), followed by 0.15 mg g^{-1} and 0.02 mg g^{-1} for 30 days and 60 days of storage, respectively. The treatments that showed the highest mean methionine values in cooked bean grains were Faa (0.31 mg g^{-1}) (Fig. 6), followed by Caa (0.25 mg g^{-1}) (Fig. 7) and FCaa (0.11 mg g^{-1}) (Fig. 8). The lowest methionine concentrations obtained in cooked bean grains could be due to the impermeability of the integument to migration of the amino acid to cotyledons, in which polyphenols may have complexed with amino acids.

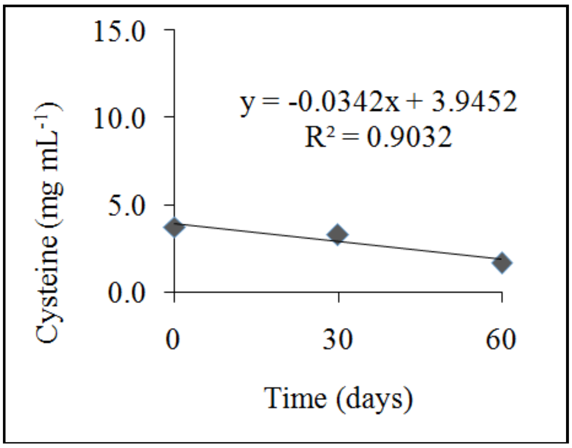

Figure 12. Averages of cysteine concentration in the cooked bean soup for the cassava starch + carnauba wax + amino acids - FCaa treatment

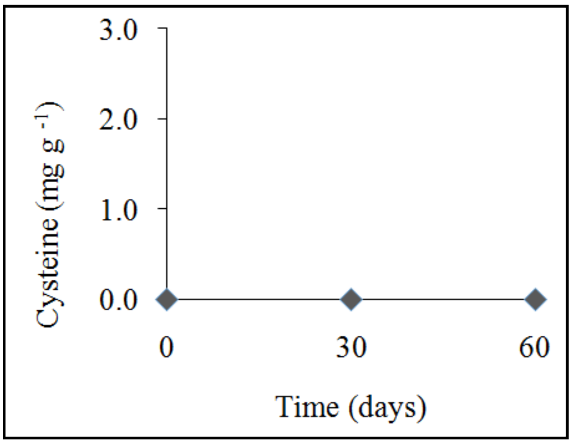

Figure 13. Averages of cysteine concentration in the cooked bean grains for the control treatment

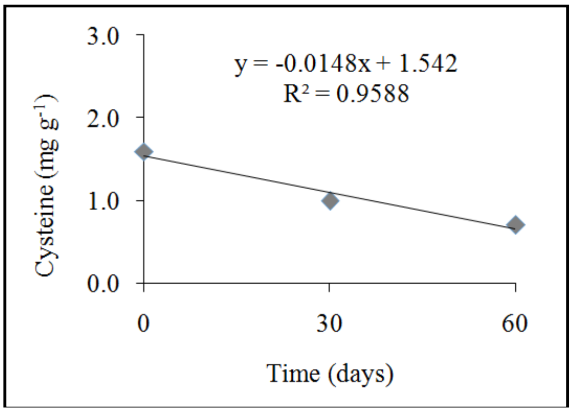

Figure 14. Averages of cysteine concentration in the cooked bean grains for the cassava starch + amino acids - Faa treatment

There was a reduction in the methionine content for treatments coated after 60 days of storage, which concentration for FCaa treatment was 0.11 mg g $^{-1}$ of cooked bean grains. Both in bean soup and bean grains, FCaa treatment showed lower concentration of this amino acid. According to literature, various bean cultivars, including Carioca, have methionine as the limiting amino acid in their protein profile, which confirmed in this study, where control treatment showed no trace of this amino acid.

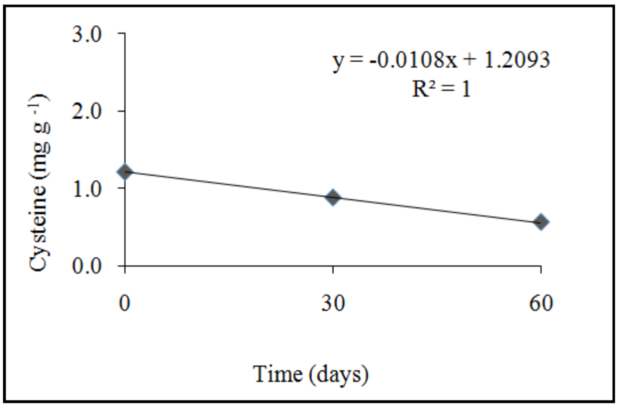

Figure 15. Averages of cysteine concentration in the cooked bean grains for the Caa treatment

