eISSN 1981-1829

Ciência e Agrotecnologia
Science and Agrotechnology

Potential use of cellulose soybean hulls as a source of carboxymethyl cellulose for coating bean seeds

Uso potencial de celulose de cascas de soja como fonte de carboximetilcelulose para revestimento de sementes de feijão

Diego Palmiro Ramirez Ascheri[1*], Patricio Javier Robles Barros[2], José Luís Ramírez Ascheri[3], Roberta Signini[4]

ABSTRACT

Implementing sustainable practices for using agricultural waste is urgent in the face of the challenges of climate change. This study aimed to investigate the application of carboxymethyl cellulose (CMC) derived from soybean hulls as a bioinput in the seed coat of beans. CMC was obtained after bleaching the pulp, alkalizing and etherifying it varying the chloroacetic acid concentration and the reaction time. The properties of CMC were compared to those of soybean hulls and bleached pulp. The CMC with the highest degree of substitution (DS) was chosen as bioinput. In addition to the control treatment, concentrations of 1%, 2%, and 3% CMC were used for coating, and the impact on the physiological quality of seeds was evaluated. CMC proved suitable as a coating agent for seeds, with a DS of 1.56 obtained with 1.2 g chloroacetic acid per gram of bleached pulp during 192 min at 63 °C. The 2% CMC solution proved to be effective, resulting in 93%, 94%, and 43.5% of germinated seeds at 5 days, 8 days, and after accelerated aging, respectively. Seedlings reached 34.2 cm in length and a dry mass of 0.05 g. Our results indicate that soybean hulls can be successfully used in the production of CMC as a coating material, improving the physiological quality of bean seeds and contributing to more sustainable agricultural practices.

Index terms: Bioinput; seed physiological quality; agrobiodiversity; sustainability; organic synthesis.

RESUMO

Este estudo aborda a necessidade de adoção de práticas sustentáveis de aproveitamento de resíduos agrícolas diante dos desafios das mudanças climáticas. O objetivo do presente estudo foi investigar a aplicação da carboximetilcelulose (CMC) obtida a partir da casca de soja como bioinsumo no revestimento de sementes de feijão. A CMC foi obtida após o branqueamento da polpa, alcalinização e eterificação, variando o ácido cloroacético e o tempo de reação. Compararam-se as propriedades da CMC com as da casca de soja e da polpa branqueada. A CMC com maior grau de substituição (GS) foi escolhida como bioinsumo. Além do tratamento controle, foram avaliadas as concentrações de 1%, 2% e 3% de CMC para o recobrimento, impactando na qualidade fisiológica das sementes. A CMC revelou-se adequada como agente de recobrimento para as sementes, com um GS de 1,56, obtido com 1,2 g de ácido cloroacético por grama de polpa branqueada, durante 192 minutos a 63 °C. A solução de 2% de CMC mostrou-se eficaz, resultando em mais de 94% de germinação aos 8 dias, 93% na primeira contagem de sementes germinadas (5° dia), 43,5% após o envelhecimento acelerado, e crescimento de plântulas de 34,2 cm de comprimento e massa seca de 0,05 g. Os resultados indicam que a casca de soja tem potencial para utilização na produção de CMC, que pode ser aplicada como recobrimento, melhorando a qualidade fisiológica das sementes de feijão e contribuindo para práticas agrícolas mais sustentáveis.

Termos para indexação: Bioinsumo; qualidade fisiológica da semente; agrobiodiversidade; sustentabilidade; síntese orgânica.

