Revista Ciência Agronômica, v. 53, e20218048, 2022
Centro de Ciências Agrárias - Universidade Federal do Ceará, Fortaleza, CE
www.ccarevista.ufc.br

Scientific Article
ISSN 1806-6690

Potential of cowpea genotypes for nutrient biofortification and cooking quality[1]

Potencial de genótipos de feijão-caupi para biofortificação de nutrientes e qualidade de cozimento

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ABSTRACT - Cowpea is a very important food for the populations of the North and Northeast regions of Brazil, representing an excellent source of proteins and minerals. The purpose of this study was to evaluate the potential of 100 cowpea genotypes for biofortification of iron, zinc, and proteins, and cooking quality of the grain. The iron and zinc contents were analyzed by x-ray fluorescence spectrometry; protein content was based on nitrogen determination, using the Kjeldahl method; and cooking quality was assessed using an electric pressure cooker and Mattson cooker. The superiority of genotypes for iron, zinc, proteins, and cooking quality was carried out using the nutritional quality and cooking index. The iron content ranged from 3.58 to 6.06 mg 100 g^{-1}, with an overall average of 4.66 mg 100 g^{-1}, while the zinc content between 2.35 and 4.57 mg 100 g^{-1} and average of 3.31 mg 100 g^{-1}. Protein range ranged from 20.82 to 26.92 g 100 g^{-1} and an average of 24.30 g 100 g^{-1}. The percentage of cooked grains ranged from 20 to 98%, with an average of 68.7%. The line MNC11-1023E-28 has the best profile of nutritional and cooking quality, showing potential as a food to meet consumer demands and reverse iron and zinc deficiency in the Brazilian population.

Key words: *Vigna unguiculata*. Protein. Iron. Zinc. Percentage of cooked grains.

RESUMO - O feijão-caupi é um alimento muito importante para as populações das regiões Norte e Nordeste do Brasil, representando uma excelente fonte de proteínas e minerais. O objetivo deste estudo foi avaliar o potencial de 100 genótipos de feijão-caupi para biofortificação de ferro, zinco e proteína, e qualidade de cozimento. Os teores de ferro e zinco foram analisados por espectrometria de fluorescência de raios X; o conteúdo de proteínas foi baseado na determinação de nitrogênio, pelo método de Kjeldahl; e a qualidade de cozimento foi avaliada com uso de panela de pressão elétrica e cozedor de Mattson. A superioridade dos genótipos para ferro, zinco, proteínas e qualidade de cozimento foi realizada utilizando-se o índice de qualidade nutricional e de cozimento. O teor de ferro variou de 3,58 a 6,06 mg 100 g^{-1}, com uma média geral de 4,66 mg 100 g^{-1}, enquanto o teor de zinco entre 2,35 e 4,57 mg 100 g^{-1} e média de 3,31 mg 100 g^{-1}. O ter de proteínas apresentou amplitude de 20,82 a 26,92 g 100 g^{-1} e média de 24,30 g 100 g^{-1}. A porcentagem de grãos cozidos variou de 20 a 98%, com média de 68,7%. Os grãos da linhagem MNC11-1023E-28 apresentam melhor perfil de qualidade nutricional e de cocção, apresentando potencial como um alimento para atender às demandas dos consumidores e reverter a deficiência de ferro, zinco e proteínas na população brasileira.

Palavras-chave: *Vigna unguiculata*. Proteína. Ferro. Zinco. Porcentagem de grãos cozidos.

DOI: 10.5935/1806-6690.20220040
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Received for publication on 05/06/2021; approved on 29/10/2021

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INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is a legume widely grown in Asia, the Americas and Africa due to the high genetic variability for agronomic, nutritional, and culinary characteristics (MAZIERO; RIBEIRO; STORCK, 2015; MOHAMMED; JAISWAL; DAKORA, 2018). It is a rustic crop due to its resistance to heat and drought, as well as its ability to grow in soils with low organic matter and fertility (IQBAL *et al*., 2018; SILVA *et al*., 2018).

Brazil is the third largest producer of cowpea in the world. In the 2019/2020 agricultural year, the crop occupied an area of 1,307,800 ha, with a production of 712,600 tons and a yield of 545 kg ha^{-1} (COMPANHIA NACIONAL DE ABASTECIMENTO, 2020). It presents grains rich in nutrients that can be used in the population's diet, avoiding deficiencies caused by the lack of minerals, such as iron and zinc (DIAS-BARBOSA *et al*., 2021). In the Northeast region, about 25 million people, consume this culture, mainly to obtain proteins and minerals, such as iron and zinc, in substitution to the high-cost sources of animal protein.

Iron and zinc are essential micronutrients in the functioning of human metabolism and their deficiency can cause malnutrition or hidden hunger, which has a negative impact on health, affecting more than 2 billion people worldwide (FOOD AND AGRICULTURE ORGANIZATION OF UNITED NATIONS, 2019; LÓPEZ-MORALES *et al*., 2020; SILVA *et al*., 2021). Iron is a component of blood hemoglobin with a role in the transport of oxygen and its deficiency can lead to diseases such as anemia, while zinc is an essential mineral for increasing immunity against diseases and its deficiency has been related to many health problems (AYENI; IKWEBE; ONYEZILI, 2018; VAN DER STRAETEN *et al*., 2020).

Recent studies have revealed that the zinc concentration in cowpea grains is higher than that found in common bean grains (COELHO *et al*., 2021; GERRANO *et al*., 2019) and that there is a great genetic variability for the protein, iron, and zinc content in the cowpea germplasm grain (CARVALHO *et al*., 2012; DIAS-BARBOSA *et al*., 2021; SANTOS; BOITEUX, 2015; WENG *et al*., 2019). The determination of the iron and zinc contents in the grains of cowpea genotypes provide information that supports the selection and development of biofortified cultivars for these micro minerals (DIAS-BARBOSA *et al*., 2020).

