



Amino Acid Content of Soybean Samples Collected in Different Brazilian States – Harvest 2003/2004

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ABSTRACT

Soybean is the most important protein source in animal nutrition and is widely used in poultry diets. Several factors influence the concentration of amino acids present in soybean grains cultivated in Brazil, such as climatic changes, genetics, topography, and soil fertility. Many technologies of soybean processing are used in order to eliminate or inactivate both heat-labile and heat-stable anti-nutritional factors. During processing, soybean by-products with different nutritional values are also produced. Processing conditions may as well affect the coefficients of digestibility of nutrients that are present in soybean protein sources. Furthermore, positive and negative effects of the environment may be masked by changes in processing conditions. This study aimed to compare the levels of crude protein and essential and non-essential amino acids in soybean samples collected in the states of Goiás, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Paraná, Rio Grande do Sul, and Santa Catarina during the harvest of 2003/2004 in Brazil. Measurements were made using NIRS (Near Infrared System of Reflectance Spectroscopy), and expressed on an "as-fed" basis. Soybeans sampled in different states evidenced nutritional differences. It is worth noting that samples collected in Minas Gerais, Rio Grande do Sul and Mato Grosso do Sul presented high levels of essential amino acids. The analyzed levels of essential amino acids were not always directly related to the protein concentrations of the samples. Because of the diversity of growing conditions in Brazil and worldwide, soybean produced in different environmental conditions is expected to show variable protein composition and quality, as demonstrated in the present study.

INTRODUCTION

The soybean chain should be noted among the many production chains that compose the agriculture and food system. Soybean production was estimated to account for approximately 20% of the GDP (Gross Domestic Product) of the Brazilian agribusiness (Fundação Getúlio Vargas, cited by Tavares, 2004), which corresponds to over US\$ 35 billions per year, demonstrating the economic importance of soybean in this country.

Soybean belongs to the family Leguminosae, subfamily Papilionoideae, and genus *Glycine L.* The cultivated form *Glycine max.* (L.) Merrill is used by the chemical and agricultural industries. Some final products that result from the processing of soybean might be used in the production of food, nutraceuticals and soy isoflavones, animal feeding and manufacturing of adhesives, fibers, lining, foams, and fertilizers.

The animal feed industry has become increasingly dependent on vegetable protein sources – a situation related for example to the



decrease in fish meal stocks. Besides, there has been strong international pressure, particularly by the European Union, to prohibit the use of offal meal and meat and bone meal as feedstuffs (Goldflus, 2004). More than 9.4 million tons of soybean meal were used in Brazil to produce close to 47 million tons of feed in 2005, which represents more than 61% of all types of meals used in the country during this period (Sindirações, 2005).

The chemical composition and average energy values of soybean samples are shown in Table 1 (Lima, 1999). Despite the low levels of sulfur amino acids, soybean is the main source of lysine in swine diets, and is complemented by corn in diet formulation. In addition, the significant oil content makes soybean an excellent energy source, although its crude energy is not highly metabolizable.

Soybean grains to be used in feeding should essentially have adequate nutritional profile, because plant origin strongly influences the quality of the final product – soybean meal, soybean concentrate, soybean protein isolate, or even processed full-fat soybeans. Besides, processing steps must be carefully controlled, since processing conditions may influence the composition of the final protein source.

Taking into consideration the diversity of agricultural, weather, and storage characteristics in Brazil, it is expected that soybeans produced in different regions have different nutritional composition and quality. Therefore, this study aimed to compare the amino acid profiles of soybeans grown in different Brazilian states during the harvest of 2003-2004. Besides, the present data might be used as a basis for comparison in further studies.

MATERIAL AND METHODS

A total number of 159 soybean samples were randomly collected by ADM do Brasil Ltda. in different soybean producing regions and states in Brazil. The different states and the number of soybean grain samples (n) collected each of them were: Goiás (20),

Mato Grosso (20), Mato Grosso do Sul (25), Minas Gerais (21), Paraná (17), Rio Grande do Sul (34) and Santa Catarina (21).

The cultivars were not individually identified during collection, not even if these could be genetically modified, and therefore this source of variation was not considered in the analyses.

Samples were collected between June and August 2004, immediately labeled and submitted for analysis in Centro de Apoio Nutricional (CEAN Adisseo), a Laboratory of Amino Acids Analysis in Santa Maria (State of Rio Grande do Sul).