Agricultural Sciences

Ciênc. Agrotec., 48:e003924, 2024
http://dx.doi.org/10.1590/1413-7054202448003924

Editor: Renato Paiva

[1]Universidade Estadual de Goiás/UEG, Programa de Pós-Graduação Stricto sensu em Engenharia Agrícola, Anápolis, GO, Brasil
[2]Grupo Jacto. Marília, São Paulo, SP, Brasil
[3]Embrapa Agroindústria de Alimentos, Laboratório de Extrusão e propriedades físicas, Guaratiba, RJ, Brasil
[4]Universidade Estadual de Goiás, Programa de Pós-Graduação Stricto Sensu em Ciências Moleculares, Anápolis, GO, Brasil

Corresponding author: diego.ascheri@ueg.br

Received in February 27, 2024 and approved in May 14, 2024

Introduction

Overcoming the challenges posed by climate change and the loss of biodiversity demands new practices for the sustainable use of agricultural and agro-industrial wastes to minimize deforestation and the production of bioinputs. Soybean hulls, which are rich in cellulose (32 - 41% on a dry basis) (Barros et al., 2020; Debiagi, Faria-Tischer, & Mali, 2020), stand out as a sustainable raw material with potential use as a bioinput.

According to estimations of the (Companhia Nacional de Abastecimento - CONAB, 2024), Brazil will produce approximately 312.3 million tons of soybean in the 2023/2024 harvest, resulting in approximately 26 million tons of soybean hulls. Part of this co-product can be used to produce cellulose derivatives. Studies such as those of Barros et al. (2020) indicate that cellulose extracted from soybean hulls can be converted into carboxymethyl cellulose (CMC) through simple processes, such as pulping and bleaching, without additional treatments with organic solvents due to the low ether content.

Considering the increasing interest in developing coatings from biodegradable materials, bioinputs have emerged as a safe option in agriculture, promoting sustainability and environmental conservation (Jahangiri, Mohanty, & Misra, 2024; Pirzada et al., 2020; Zhang et al., 2022). This approach contributes to reducing raw material waste and optimizing the utilization of resources such as soybean hulls.

Among the physicochemical properties and applications of CMC, the average degree of substitution (DS) is fundamental. This parameter is defined as the average number of substituted carboxymethyl groups per monomeric unit of cellulose (Nóbrega & Amorim 2015; Nóbrega et al., 2019). DS could directly influence the effectiveness of CMC as a seed coating agent, as together with the solubility of CMC, it plays a crucial role in the viscosity of the polymer (Nóbrega & Amorim, 2015; Nóbrega et al., 2019).

In seed coating, the surface of the seed is coated with polymers in solution (Rocha et al., 2019) that adhere to chemicals (Camargo et al., 2017, Ludwig et al., 2020) or microbiological products. CMC is used alone or in combination with other polymers or biological agents (Paravar et al., 2023).

Chin, Lim and Yien (2021) explored various approaches, including using a CMC solution combined with a fungal bioagent (*Trichoderma asperellum*) as a coating for pak choi. Abeywardana et al. (2023) used a mixture of sodium alginate and CMC, incorporating hydroxyapatite urea nanoparticles doped with zinc, to coat corn seeds.

In a similar context, Moeljani, Priyadarshini and Wuryandari (2023) adopted CMC as a coating agent for rice, onion, pepper, and cucumber seeds, enriching the solution with liquid smoke and carbonates. However, these approaches do not offer a comprehensive assessment of CMC coating capacity, as CMC is often used as an adhesive agent for other products rather than being the main coating substance.

Preliminary studies are crucial to ensure that the coating agent is compatible and does not affect the physiological quality of treated seeds. Scarsi et al. (2020) observed that increasing the concentration of a polymer reduces seedling emergence, and highly hydrophilic polymers can reduce seed germination and vigor. In this sense, there is still no consensus on the ideal concentration of CMC for coating seeds. Camargo et al. (2017) obtained satisfactory results in coating soybean seeds using different CMC concentrations mixed with fungicide. In addition, Bouri et al. (2022) obtained promising results by combining two active ingredients (zinc pyrithione and triclosan) associated with 0.5% CMC to control seed-borne pathogens.

To date, there is a lack of consolidated research using CMC derived from soybean hulls as a coating agent, especially concerning its concentration. This study aimed to assess the physiological quality of bean seeds from the Pérola cultivar coated with CMC derived from soybean hulls.