In this sense, EMBRAPA, through the BioFort and HarvestPlus programs, has developed biofortified cowpea cultivars (BRS Aracê, BRS Tumucumaque, and BRS Xiquexique), which have high levels of iron and zinc in the grains (ROCHA; DAMASCENO-SILVA; MENEZES-JÚNIOR, 2017). The grains of biofortified cowpea cultivars have iron and zinc contents higher than 6.00 and 4.00 mg 100 g^{-1}, respectively, while conventional cultivars presented lower contents for both micronutrients (FREIRE-FILHO, 2011). The new cowpea cultivars biofortified are a potential vehicle for improving the iron and zinc status in groups in which the micronutrient deficiency is prevalent (COELHO *et al*., 2021).

The incorporation of cooking quality in the evaluation of cultivars has contributed to improve culinary quality and consumer acceptance (CARVALHO *et al*., 2017). A long cooking time of cowpea leads to loss of nutrients, loss of useful time and greater energy expenditure (gas or firewood), whereas fast cooking has the potential to provide a highly nutritious food in less preparation time and less energy expenditure (ADDY *et al*., 2020; ROCHA; DAMACENO-SILVA; MENEZES-JÚNIOR, 2017).

In order to identify a food cowpea with best nutritional and cooking attributes to serve the consumer, the purpose of this study was to evaluate the potential of cowpea genotypes for iron, zinc, and protein biofortification and cooking quality.

MATERIAL AND METHODS

Raw material origin

Grain samples from 100 cowpea genotypes were provided by Embrapa Meio-Norte, in Teresina, PI, Brazil, from a cultivation carried out under irrigated conditions from April to June 2019.

Sample preparation

After the cultivation and harvesting of the assay, samples of grains from each genotype were taken at random, packed in plastic bags and then kept in a refrigerator until the time of analysis. Grain samples of 15 grams were ground in a zirconium ball mill and the resulting flour was used in the analysis of protein, iron, and zinc contents, carried out in triplicate. For the evaluation of cooking quality, samples of 50 grains per genotype were used, conducted in duplicate.

Laboratory analysis

Protein and cooking quality analyzes were carried out at the Bromatology Laboratory of Embrapa Meio-Norte, in Teresina, PI, Brazil, while analyzes of iron and zinc contents were conducted at the Physical-Chemical Analysis and Minerals Laboratory of Embrapa Agroindústria de Alimentos, in Rio de Janeiro, RJ, Brazil.

Experimental design

A completely randomized block design was adopted, with three replications for protein, iron, and zinc contents. For the analysis of the percentage of cooked grains, only two replications were used, due to the limited space of the electric pressure cooker used in the first step of evaluation of the cooking quality.

Analysis of protein content

The protein content was based on nitrogen determination, by Kjeldahl method, using the conversion factor 6.25 (ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS, 2008). About 0.2 g of the sample was weighed on parchment paper and then the sample was transferred to digestion tubes. The amount of 5 mL of concentrated sulfuric acid and 2 g of catalytic mixture (96% of potassium sulphate and 4% of copper sulphate) were added, followed by heating in a digesting block at a temperature of 400 °C, until the solution become blue-green, free of undigested material (black dots). After cooling the tubes, the amount of 10 mL of distilled water was added and the tube was coupled to the distiller. The amount of 10 mL of boric acid solution and indicators were added and coupled In an Erlenmeyer to collect the distillate.

From a 50% sodium hydroxide solution, the amount of 10 mL was removed and added to the flask with the digested sample, using a funnel with a tap until a slight excess of base was guaranteed. After boiling, distillation took place until 100 mL of the distillate was obtained. Then, the distilled solution was titrated with 0.02 N hydrochloric acid of known factor.

Analysis of iron and zinc contents

The iron and zinc contents were determined using the technique of x-ray fluorescence spectrometry (XRF). The analyzes were performed on the XRF equipment (S2 Ranger Bruker). A sample of flour from each genotype was used for the analyzes. Before starting the analyzes, the XRF equipment was checked for calibration, where the FLX-K04 (BAX) and FLX-C3 standards were placed in the sampler positions E5 and F5, respectively, in the sample chamber.

The samples were placed in a sample holder, previously prepared using plastic film stretched over them, which were filled with the samples up to half. The sample holders were then placed in the sample chamber, in positions according to the identifications entered in the equipment's control panel. The reading of the iron and zinc contents was performed by the XRF spectrophotometer, and the results were accessed via the Spectra EDX Launcher software from the computer associated with the equipment.

Cooking quality evaluation

The evaluation of cooking quality was carried out using the methodology proposed by Carvalho *et al*. (2017), with adaptations for cowpea. Two samples of 50 grains of each genotype without mechanical damage were placed in organza bags and identified. Two bags were prepared per genotype, that is, two replications.

The bags were placed in distilled water for 60 minutes. Later, for cooking, the bags were placed at the bottom of an electric pressure cooker (Eletrolux) with a capacity of 5 L. The water level used was 3/5 of the pot's capacity; keeping the water in which the bags were soaked. The grains were cooked for 30 minutes. After that, the samples were immediately removed from the pot and the grains placed on a counter for cooling for five minutes.

The evaluation of the percentage of cooked grains was carried out with the aid of the Mattson cooker (MATTSON, 1946). Twenty-five grains per sample were used, chosen at random and the pins placed on the grains. The number of pins that immediately drilled through the grains were recorded. The higher the percentage of grains with fully perforated pins, the higher the cooking quality. The sauce and cooking times were pre-established in preliminary tests.

Estimation of nutritional and cooking quality index

The superiority of cowpea genotypes for iron, zinc, and proteins contents and cooking quality was performed using the nutritional and cooking quality index (NCQI), which was based on the nutritional quality index adopted by Carvalho *et al.* (2012), with some modifications. It was established a minimum value that the genotype should have to be selected, which in the case of the present work was the general average of each characteristic; larger quantities than the general average counted positively, while smaller quantities than the general average counted negatively.