The total amino acid contents of samples were determined using Near Infrared Reflectance Spectroscopy (NIRS). Currently, NIRS is widely used in the feed milling industry to estimate the composition of feed ingredients (Pesti *et al.*, 2005). The prediction curves were defined according to the traditional method of High Performance Liquid Chromatography (HPLC) using a significant number of soybean samples (>100). Ten amino acid profiles of the collected samples were obtained by HPLC and added to these prediction curves in order to ensure accuracy of the results. Soybean samples were ground to a 1-mm diameter particle size before analyses, according to the pre-established standard of Adisseo.

Information on the main principles of NIRS and how to perform analyses may be found elsewhere (Van Kempen and Jackson, 1996). Briefly, the bonds between organic molecules generally absorb specific frequencies (or wavelength) of light in the near infrared region Bodin and Aubret (2005). Therefore, the absorption of color in the near infrared region provides information about the organic composition of the sample. NIRS technology also enables to analyze the absorbance of different wavelengths by the sample. The resulting spectrum will provide information on the identity of the sample, based on prediction curves that are built previously using the spectra of specific samples analyzed using the reference methodology. Therefore, it is possible to calculate the amino acid content of a sample by comparison. The system uses a different

Table 1 - Analyzed mean values of nutrients in samples of soybean grains (Lima, 1999).

Nutrient	N	Mean	Nutrient	N	Mean
<i>Energy, kcal/kg (swine)</i>			<i>Protein/Amino acid, %</i>		
Crude energy	14	5155	Crude protein	40	36.63
Digestible energy	4	4025	Lysine	6	2.34
Metabolizable energy	4	3824	Methionine	5	0.47
<i>Others, %</i>			Methionine + Cystine	5	1.09
Ether extract	28	15,62	Threonine	6	1.32
Crude fiber	15	8,54	Tryptophan	6	0.45

N: number of samples.



deviation coefficient (2-7%) for each analyzed amino acid, and determines the global H value (GH) of the analyzed sample in comparison to the standard of the prediction curve. Global H indicates the mean proximity to the standard spectrum. The calculation of mean amino acid levels in the sample only considers results with GH values lower than 3.0. All analyzed soybean samples presented GH values lower than 2.2, evidencing high similarity between the samples and those used to plot NIRS curve. This also indicates high reliability in the results obtained in the present study.

The total content of the following amino acids were quantified in the soybean samples using NIRS: Glutamic Acid (Glu), Aspartic Acid (Asp), Alanine (Ala), Arginine (Arg), Phenylalanine (Phe), Glycine (Gly), Histidine (His), Isoleucine (Ile), Leucine (Leu), Lysine (Lys), Methionine (Met), Methionine and Cystine, Proline (Pro), Serine (Ser), Tyrosine (Tyr), Threonine (Thr) and Valine (Val). Crude Protein (CP) levels were also determined using this technique. Total cystine (Cys) in the samples was calculated as the difference between methionine and cystine contents. The analysis of amino acid contents in feedstuffs using NIRS allows a reduction of 50% in the amino acid content in comparison to the use of mean values of nutritional recommendation tables (Brugalli, 2002).

For statistical analysis purposes, essential amino acids (EAA) were considered the sum of arginine, phenylalanine, histidine, isoleucine, leucine, lysine, methionine, threonine, and valine. The content of tryptophan in the samples was not determined. Non-essential amino acids (NEA) comprised alanine, aspartic acid, cystine, glutamic acid, glycine, proline, serine, and tyrosine. Total amino acids (TAA) were considered as the sum of the analyzed essential and non-essential amino acids.

Statistical analyses were carried out using the software ESTAT (1995). The mean amino acid levels in soybean grains per region were compared by the test of Tukey at 1, 2 and 5% of probability.

RESULTS AND DISCUSSION

One of the factors that affect the composition and quality of feedstuffs derived from soybean processing is the nutritional value of the available grain. The soybean grain must present an adequate nutrient profile, in addition to low levels of anti-nutritional factors, in order to ensure the intrinsic value of this ingredient. Due to the diversity of climatic and soil conditions, and to the different cultivars of soybean

grains available in the large geographic region where soybean is grown in Brazil, it is assumed that soybean produced under different environmental conditions and harvested in different years present considerable variation in their composition and nutritional quality.