Material and Methods

The Brejeiro soybean oil company, located in Anápolis, GO, kindly supplied the soybean hulls. Preparing the hulls (Figure 1) involved drying them in an oven with forced air recirculation (Marconi, MA-035) at 105 ± 3 °C for approximately 12 h. Afterward, the soybean hulls were ground in a Willy-type vertical rotor mill equipped with four movable knives and four fixed knives (SPlabor, SP-31) with a 1 mm mesh opening, and were referred to as ground soybean hulls (GSH).

The extraction and purification of the cellulose pulp, as well as the production of CMC, followed the method described by Barros et al. (2020). Cellulose pulp was obtained from ground soybean hulls using a 1% NaOH solution with a GSH/NaOH ratio of 1/20 (m/v). The temperature was maintained at 90 ± 3 °C for 2 h with constant stirring. After pulping, the mixture was cooled in an ice bath until it reached 25 ± 3 °C and filtered through a Buchner nº 3 glass funnel using distilled water until the pH reached neutrality. The raw pulp was then dried in a forced-air oven at 60 ± 3 °C for 15 h.

The raw pulp was mixed with a bleaching solution [27 g of NaOH and 75 mL of a 50% glacial acetic acid (CH_3COOH) solution diluted in 1 L of distilled water and 2% sodium chlorite ($NaClO_2$)] in the ratio of 1/75 (m/v). The reaction was maintained at 95 ± 3 °C under stirring for 4 h and was immediately cooled in an ice bath until it reached 25 ± 1 °C. The pulp was then filtered through a Buchner funnel n° 3 using distilled water until it reached a neutral pH. The bleached pulp, known as soybean hull pulp (SHP), was dried in a forced circulation oven at 60 ± 3 °C for 12 h.

CMC was produced by acetylating cellulose from soybean hulls using two main steps: alkalinization and etherification. First, 3 g of soybean hull pulp was homogenized in 80 mL of isopropyl alcohol with constant stirring for 30 min. Subsequently, 10 mL of a 50% NaOH solution was added, and stirring continued for another 30 min. The experimental design for the etherification stage was conducted randomly, following a double factorial scheme (2 x 2, in triplicates), varying the amount of chloroacetic acid ($C_2H_3ClO_2$) added to 1.0 g soybean hull pulp (1.2 and 2.1 g) and the reaction time (192 and 228 min). The temperature of the reaction was kept constant at 63 °C. At the end of the reaction, the resulting mixture was filtered and washed three times with 300 mL of 70% ethyl alcohol and then neutralized with 90% glacial acetic acid. The liquid resulting from the etherification step was purified according to Mondal, Yeasmin and Rahman (2015). For that, 150 mL of 80% ethanol was added to the mixture, with constant stirring for 30 min, followed by vacuum filtration. This procedure was repeated using absolute ethyl alcohol and then methyl alcohol. The product obtained was dried at 50 °C in an oven with forced air recirculation until it reached a constant mass, resulting in the final CMC.