The NCQI was calculated by multiplying each value in excess (above the general average) or scarcity (below the general average) by the respective arbitrary weight. The following weights were adopted for the characteristics according to the nutritional and culinary importance of the cowpea: 4 for the protein content; 3 for iron and zinc content; and 2 for cooking quality; these were positive because they are characteristics that seek to increase the genetic improvement of the cowpea grain. The algebraic sum of each term (product between the maximum or excess and the respective weight of each characteristic) was then divided by the sum of the weights, according to the following equation 1:

$$NCQI_i = \frac{\Sigma\left[\left(dvm_i \ x \ p_i\right)\right] + \ldots + \left[\left(dvm_n \ x \ p_n\right)\right]}{P_i \ + \ldots + P_n} \tag{1}$$

where $NCQI_i$ is the nutritional and cooking quality of the i-th genotype; dvm_i is the difference between the mean of the i-th genotype and the general mean for the n-th characteristic; and p_i is the arbitrary weight adopted for the n-th characteristic. The higher the NCQI, the better the genotype in terms of nutritional and cooking quality.

Statistical analysis

Data were analyzed according to a completely randomized block design, with three replications for iron, zinc and protein content and two replications for the percentage of cooked grains. Data were subjected to analysis of variances and the averages were grouped by the Scott-Knott test at the level of 5% probability. Statistical analyzes were performed using the SAS software (STATISTICAL ANALYSIS SYSTEM, 2011).

RESULTS AND DISCUSSION

The analysis of variance of the characteristics iron, zinc, and protein contents, and the percentage of cooked grains (cooking quality) is shown in Table 1.

The genotypes showed statistically significant differences ($p < 0.01$) for all characteristics evaluated (Table 1), showing variability between genotypes for the nutritional and cooking quality in the grain. Dias-Barbosa *et al.* (2021), evaluating 33 cowpea genotypes, also found genetic variability for the iron, zinc, and proteins contents and cooking time.

The mean and standard deviation of the genotypes studied for the iron, zinc, and proteins contents and the percentage of cooked grains are shown in Table 2.

Iron content

The iron content varied from 3.58 to 6.06 mg 100 g^{-1}, with an overall average of 4.66 mg 100 g^{-1} (Table 2). Similar values were reported by Pereira *et al.* (2014), who assessed the bioaccessibility of iron and zinc in cowpea cultivars, found that the iron content ranged from 5.8 mg 100 g^{-1} to 6.4 mg 100 g^{-1}. Gunathilake, Herath and Wansapala (2016) found values for the iron content between 2.26 mg and 3.54 mg 100 g^{-1} in a cowpea cultivar in Sri Lanka, contents lower than those found in the present work. In a study with mutant cowpea cultivars carried out in India, Raina *et al.* (2020) found higher contents between 8.17 and 9.32 mg 100 g^{-1}.

Table 1 - Summary of the analysis of variance of the characteristics iron content, zinc content, and protein contents, and the percentage of cooked grains of 100 cowpea genotypes

Variation sources	Degrees of freedom	Medium squares			
		Iron content (mg 100 g^{-1})	Zinc content (mg 100 g^{-1})	Protein content (g 100 g^{-1})	Percentage of cooked grains (%)
Genotypes	99	82.96**	67.84**	6.09**	1096.00**
Error		1.96	0.28	0.37	27.26
Variation coefficient (%)		3.00	1.61	2.52	7.56

** Significant at 1% probability by the F-test

Table 2 - Means and standard deviation (SD) for the iron content, zinc content, and protein content and the percentage of cooked grains of 100 cowpea genotypes

Genotype	Iron content (mg 100 g^{-1}) ± SD	Zinc content (mg 100 g^{-1}) ± SD	Protein content (g 100 g^{-1}) ± SD	Percentage of cooked grains (%)
MNC11-1005E-20	4.97 ± 0.75 f	2.87 ± 0.36 p	23.94 ± 1.04 c	98 ± 2.83 a
MNC11-1005E-28	4.45 ± 0.61 h	3.09 ± 0.40 n	25.78 ± 0.57 b	96 ± 5.66 a
MNC11-1005E-37	3.79 ± 1.33 j	2.71 ± 0.72 r	26.60 ± 0.10 a	92 ± 0.00 a
MNC11-1006E-10	3.58 ± 1.52 k	2.69 ± 0.84 r	23.72 ± 0.60 d	98 ± 2.83 a
MNC11-1008E-9	4.46 ± 2.82 h	2.82 ± 0.46 q	24.68 ± 0.26 c	94 ± 2.83 a
MNC11-1012E-7	4.69 ± 0.61 g	3.21 ± 0.21 m	23.66 ± 0.03 d	90 ± 2.83 a
MNC11-1013E-18	4.83 ± 1.25 f	3.89 ± 0.87 f	23.32 ± 0.24 d	94 ± 2.83 a
MNC11-1013E-33	4.82 ± 1.14 f	3.59 ± 0.53 i	24.42 ± 0.23 c	27 ± 9.90 g
MNC11-1013E-27	4.12 ± 0.70 i	2.70 ± 0.36 r	26.05 ± 0.23 b	96 ± 0.00 a
MNC11-1013E-8	4.65 ± 1.07 g	2.90 ± 0.00 p	24.30 ± 0.34 c	98 ± 2.83 a
MNC11-1013E-16	4.66 ± 1.48 g	3.49 ± 0.44 j	24.55 ± 0.59 c	88 ± 0.00 a
MNC11-1013E-25	4.19 ± 2.00 i	2.88 ± 0.21 p	24.31 ± 0.08 c	92 ± 5.66 a
MNC11-1015E-2	5.06 ± 1.26 e	2.84 ± 0.25 p	22.70 ± 0.08 e	94 ± 2.83 a
MNC11-1015E-5	4.50 ± 1.05 h	3.29 ± 0.23l	20.90 ± 1.35 f	60 ± 5.66 d