It is essential to understand the effects of the environment on the nutritional quality of the main agricultural commodities, and consequently, of any products derived from processing. As soybean is the primary source of crude protein in feeds, it is important to study some of the external factors affecting its protein content and quality. Westgate *et al.* (2000) demonstrated that high environmental temperatures favor protein synthesis in the soybean grain in detriment of oil production. In temperate regions, where average temperature during the grain filling phase is lower than 24°C, this negative correlation is more pronounced. Maximum oil accumulation in the soybean grain occurs at 25-28°C, according to experiments carried out by Piper & Boote (1999). Under high environmental temperatures, higher deposition of sulfur amino acids (Wolf *et al.*, 1982) and glycine (Barbour *et al.*, 1998) were observed, in addition to an increase in the ratio glycine: α -conglycinine (Barbour *et al.*, 1998).

There are several studies showing that soybean cultivars may also vary in their capacity of N₂ fixation (Bohrer & Hungria, 1998), which might affect the crude protein and amino acid contents in the grains. Burgos *et al.* (1973) compared 13 different soybean varieties and found protein digestibility coefficients varying from 66.74 to 86.09%.

Assay results were expressed on an as fed-basis. Dry matter was not determined in the present study, in order to compare the nutritional values regularly used by nutritionists in feed formulation programs. Although there are differences in the dry matter concentration of soybean samples within each country, such differences are generally quite small (Grieshop and Fahey, 2001). This similarity implies that in despite of the environmental conditions during the growing season, soybean at harvest contains a fairly constant moisture level (Grieshop and Fahey, 2001). Therefore, some differences in the contents of protein and amino acid may have been caused by differences in dry matter levels. No significant differences ($p > 0.05$) were seen in dry matter of samples collected in the states of Mato Grosso do Sul, Rio Grande do Sul, Santa Catarina, or São Paulo (Grieshop and Fahley, 2001). However, dry matter content of samples from the state of Paraná was significantly higher ($p < 0.05$) in comparison to the other states.



The individual amino acid compositions of the soybean samples collected in different states of Brazil showed significantly different amino acid mean levels ($p < 0.01$ and $p < 0.02$) (Tables 2 and 3).

It is also important to evaluate the individual results of the limiting essential amino acids in corn- and soybean-based poultry and swine feeds, i.e., methionine, lysine, and threonine.

Methionine levels in soybean samples varied among states (Table 2). The average methionine level obtained in the state of Mato Grosso do Sul, albeit not significantly different ($p > 0.05$) from that of Paraná State, was lower ($p < 0.01$) compared to other states. Besides, the means of the states Goiás, Mato Grosso, Rio Grande do Sul and Santa Catarina were higher ($p > 0.01$) than those calculated for the state of Paraná.

Soybean grown in Santa Catarina presented a significantly higher lysine level ($p < 0.01$) as compared to samples from Goiás, Mato Grosso and Paraná. On the other hand, lysine was not different ($p > 0.05$) among samples collected in Santa Catarina, Minas Gerais, Mato Grosso do Sul, and Rio Grande do Sul, as shown in Table 2.

Average threonine level in soybean harvested in Rio Grande do Sul was significantly higher ($p < 0.01$) than those from Mato Grosso do Sul, Paraná, and Santa Catarina, which were not different between each other ($p > 0.05$). Samples from Mato Grosso do Sul and Paraná presented lower average threonine levels ($p < 0.01$) as compared to Goiás, Minas Gerais, and Mato Grosso (Table 2).

Soybean importance as arginine source should not be disregarded, since arginine is a conditionally functional essential amino acid, as stated by Enting *et al.* (2005) and Machado & Fontes (2005). Therefore, according to the results presented in Table 2, despite the significantly higher arginine mean levels ($p < 0.01$) of soybean samples from Mato Grosso do Sul in comparison to the other states, it was not different ($p > 0.05$) from Goiás. The lowest arginine content ($p < 0.01$) was detected in soybean samples collected in Santa Catarina.

Unfortunately, only few studies have been carried out to compare the nutritional values of soybeans within and among different geographic regions in the world. The variation in amino acid composition of soybean

Table 2 - Mean levels of individual essential amino acids of soybean samples collected in different Brazilian states¹.