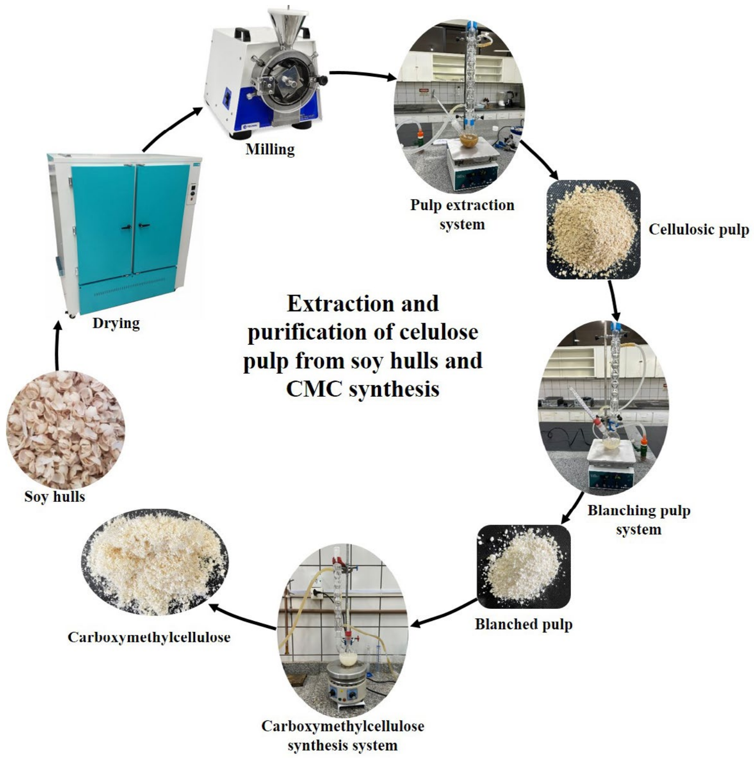

Figure 1: Flowchart depicting the processes for extracting and purifying cellulosic pulp, as well as synthesizing carboxymethylcellulose from cellulose extracted from soybean hulls.

Determinations of the degree of substitution (DS) and apparent viscosity were conducted in triplicates to assess the purity of the CMC, which was used for coating the bean seeds. DS was determined following the ASTM 1439-03 procedure (American Society for Testing and Materials - ASTM, 2003). Initially, 3 g CMC was immersed in 75 mL of 95% ethyl alcohol, with constant stirring for 5 min. Then, 5 mL of nitric acid (HNO_3) was added to the mixture and heated to boiling (~80 °C) for 5 min while stirring. The temperature was then reduced to 25 °C while stirring for 15 min. The mixture was filtered, washed with 150 mL of 80% ethanol and 15 mL methyl alcohol, and the filtered product was dried at 105 °C for 3 h.

After drying, 1.0 g of the treated CMC was mixed with 100 mL of distilled water and 25 mL of 0.4 N NaOH. The reaction was constantly stirred until boiling (~100 °C) for 15 min. While still hot, the mixture was titrated with 0.4 N HCl until it became colorless, using phenolphthalein as an indicator. DS was calculated according to Equations 1 and 2.

$$A = \frac{(B\ C) - (D\ E)}{F} \quad (1)$$

Where: A = milliequivalents of HCl consumed/g of sample; B = volume of NaOH used (mL); C = normality of NaOH solution (N); D = volume of HCl (mL); F = mass of sample (g).

$$\text{DS} = \frac{0.162A}{1 - (0.058A)} \quad (2)$$

The apparent viscosity of CMC was measured following the methodology described by Barros et al. (2020). Before the measurements, preliminary studies were conducted to understand the behavior of 2% (w/v) CMC in distilled water with constant stirring for 20 min at 1300 rpm.

The viscosity measurements were conducted using a concentric tube viscometer (Brookfield, model DVII+) equipped with a sample adapter and pin n° 18. Samples (8 mL of the solutions) were subjected to variations in the speed of the pin from 3 to 60 rpm. The temperature was maintained at 25 °C by circulating water in a thermostatic bath (Brookfield, SC4-45Y) fitted with a cooling unit (Tecnal, TE-183). The viscosity was measured in cP for 10 min for each speed selected.

We compared the microscopic and infrared spectroscopy analyses of CMC with the samples of ground soybean hulls, cellulose pulp, and bleached pulp, and the CMC with the highest degree of substitution and apparent viscosity was chosen as a coating material for the bean seeds.

To assess the surface morphology of the samples, scanning electron microscopy was conducted using a Hitachi TM3030 plus benchtop scanning electron microscope. Samples were previously dried for 12 h at 60 °C, as described by Santos et al. (2015).