Continuation Table 2

MNC11-1015E-7	4.35 ± 0.50 h	2.97 ± 0.36º	23.62 ± 0.23 d	98 ± 2.83 a
MNC11-1015E-15	4.88 ± 1.72 f	3.88 ± 0.60 f	25.10 ± 0.17 c	76 ± 5.66 c
MNC11-1015E-28	4.98 ± 0.75 f	3.55 ± 0.67 i	23.43 ± 0.05 d	68 ± 0.00 c
MNC11-1015E-29	4.10 ± 0.25 i	2.63 ± 0.20 s	23.71 ± 0.13 d	66 ± 2.83 d
MNC11-1015E-35	4.59 ± 1.81 g	3.41 ± 0.56 k	23.61 ± 1.46 d	88 ± 5.66 a
MNC11-1016E-12	4.43 ± 1.05 h	3.66 ± 0.17 h	24.31 ± 0.31 c	96 ± 0.00 a
MNC11-1016E-16	5.33 ± 1.40 d	3.73 ± 0.64 g	24.52 ± 0.34 c	72 ± 5.66 c
MNC11-1017E-3	4.54 ± 0.38 h	2.85 ± 0.78 p	24.02 ± 0.36 c	46 ± 2.83 e
MNC11-1017E-8	4.44 ± 2.21 h	2.96 ± 0.80º	24.64 ± 0.61 c	82 ± 2.83 b
MNC11-1017E-10	3.84 ± 0.35 j	2.35 ± 0.31 u	22.65 ± 0.31 e	94 ± 2.83 a
MNC11-1017E-26	4.09 ± 0.75 i	3.13 ± 0.53 n	24.99 ± 0.15 c	86 ± 2.83 b
MNC11-1017E-30	4.34 ± 1.44 h	2.52 ± 0.50 t	24.16 ± 0.39 c	46 ± 2.83 e
MNC11-1017E-31	4.68 ± 1.80 g	3.20 ± 0.36 m	24.30 ± 0.10 c	76 ± 5.66 c
MNC11-1017E-33	4.41 ± 1.25 h	3.09 ± 1.02 n	22.70 ± 0.09 e	94 ± 2.83 a
MNC11-1017E-37	3.69 ± 0.84 k	2.87 ± 0.55 p	26.22 ± 0.82 a	86 ± 2.83 b
MNC11-1018E-2	3.77 ± 1.53 j	3.26 ± 0.42l	23.07 ± 0.07 d	50 ± 2.83 e
MNC11-1018E-4	4.17 ± 1.39 i	2.89 ± 0.65 p	22.79 ± 0.20 e	96 ± 0.00 a
MNC11-1018E-17	5.02 ± 1.11 f	3.32 ± 0.70l	22.48 ± 0.23 e	56 ± 5.66 d
MNC11-1018E-20	4.63 ± 0.90 g	2.86 ± 0.21 p	25.79 ± 0.65 b	62 ± 2.83 d
MNC11-1019E-8	4.72 ± 1.21 g	3.62 ± 0.20 h	24.59 ± 0.14 c	84 ± 5.66 b
MNC11-1019E-12	4.28 ± 0.45 i	3.51 ± 0.35 j	24.75 ± 0.09 c	64 ± 5.66 d
MNC11-1019E-15	5.29 ± 1.17 e	3.51 ± 0.64 j	26.27 ± 0.36 a	82 ± 2.83 b
MNC11-1019E-16	4.31 ± 1.66 h	3.01 ± 0.25º	23.08 ± 0.71 d	90 ± 2.83 a
MNC11-1019E-40	4.45 ± 1.21 h	3.23 ± 0.56 m	25.86 ± 1.35 b	38 ± 2.83 f
MNC11-1019E-46	4.65 ± 0.60 g	3.25 ± 0.32l	26.24 ± 0.35 a	45 ± 7.07 e
MNC11-1020E-29	4.76 ± 0.15 g	3.09 ± 0.65 n	26.42 ± 0.34 a	90 ± 8.49 a
MNC11-1020E-18	4.65 ± 1.05 g	3.13 ± 0.30 n	24.30 ± 0.66 c	28 ± 5.66 g
MNC11-1020E-16	4.13 ± 2.00 i	2.77 ± 0.61 q	23.55 ± 0.39 d	86 ± 2.83 b
MNC11-1020E-5	5.14 ± 1.37 e	3.17 ± 0.67 m	23.29 ± 0.39 d	60 ± 5.66 d
MNC11-1020E-36	4.56 ± 0.23 g	4.01 ± 0.21 e	24.72 ± 0.50 c	84 ± 5.66 b
MNC11-1020E-6	4.32 ± 1.11 h	3.08 ± 0.46 n	24.30 ± 0.12 c	56 ± 5.66 d
MNC11-1021E-27	4.68 ± 1.31 g	3.30 ± 0.65l	25.40 ± 0.12 b	60 ± 5.66 d
MNC11-1021E-17	4.18 ± 0.60 i	2.78 ± 0.38 q	24.77 ± 0.14 c	78 ± 2.83 c
MNC11-1022E-1	4.66 ± 1.68 g	3.40 ± 0.61 k	24.11 ± 0.10 c	40 ± 5.66 f
MNC11-1022E-9	5.36 ± 0.95 d	4.57 ± 1.05 a	25.63 ± 0.41 b	49 ± 9.90 e
MNC11-1022E-58	4.91 ± 2.03 f	4.26 ± 0.47 d	26.92 ± 0.11 a	66 ± 8.49 d
MNC11-1023E-28	6.06 ± 2.84 a	3.45 ± 1.18 j	25.67 ± 0.06 b	92 ± 5.66 a
MNC11-1023E-60	4.63 ± 1.76 g	3.23 ± 0.38 m	24.82 ± 0.41 c	94 ± 2.83 a
MNC11-1023E-48	4.77 ± 1.50 g	3.40 ± 0.17 k	24.47 ± 1.39 c	75 ± 7.07 c
MNC11-1023E-26	4.48 ± 0.59 h	3.37 ± 0.15 k	23.16 ± 1.51 d	84 ± 5.66 b
MNC11-1024E-18	4.05 ± 1.50 i	2.51 ± 0.31 t	22.35 ± 1.26 e	28 ± 5.66 g
MNC11-1024E-1	5.39 ± 1.59 d	3.43 ± 0.70 k	24.10 ± 0.80 c	24 ± 5.66 g
MNC11-1024E-16	4.14 ± 1.64 i	3.16 ± 0.36 m	26.72 ± 0.08 a	24 ± 5.66 a
MNC11-1026E-15	5.37 ± 2.95 d	3.74 ± 0.35 g	24.97 ± 0.63 c	90 ± 8.49 a
MNC11-1026E-5	3.91 ± 1.18 j	2.89 ± 0.20 p	25.53 ± 0.60 b	52 ± 5.66 e
MNC11-1026E-19	5.57 ± 0.67 c	4.14 ± 0.60 d	23.09 ± 0.81 d	52 ± 5.66 e
MNC11-1028E-16	5.26 ± 0.64 e	3.40 ± 0.68 k	24.75 ± 0.14 c	94 ± 2.83 a