State/ Nutrient, %	Arg	Phe	His	Ile	Leu	Lys	Met	Thr	Val
GO	2.786 ^{cd}	1.880 ^a	0.972 ^{abc}	1.574 ^{ab}	2.798 ^a	2.275 ^a	0.410 ^c	1.323 ^{bc}	1.661 ^{ab}
MG	2.661 ^b	1.950 ^c	0.975 ^c	1.625 ^{cd}	2.868 ^c	2.305 ^{abc}	0.402 ^{bc}	1.339 ^{bc}	1.695 ^c
MS	2.867 ^d	1.904 ^{bc}	0.971 ^{bc}	1.561 ^a	2.820 ^{ab}	2.321 ^{bc}	0.375 ^a	1.274 ^a	1.670 ^{bc}
MT	2.669 ^b	1.925 ^{bc}	0.966 ^a	1.605 ^{bc}	2.831 ^{abc}	2.299 ^a	0.412 ^c	1.328 ^{bc}	1.650 ^{ab}
PR	2.670 ^b	1.896 ^{ab}	0.953 ^a	1.575 ^{ab}	2.806 ^{ab}	2.281 ^a	0.386 ^{ab}	1.256 ^a	1.628 ^a
RS	2.735 ^{bc}	1.953 ^c	0.977 ^c	1.635 ^d	2.869 ^c	2.330 ^{bc}	0.420 ^c	1.355 ^c	1.680 ^{bc}
SC	2.499 ^a	1.952 ^c	0.955 ^{ab}	1.647 ^d	2.848 ^{bc}	2.339 ^c	0.421 ^c	1.303 ^{ab}	1.628 ^a
Mean, %	2.706	1.926	0.968	1.606	2.838	2.310	0.405	1.315	1.662
Standard deviation	0.144	0.050	0.020	0.047	0.053	0.046	0.030	0.063	0.042
CV, %	5.323	2.576	2.088	2.916	1.887	1.991	7.326	4.762	2.538
SEM	0.011	3.934	1.603	3.713	4.246	3.648	2.352	4.967	3.345

Mean: overall mean (%); CV: coefficient of variation (%); SEM: standard error of the mean; Means followed by different letters in the column are significantly different ($p < 0.01$) by the test of Tukey; ¹Levels are expressed on as-fed basis.

Table 3 - Mean levels of individual non-essential amino acids of soybean samples collected in different Brazilian states¹.

State/ Amino acid, %	Ala [*]	Asp ^{**}	Cys [*]	Glu [*]	Gly ^{**}	Pro ^{**}	Ser [*]	Tyr [*]
GO	1.690 ^{bcd}	4.387 ^{ab}	0.4133 ^{abc}	5.951 ^a	1.666 ^{bc}	1.970 ^{cd}	2.065 ^b	1.345 ^b
MG	1.716 ^{de}	4.4278 ^{ab}	0.3933 ^{ab}	6.479 ^b	1.628 ^{ab}	1.968 ^{cd}	1.974 ^a	1.330 ^{ab}
MS	1.728 ^e	4.814 ^c	0.3992 ^{ab}	6.492 ^b	1.681 ^{cd}	1.991 ^d	2.027 ^{ab}	1.378 ^c
MT	1.674 ^{bc}	4.345 ^a	0.3880 ^a	6.356 ^{ab}	1.648 ^{bc}	1.937 ^{bc}	2.048 ^{ab}	1.342 ^b
PR	1.663 ^b	4.552 ^b	0.4035 ^{abc}	5.936 ^a	1.669 ^{bcd}	1.927 ^b	1.972 ^a	1.325 ^{ab}
RS	1.700 ^{cde}	4.405 ^{ab}	0.4159 ^{bc}	6.535 ^b	1.705 ^d	1.981 ^d	2.054 ^b	1.347 ^b
SC	1.598 ^a	4.331 ^a	0.4276 ^c	6.572 ^b	1.592 ^a	1.827 ^a	2.029 ^{ab}	1.307 ^a
Mean	1.685	4.469	0.407	6.362	1.660	1.948	2.028	1.341
Standard deviation	0.053	0.234	0.034	0.496	0.056	0.065	0.085	0.034
CV, %	3.178	5.243	8.261	7.791	3.400	3.355	4.214	2.599
SEM	4.246	0.019	2.664	0.039	4.477	5.183	6.777	2.764

Mean: overall mean (%); CV: coefficient of variation (%); SEM: standard error of the mean; *Means followed by different letters in the column are significantly different ($p < 0.01$) by the test of Tukey; **Means followed by different letters in the column are significantly different ($p < 0.02$) by the test of Tukey; ¹Levels expressed on an as-fed basis.



samples between the states, and the resulting soybean meal and heated full-fat soybean, might be explained by the conjunction of different factors

The mean individual levels of non-essential amino acids are shown in Table 3, although these have relatively low importance in exogenous animal nutrition.