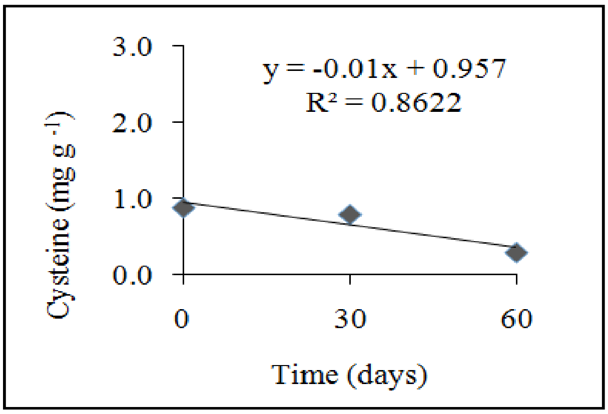

Figure 16. Averages of cysteine concentration in the cooked bean grains for the cassava starch + carnauba wax + amino acids - FCaa treatment

Cysteine content in bean soup and bean grains: The quantification of cysteine added to the coatings of Faa, Caa and FCaa treatments, as well as the control treatment in bean soup and cooked bean grains are shown in Figures 9, 10, 11 and 12. All treatments and storage times showed significant differences in relation to the cysteine concentration in the bean soup. According to data obtained from the analysis of variance, it observed that there was a significant interaction between treatments *versus* storage time. The Figure 10 shows the content of cysteine in cooked bean soup of the Faa treatment during 60 days of storage. The Figure 11 shows the cysteine concentration in cooked bean soup for the treatment with carnauba wax and amino acids. The content of cysteine in cooked bean soup is shows in Figure 12 to FCaa treatment. Using the comparison of means, it was observed that Faa treatment (Figure 10) showed higher mean cysteine concentration value in the bean soup (9.38 mg ml $^{-1}$), followed by Caa (5.87 mg ml $^{-1}$) (Figure 11) and FCaa treatments (2.92 mg ml $^{-1}$) (Figure 12). It was found that treatments containing carnauba wax coating (Caa and FCaa) had lower cysteine concentrations, indicating a probable complexation of this amino acid with some wax constituent or its possible degradation (Figures 11 and 12). The results obtained by analysis of variance for cysteine in bean grains showed significant interaction between treatments *versus* time. The Figure 13 shows the content of cysteine in cooked beans grains in the control treatment. The content of cysteine in the cooked bean grains is showed in the Figure 14 for the Faa treatment. In the Figure 15 we can see the concentration of cysteine in cooked bean grains of Caa treatment. The Figure 16 show the content of cysteine in cooked bean grains in the FCaa treatment during the storage period. All treatments and storage times showed differences, where the highest mean among all treatments was for Faa treatment at time zero (1.10 mg g $^{-1}$), followed by Caa (0.91 mg g $^{-1}$) and FCaa treatments (0.66 mg g $^{-1}$). Due to the hydrophilic nature of amino acids added to coatings, the carnauba wax emulsion may have formed an insoluble complex with amino acids, preventing their migration both the bean soup as for bean grains. Candela *et al*. (1997), analyzed dried bean seed samples and observed that after cooking, there was a decrease in the dry matter content, and that all amino acids were affected, particularly tyrosine, methionine and threonine. Toledo *et al*. (2008) studied the nutritional and proximate composition of beans variety Carioca and different cooking methods and observed that the cooking of beans in microwave oven preserved more the availability of amino acids lysine and methionine. For instance, a male child of school age (two to five years), with average weight of 15 kg, would need to ingest 25 mg kg $^{-1}$ body weight day $^{-1}$ the sum of amino acids methionine + cysteine, as recommended by the FAO / WHO (2007). Thus, he will need to consume 375 mg of these amino acids per day. Therefore, to meet the daily requirement, one must consume a portion of 16.45 g of beans submitted to Faa treatment, the portion for Caa treatment is 32.16 g of beans and the portion for FCaa treatment is 75.15 g of beans, corresponding to 100 % of the intake of legumes and amino acids, as recommended by the RDC 359, December 23, 2003 (Brazil, 2003).

Conclusions

The levels of amino acids methionine and cysteine in the control treatment (no covering), both in raw as cooked grains was zero, so, these amino acids were limiting in the protein composition of common beans analyzed. Treatments containing cassava starch in the coating showed higher concentrations of amino acids methionine and cysteine both in bean soup as in cooked bean grains. The addition of sulphur amino acids (methionine and cysteine) limiting in common bean in edible coatings can be a viable alternative for the daily ingestion of these amino acids in order to obtain protein of good quality and high biological value in the diet.

Acknowledgments

The Coordination of Improvement of Higher Education (CAPES) by the research scholarship.

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