Continuation Table 2

MNC11-1028E-34	4.92 ± 1.16 f	3.54 ± 0.50 i	23.24 ± 0.88 d	94 ± 2.83 a
MNC11-1028E-95	4.43 ± 1.91 h	3.23 ± 0.42 m	24.71 ± 0.83 c	86 ± 2.83 b
MNC11-1029E-9	4.80 ± 1.71 f	3.53 ± 0.25 i	25.96 ± 0.14 b	40 ± 0.00 f
MNC11-1029E-13	5.45 ± 0.55 d	3.80 ± 0.26 g	25.54 ± 0.44 b	34 ± 8.49 f
MNC11-1029E-15	4.72 ± 1.54 g	3.44 ± 0.35 j	23.04 ± 0.04 d	82 ± 2.83 b
MNC11-1031E-5	4.64 ± 0.97 g	2.60 ± 0.32 s	24.88 ± 0.24 c	30 ± 8.49 g
MNC11-1031E-8	4.99 ± 0.61 f	2.80 ± 0.61 q	23.31 ± 0.55 d	51 ± 7.07 e
MNC11-1031E-9	4.14 ± 1.39 i	3.11 ± 0.56 n	22.47 ± 0.16 e	90 ± 8.49 a
MNC11-1031E-11	4.86 ± 0.57 f	4.06 ± 1.03 e	23.37 ± 0.23 d	45 ± 7.07 e
MNC11-1031E-13	4.41 ± 0.96 h	3.79 ± 0.31 g	23.60 ± 0.05 d	34 ± 2.83 f
MNC11-1031E-15	5.76 ± 1.22 b	3.54 ± 0.35 i	22.69 ± 0.49 e	86 ± 2.83 b
MNC11-1033E-14	4.69 ± 2.70 g	2.63 ± 0.46 s	24.34 ± 0.32 c	47 ± 7.07 e
MNC11-1033E-30	5.77 ± 1.15 b	3.10 ± 0.66 n	24.91 ± 0.55 c	92 ± 5.66 a
MNC11-1034E-1	4.55 ± 1.45 h	2.99 ± 0.25º	25.70 ± 0.60 b	98 ± 2.83 a
MNC11-1034E-2	5.57 ± 1.13 c	3.39 ± 0.06 k	25.70 ± 0.36 b	31 ± 7.07 g
MNC11-1036E-3	4.46 ± 0.56 h	3.23 ± 0.47 m	25.48 ± 0.18 b	34 ± 8.49 f
MNC11-1036E-4	4.53 ± 0.10 h	3.09 ± 0.91 n	23.87 ± 2.21 d	26 ± 2.83 g
MNC11-1036E-5	4.85 ± 1.82 f	3.56 ± 0.21 i	24.33 ± 0.18 c	58 ± 2.83 d
MNC11-1037E-1	4.24 ± 1.72 i	3.35 ± 0.57 k	25.15 ± 2.00 c	78 ± 2.83 c
MNC11-1037E-4	4.53 ± 0.84 h	2.98 ± 0.21º	24.14 ± 0.32 c	46 ± 8.49 e
MNC11-1037E-5	5.10 ± 1.10 e	4.15 ± 0.50 d	24.32 ± 0.45 c	86 ± 2.83 b
MNC11-1039E-4	4.26 ± 0.38 i	3.09 ± 0.38 n	25.11 ± 0.35 c	39 ± 7.07 f
MNC11-1042E-1	3.93 ± 0.75 j	2.99 ± 0.32º	23.55 ± 0.31 d	72 ± 5.66 c
MNC11-1042E-4	5.58 ± 4.46 c	4.18 ± 0.70 d	25.11 ± 0.29 c	80 ± 5.66 b
MNC11-1043E-4	4.87 ± 0.80 f	3.57 ± 1.04 i	22.67 ± 0.82 e	80 ± 5.66 b
MNC11-1044E-8	4.01 ± 0.83 i	2.97 ± 0.21º	21.39 ± 0.10 f	90 ± 8.49 a
MNC11-1046E-3	5.14 ± 0.60 e	4.48 ± 0.32 b	21.44 ± 0.07 f	32 ± 5.66 g
MNC11-1046E-8	4.09 ± 1.54 i	3.33 ± 0.60l	22.35 ± 0.21 e	58 ± 2.83 d
MNC11-1046E-9	4.25 ± 1.04 i	2.99 ± 0.60º	21.31 ± 0.14 f	58 ± 8.49 d
MNC11-1047E-4	5.20 ± 1.10 e	3.97 ± 0.25 e	20.82 ± 0.13 f	57 ± 9.90 d
MNC11-1047E-6	4.30 ± 2.14 h	3.28 ± 0.71l	21.89 ± 0.27 f	48 ± 0.00 e
MNC11-1048E-2	4.42 ± 0.95 h	2.98 ± 0.78º	20.99 ± 0.27 f	82 ± 2.83 b
MNC11-1052E-3	5.17 ± 0.64 e	4.39 ± 0.32 c	23.13 ± 0.06 d	20 ± 0.00 g
MNC11-1052E-4	4.47 ± 2.07 h	2.82 ± 0.35 q	22.43 ± 0.11 e	48 ± 5.66 e
MNC11-1053E-3	4.43 ± 1.14 h	3.31 ± 0.38l	20.91 ± 0.15 f	56 ± 0.00 d
BRS Tumucumaque	5.95 ± 0.81 a	4.18 ± 0.55 d	23.35 ± 1.48 d	96 ± 5.66 a
BRS Pajeú	5.89 ± 2.05 a	4.17 ± 0.80 d	21.81 ± 0.18 f	55 ± 7.07 d
Inhuma	5.13 ± 1.07 e	4.06 ± 0.45 e	23.03 ± 0.35 d	42 ± 2.83 f
Pingo de Ouro 1-2	3.90 ± 0.06 j	3.47 ± 0.46 j	22.09 ± 0.06 f	98 ± 2.83 a
Overall mean	4.66	3.31	24.15	69.09