As demonstrated in Figures 1, 2, and 3, the total content of amino acids does not necessarily present an increasing linear response as the protein content increases in the soybean grain. In applied nutrition, the amino acid profiles of de-activated full-fat soybeans and soybean meals are often inaccurately corrected using direct correlations with the analyzed crude protein contents.

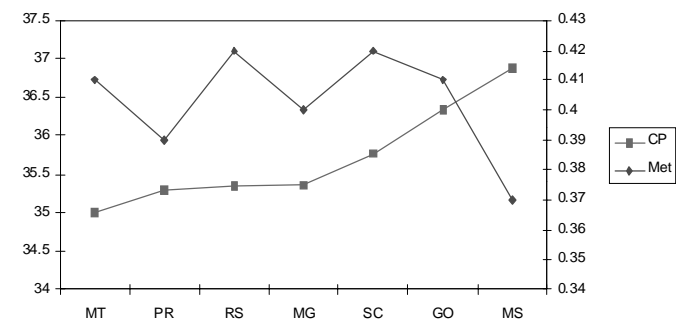


Figure 1 - Crude protein and methionine levels in soybean samples.

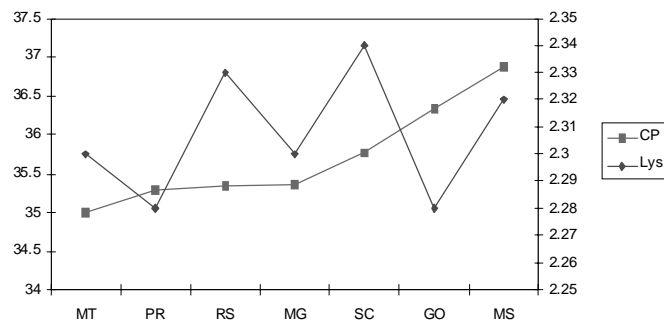


Figure 2 - Crude protein and lysine levels in soybean samples.

The average protein content of the sampled soybeans is shown in Table 4. Soybean sampled in Mato Grosso do Sul showed non-significantly higher ($p>0.05$) protein than those of other states, except from Goiás ($p<0.05$). Average crude protein level of Mato Grosso samples was significantly lower ($p<0.05$) in comparison to samples from Goiás and Santa Catarina, which were not different from each other ($p>0.05$). Nevertheless, this information is of little practical importance since most diets are formulated to supply amino acid needs and not protein needs. Mieth *et al.* (1988) reported

differences in the crude protein contents of different soybean cultivars grown in Central Europe. Although Grieshop and Fahey (2001) have not detected any statistical differences ($p>0.05$) in the crude protein concentrations of soybean sampled in China and the USA, soybeans from the latter have been reported to have higher contents of most amino acids in comparison to samples from Brazil or China.

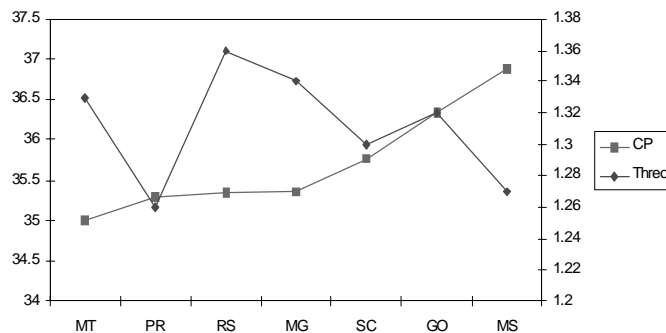


Figure 3 - Crude protein and Threonine levels in soybean samples.

Table 4 - Mean values of crude protein (CP), and essential (EAA), non-essential (NEA), and total (TAA) amino acids of soybean samples collected in different Brazilian states¹.