Averages followed by the same capital letter in the column belong to the same group, according to the Scott-Knott grouping criterion ($p < 0.05$)

The averages of the iron content were broken down into eleven groups by the Scott-Knott grouping ($p < 0.05$) (Table 2), showing a great contrast for this mineral among the evaluated genotypes. According to Rocha, Damasceno-Silva and Menezes-Júnior (2017), genetic biofortification for iron content is one of the objectives of the cowpea breeding programs. Unlike food fortification, which occurs during processing, genetic biofortification occurs by increasing the micronutrient content of the plant. It benefits both farming families who produce for their own consumption, as well as urban and rural families who buy biofortified food.

Group A stood out from the others, including genotypes with high iron content: the line MNC11-1023E-28, with 6.06 mg 100 g^{-1} and the cultivars BRS Tumucumaque and BRS Pajeú, with 5.95 and 5.89 mg 100 g^{-1}, respectively (Table 2). It was observed that the line MNC11-1023E-28, with 6.06 mg 100 g^{-1} present iron content in the grain greater than 6.00 mg 100 g^{-1}, showing that they fall into the group of biofortified genotypes (FREIRE-FILHO, 2011).

The iron content found for the BRS Tumucumaque cultivar in the present study confirms its status as a biofortified cultivar for this mineral (COELHO *et al*., 2021; ROCHA; DAMASCENO-SILVA; MENEZES-JÚNIOR, 2017). These first authors, evaluating three biofortified cowpea cultivars (BRS Aracê, BRS Taumucumaque, and BRS Xiquexique), concluded that the biofortification process was effective not only in increasing the levels, but also in the bioaccessibility of iron in the grains of these cultivars.

The variation among the iron contents found in cowpea can be explained by the genetic variability of the crop, edaphoclimatic conditions, and the existence of the genotype x environment interaction (SILVA *et al*., 2012). The genotypic difference for iron content may be associated with the additive inheritance that controls the expression of this characteristic and the genetic divergence between parents, used in the crossings that gave rise to the evaluated genotypes (SANTOS; BOITEUX, 2015).

The Resolution of the Collegiate Board (RDC) nº 269 of the National Health Surveillance Agency (AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA, 2005) determines that the reference daily intake (RDI) of iron for adults is 14 mg day^{-1}. Considering that the average for the iron content of the genotypes analyzed in this study was 4.66 mg 100 g^{-1}, the consumption of 100 g day^{-1} of cowpea meets 33.3% of the iron RDI. Consumption of 100 g day^{-1} of the best genotype, the line MNC11-1023E-28 (6.06 mg 100 g^{-1}), meets 43.3% of the iron RDI, helping to fight anemia resulting from iron deficiency in the population.

Zinc content

The studied genotypes showed a variation for the zinc content between 2.35 mg 100 g^{-1} and 4.57 mg 100 g^{-1}, with an average of 3.31mg 100 g^{-1} (Table 2). A similar result was found by Dias-Barbosa *et al.* (2020), evaluating 16 cowpea genotypes, which found an average for the zinc content of 4.17 mg 100 g^{-1}; as well as Rios *et al.* (2018), evaluating commercial cowpea cultivars, which had zinc content ranging from 3.17 to 5.14 mg 100 g^{-1}; and Gunathilake, Herath and Wansapala (2016), who studied cowpea cultivars in Sri Lanka, observed zinc contents between 2.04 and 2.82 mg 100 g^{-1}. On the other hand, Oliveira *et al.* (2017), evaluating the zinc content in 12 cowpea genotypes in three locations in Maranhão and Piauí, Brazil, found variation and mean of 3.66 to 5.66 mg 100 g^{-1}, being higher than those obtained in the present study.

The genotypes were classified by the Scott-Knott test ($p < 0.05$) in 21 groups (Table 2), showing a high genetic variability among them for the zinc content in the grain, in relation to the other characteristics evaluated in the present study. Dietary zinc deficiency is widespread globally and is particularly prevalent in low- and middle-income countries, resulting in a public health problem, and increasing the nutrient's content in the edible parts of plants can help mitigate it (LÓPEZ-MORALES *et al*., 2020; SILVA *et al*., 2021). One of the objectives of cowpea breeding is to increase the zinc content in the grain (ROCHA; DAMASCENO-SILVA; MENEZES-JÚNIOR, 2017). Genetic variability for zinc is a condition for the development of biofortified cultivars in this mineral.

Groups A and B highlighted, with the highest zinc content, which comprised only one genotype each, respectively the lines MNC11-1022E-9, with 4.57 mg 100 g^{-1}, and the line MNC11-1046E-3, with 4.48 mg 100 g^{-1} (Table 2). The cultivar BRS Tumucumaque, considered to be biofortified for zinc, had a content of 4.18 mg 100 g^{-1}, lower than the contents observed for the lines above. Coelho *et al*. (2021), evaluating the zinc content in biofortified cowpea cultivars, observed a zinc content of 46.0 mg 100 g^{-1} for this cultivar, which was higher than that found in the present study. This zinc content is above 4.00 mg 100 g^{-1}, higher than that presented by non-biofortified cowpea cultivars (FREIRE-FILHO, 2011).

The variation observed for the zinc content in the grains of the cultivar BRS Tumucumaque in different studies, in addition to the genotype, can be attributed to factors such as the place of cultivation and environmental factors (CARVALHO *et al*., 2012; PEREIRA *et al*., 2016), such as temperature and rainfall, which can influence the value of this characteristic.