State/Nutrient, %	CP*	EAA**	NEA**	TAA*
GO	36.33 ^{cd}	15.680 ^{ab}	19.487 ^a	35.168 ^{ab}
MG	35.36 ^{ab}	15.821 ^{bc}	19.917 ^{ab}	35.738 ^{bc}
MS	36.89 ^d	15.764 ^{bc}	20.510 ^c	36.274 ^c
MT	34.99 ^a	15.688 ^{ab}	19.742 ^{ab}	35.431 ^{ab}
PR	35.21 ^{ab}	15.453 ^a	19.447 ^a	34.899 ^a
RS	35.33 ^{ab}	15.957 ^c	20.144 ^{bc}	36.101 ^c
SC	35.76 ^{bc}	15.593 ^{ab}	19.685 ^{ab}	35.278 ^{b^a}
Mean, %	35.71	15.737	19.899	35.410
Standard deviation	0.8944	0.329	0.665	0.944
CV, %	2.5046	2.088	3.341	2.620
SEM	-	0.026	0.053	0.075

Mean: overall mean (%); CV: coefficient of variation (%); SEM: standard error of the mean; *Means followed by different letters in the column are significantly different ($p<0.01$) by the test of Tukey; **Means followed by different letters in the column are significantly different ($p<.02$) by the test of Tukey; ¹Levels expressed on an as-fed basis.

Although the crude protein content of the samples collected in Rio Grande do Sul was significantly lower ($p<0.05$) than those of Goiás and Mato Grosso do Sul, it was similar to other states; the mean total essential amino acid content was higher than those from Goiás, Mato Grosso, Paraná, and Santa Catarina (Table 4). The higher quality of soybean sampled in Rio Grande do Sul may also be observed in Table 3: in addition to higher concentration of essential amino acids in general, there were considerable levels of amino acids considered essential in swine and poultry feeding.



Moreover, the levels were often above the average. On the other hand, soybean sampled in Paraná not only presented lower essential amino acid levels ($p < 0.01$) compared to Minas Gerais and Mato Grosso do Sul, but lower levels of the most important essential amino acids. In comparison, Grieshop and Fahey (2001) have reported that soybeans from Mato Grosso do Sul, Rio Grande do Sul and São Paulo showed greater concentrations of crude protein than samples from Santa Catarina. Only soybeans from Paraná presented lower essential amino acid content in comparison to five other Brazilian states, i.e., Mato Grosso do Sul, Paraná, Rio Grande do Sul, Santa Catarina and São Paulo. However, soybeans from Paraná have also shown lower levels of total essential, total non-essential and total amino acids compared to soybeans from other Brazilian states.

It is worth noting that the content of non-essential amino acids, in spite of being less important to animal nutrition, has markedly contributed to the total sum of amino acids as compared to the essential amino acids (Table 4).

No significant differences were detected ($p > 0.05$) in the contents of total amino acids between samples from Mato Grosso do Sul and Rio Grande do Sul, but these were higher ($p < 0.01$) than those found in soybeans from Goiás, Mato Grosso, Paraná, and Santa Catarina. The average value of total amino acids obtained in soybeans from Minas Gerais was not different from that of the states of Mato Grosso do Sul and Rio Grande do Sul.

It is not possible to compare the present data with the national nutritional tables of foodstuffs, because the present samples have not been heat treated, contrarily to the data presented in such tables.

CONCLUSION

It was concluded that soybean grains sampled in different Brazilian states during the harvest of 2003/2004 showed significant differences in regard to their content of crude protein, essential amino acids and non-essential amino acids. Although soybeans from Minas Gerais and Rio Grande do Sul have shown low average crude protein values, their individual essential amino acid levels have markedly influenced the total amino acid content of the protein. The samples derived from Mato Grosso are also highlighted. Amino acid profiles may be different in soybeans cultivated in different production regions. Other factors, such as weather conditions, typical characteristics of the

sampled soybean cultivars, and agricultural practices possibly affect soybean nutritional values. Information such as that generated in this experiment is indispensable, due to the importance of international soybean trading. Therefore, further analytical studies are needed to determine if the different Brazilian geographic regions have real and specific influences on the nutritional quality of soybeans in different agricultural years. A database comprising the samples from these different regions and in different years might be created to support a discussion on this issue, and thus research in vegetal physiology. On the other hand, it is possible to actually determine the amino acid composition of each sample efficiently.

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Adquira já!

Livro

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