The RDC nº 269 of the National Health Surveillance Agency determines that the zinc RDI for adults is 7.0 mg day^{-1} (AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA, 2005). Considering that the average for the zinc content of the genotypes analyzed in this study was 3.31 mg 100 g^{-1}, the consumption of 100 g day^{-1} of cowpea meets 47.3% of the daily zinc requirement. Consumption of 100 g day^{-1} of the best genotype, the line MNC11-1022E-9 (4.57 mg 100 g^{-1}), meets 65.3% of the zinc RDI. The adoption of this line in the population's diet represents a strategy to combat malnutrition resulting from zinc deficiency.

Protein content

Protein contents varied from 20.82 to 26.92 g 100 g^{-1}, with a general average of 24.30 g 100 g^{-1} (Table 2). The Scott-Knott test ($p < 0.05$) classified the genotypes into six groups. Group A included seven genotypes: the lines

MNC11-1022E-58 (26.92 g 100 g^{-1}), MNC11-1024E-16 (26.72 g 100 g^{-1}), MNC11-1005E-37 (26.60 g 100 g^{-1}), MNC11-1020E-29 (26.42 g 100 g^{-1}), MNC11-1019E-15 (26.27 g 100 g^{-1}), MNC11-1019E-46 (26.24 g 100 g^{-1}), and MNC11 -1017E-37 (26.22 g 100 g^{-1}), which highlighted in relation to the other evaluated genotypes, with the highest protein contents. This cowpea lines with high protein contents in the grains is crucial for human health, especially in the diet of the low-income population in the Brazilian semi-arid zone.

Carvalho *et al*. (2012), studying 30 Brazilian cowpea genotypes, found variation (17.4 to 28.3 g 100 g^{-1}) and average (21.6 g 100 g^{-1}) for protein content higher than those observed in the present study. Likewise, Weng *et al*. (2019), evaluating 173 USDA cowpea accessions, observed a variation from 22.8 to 28.9 g 100 g^{-1} and an average of 25.6 g 100 g^{-1}. Several studies carried out on cowpea have found similar results for protein content to those obtained in the present study, such as the studies by Bezerra *et al*. (2019), with contents between 20.66 and 26.06 g 100 g^{-1}; Gomes, Reis and Silva (2012), average content of 26.4 g 100 g^{-1} in whole flour; and Kanda *et al*. (2020), who reported an average content of 24.33 g 100 g^{-1}.

The RDC nº 269 of the National Health Surveillance Agency (AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA, 2005) determines that the RDI of proteins for adults is 50 g day^{-1}. Considering that the average for the protein content of the genotypes analyzed in this study was 24.15 g 100 g^{-1}, the consumption of 100 g day^{-1} of cowpea meets 48.30% of the protein RDI. Protein contents above 25 g 100 g^{-1} are considered quite high for legumes (ÇAKIR *et al*., 2019), as observed in the seven cowpea lines with higher protein content in this study. The average protein content obtained in this study is also higher than the average presented by the latest cowpea cultivars released on the market (FREIRE-FILHO, 2011).

Cooking quality

The cooking quality, assessed from the percentage of cooked grains, varied from 20 to 98% (Table 2), where samples from 72 genotypes showed good cooking quality, since when 13 of the 25 sticks pierce the grains, they are considered if the sample is cooked (MATTSON, 1946). The general average was 68.7% of cooked grains. Carvalho *et al*. (2017), evaluating 252 common bean progenies and three locations in Minas Gerais, Brazil, used the same methodology and found an average percentage of cooked grains of 36.71%, lower than the average obtained in the present study.

The genotypes were organized into seven groups according to the Scott-Knott test ($p < 0.05$) (Table 2). Group A comprised 32% of the genotypes, which had the highest percentage of cooked grains, constituting the group of genotypes with the best cooking quality. The lines MNC11-1005E-20, MNC11-1006E-10, MNC11-1013E-8, MNC11-1015E-7, MNC11-1034E-1, and Pingo de Ouro 1-2 showed an average of 24.5 perforated grains, equivalent to 98% of cooked grains, constituting as the genotypes with the best cooking quality. According to Rocha, Damasceno-Silva and Menezes-Júnior (2017) and Addy *et al*. (2020), fast cooking has the potential to provide a highly nutritious food in less preparation time and less energy expenditure.

On the other hand, the lines allocated to group G, MNC11- 1052E-3 and MNC11- 1024E-1, with an average of five perforated grains, making up between 20 and 24% of cooked grains, presented the worst cooking quality (Table 2), indicating that there is the need for a longer cooking time for these genotypes, which is a negative aspect for the consumer. According to Addy *et al*. (2020), a long cooking time of cowpea leads to loss of nutrients, loss of useful time and greater expenditure of energy in the preparation of the meal.

Therefore, lines with a high percentage of cooked grains are promising for the development of new cowpea cultivars with high cooking quality. It is important to note that a fixed time was used for cooking the grains (30 minutes), so some samples were much more cooked than others, not allowing the ideal cooking time to be determined. Therefore, the grains of the genotypes that showed the highest percentage of cooking, probably have cooking time of less than 30 minutes.

Nutritional and cooking quality index

The nutritional and cooking quality indexes (NCQI) of the evaluated genotypes, based on the iron, zinc, and protein contents and the cooking quality are shown in Table 3. It is observed that the highest values of NCQI were presented by the genotypes MNC11-1023E-28 (12.48), followed of BRS Tumucumaque (10.50), MNC11-1013E-27 (9.55), MNC11-1005E-37 (9.35), and MNC11-1042E-4 (9.04). These genotypes had the best nutritional profile and culinary quality and, therefore, can be used to combat malnutrition in populations with iron and zinc deficiency, as a complementary intervention to food fortification and drug supplementation.

The result obtained in this study confirms the superiority of biofortification of the cultivar BRS Tumucumaque for iron and zinc contents (COELHO *et al*., 2021; ROCHA; DAMASCENO-SILVA; MENEZES-JÚNIOR, 2017) and shows that it also presents high cooking quality. These last authors highlight the BRS Tumucumaque as the cowpea cultivar with the fastest cooking time (13’23”, after soaking in water for two hours).

Table 3 - Nutritional and cooking quality indexes (NCQI) of 100 cowpea genotypes, based on iron, zinc, and protein contents and cooking quality

Genotype	NCQI	Genotype	NCQI	Genotype	NCQI
MNC11-1005E-20	6.76	MNC11-1019E-8	3.81	MNC11-1031E-5	-5.21
MNC11-1005E-28	7.72	MNC11-1019E-12	7.76	MNC11-1031E-8	-5.72
MNC11-1005E-37	9.35	MNC11-1019E-15	6.16	MNC11-1031E-9	1.65
MNC11-1006E-10	5.81	MNC11-1019E-16	-0.61	MNC11-1031E-11	-6.85
MNC11-1008E-9	6.23	MNC11-1019E-40	-3.53	MNC11-1031E-13	-4.07
MNC11-1012E-7	3.53	MNC11-1019E-46	0.17	MNC11-1031E-15	0.95
MNC11-1013E-18	-9.46	MNC11-1020E-29	7.71	MNC11-1033E-14	-1.10
MNC11-1013E-33	-8.56	MNC11-1020E-18	-9.46	MNC11-1033E-30	6.92
MNC11-1013E-27	9.55	MNC11-1020E-16	-0.30	MNC11-1034E-1	7.70
MNC11-1013E-8	6.72	MNC11-1020E-5	-2.28	MNC11-1034E-2	7.78
MNC11-1013E-16	5.36	MNC11-1020E-36	4.69	MNC11-1036E-3	-7.69
MNC11-1013E-25	-0.16	MNC11-1020E-6	-3.93	MNC11-1036E-4	-9.55
MNC11-1015E-2	5.06	MNC11-1021E-27	-3.29	MNC11-1036E-5	-4.96
MNC11-1015E-5	-5.39	MNC11-1021E-17	0.26	MNC11-1037E-1	2.09
MNC11-1015E-7	5.97	MNC11-1022E-1	-7.65	MNC11-1037E-4	-5.75
MNC11-1015E-15	3.05	MNC11-1022E-9	8.60	MNC11-1037E-5	5.21
MNC11-1015E-28	-3.43	MNC11-1022E-58	8.49	MNC11-1039E-4	-5.24
MNC11-1015E-29	0.33	MNC11-1023E-28	12.48	MNC11-1042E-1	-5.65
MNC11-1015E-35	-0.88	MNC11-1023E-60	6.53	MNC11-1042E-4	9.04
MNC11-1016E-12	6.89	MNC11-1023E-48	3.01	MNC11-1043E-4	2.11
MNC11-1016E-16	-0.12	MNC11-1023E-26	-0.08	MNC11-1044E-8	0.23
MNC11-1017E-3	-5.60	MNC11-1024E-18	-15.81	MNC11-1046E-3	-9.67
MNC11-1017E-8	3.23	MNC11-1024E-1	-8.62	MNC11-1046E-8	-6.32
MNC11-1017E-10	3.03	MNC11-1024E-16	-7.23	MNC11-1046E-9	-4.61
MNC11-1017E-26	2.66	MNC11-1026E-15	7.74	MNC11-1047E-4	0.10
MNC11-1017E-30	-8.31	MNC11-1026E-5	0.06	MNC11-1047E-6	-9.99
MNC11-1017E-31	1.17	MNC11-1026E-19	7.75	MNC11-1048E-2	-0.99
MNC11-1017E-33	4.21	MNC11-1028E-16	7.73	MNC11-1052E-3	-9.85
MNC11-1017E-37	5.21	MNC11-1028E-34	6.30	MNC11-1052E-4	-3.12
MNC11-1018E-2	-8.25	MNC11-1028E-5	2.88	MNC11-1053E-3	-6.38
MNC11-1018E-4	-1.31	MNC11-1029E-9	-3.79	BRS Tumucumaque	10.50
MNC11-1018E-17	-6.33	MNC11-1029E-13	-5.78	BRS Pajeú	7.83
MNC11-1018E-20	0.87	MNC11-1029E-15	2.44	Inhuma	-7.19
				Pingo de Ouro 1-2	8.76

Carvalho *et al.* (2012), evaluating 30 Brazilian cowpea genotypes, used a nutritional quality index and, through this, identified genotypes with high iron, zinc, and protein contents, however, the cultivars BRS Tumucumaque and BRS Pajeú and the line Pingo de Ouro 1-2 did not present the best attributes in relation to these nutrients, when compared with the other evaluated genotypes. These differences in the behavior of these characteristics may be associated with the origin of the samples of the genotypes, which depend on the location and agricultural year of cultivation, edaphoclimatic conditions of cultivation and number of days and post-harvest storage conditions.

Among all cowpea lines evaluated, the grain of the line MNC11-1023E-28 presented the best nutritional and cooking attributes, constituting a food of great nutritional value and quick to prepare for the consumer.

CONCLUSIONS

1. Among the 100 cowpea genotypes evaluated, 83% have a high iron content and a zinc content above 30% of the reference daily intake;
2. The protein content of the cowpea genotypes evaluated supplies more than 40% of the reference daily intake, constituting an excellent source of this nutrient;
3. Most of the cowpea genotypes evaluated (72%) serve the consumer in terms of cooking quality;
4. Among the cowpea lines evaluated, MNC11-1023E-28 has the best nutritional and cooking quality profile, constituting an excellent food option for consumers who demand quick meal preparation, as well as an alternative to combat iron deficiency and zinc in the Brazilian population.

ACKNOWLEDGMENTS

The authors would like to thank the Empresa Brasileira de Pesquisa Agropecuária (Projects 20.18.01.022.00.04 and 20.19.00.118.00.06), Agreement EMBRAPA/HARVESTPLUS/FUNARBE-4370 (IFPRI 6332 306363.006.032 - TASK 5), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for funding this research.

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