To identify the functional groups in the molecular structures of the samples, they were first dried in an oven at 60 °C for 12 h, ground, and mixed with KBr in a ratio of 1/100 (m/m). The resulting mixtures were pressed into pellets and dried in a vacuum oven at 60 °C for 24 h. Spectra were acquired using a Perkin Elmer Spectrum Frontier FT-IR/NIR infrared spectrophotometer (Perkin Elmer, Norwalk, CT) in the region between 4000 and 400 cm^{-1}, with a resolution of 4 cm^{-1}, following the methodology described by Ascheri et al. (2014).

The seed coating process was adapted from the methodology of Bertoldo et al. (2010). CMC solutions were prepared in 1, 2, and 3% (m/v) concentrations under constant stirring for 1 h. The coating experiment was conducted randomly with four treatments (no coating – control, 1, 2, and 3% CMC) and four replications. Each experimental unit consisted of a 150 mL beaker containing 200 bean seeds with the CMC solution which were left to stand for 5 min.

The seeds soaked in the CMC solution (Figure 2) were poured onto 11 x 11 cm stainless steel wire mesh to remove excess liquid and dried in a forced-air oven at 35 ± 3 °C for 1 h.

After coating, the coated seeds were subjected to a physiological quality assessment. Samples of 200 seeds per treatment were divided into four replicates of 50 seeds, placed on germitest paper previously moistened with distilled water equivalent to 2.5 times the weight of the paper. The germination boxes were placed in a germinator at 25 °C. The evaluation was conducted on the 8^{th} day after the test was implemented. The percentage of normal seedlings (G) was considered (Brasil, 2009) according to Equation 3.

$$G(\%) = \left[100 \times \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}}\right]_{8°\text{ day}} \quad (3)$$

The first count of germinated seeds (FC) was conducted along with the germination test to assess vigor, considering the percentage of normal seedlings present on the 5^{th} day after the start of the germination test (Brasil, 2009), according to Equation 4.

$$FC(\%) = \left[100 \times \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}}\right]_{5°\text{ day}} \quad (4)$$

The accelerated aging test was conducted according to the methodology of Krzyzanowski, França-Neto and Henning (1991) to complement the vigor analysis. Two hundred seeds from each treatment were distributed in a single layer on a stainless-steel wire mesh and placed in germination boxes measuring 11 x 11 x 3.5 cm, containing 40 mL of distilled water. The seeds were maintained at 42 ± 3 °C for 48 h. After this period, the seeds were subjected to the germination test as described above for five days at 25 °C (Brasil, 2009). Vigor was determined using Equation 4.

The seedling length was determined by sowing four sub-samples of 10 seeds per treatment on the germiest paper moistened 2.5 times the weight of the paper. The rolls were kept for 10 days in a germination chamber, in the dark, at 25 °C. Afterward, the primary root of the germinated seedlings was measured using a ruler, and the results were expressed in centimeters (Nakagawa, 1999).

To obtain the dry mass, seedlings were placed in labeled paper bags and taken to an oven with forced air circulation, maintained at a temperature of 80 °C for 24 h (Nakagawa, 1999). Dry mass was evaluated on a scale with a precision of 0.001 g, and the average results were expressed in milligrams per seedling.

The results obtained at the end of the bleached cellulose pulp carboxymethylation were subjected to variance analysis at a 5% probability level to verify the interaction between the proportion of chloroacetic acid and reaction time.

The polynomial model used to predict these responses is shown in Equation 5 (Neto, Scarminio, & Bruns, 2010):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \varepsilon \quad (5)$$

Where: X_1 and X_2 are the variables referring to the factors; β_0 is the intersection point; β_1 and β_2 refer to linear effects; β_{12} refers to double interaction effects; ε = experimental error.

The mathematical model was chosen using analysis of variance and the adjusted coefficient of determination ($R^2_{aj.}$). The regression coefficients were estimated using the least squares method, and their significance was assessed by the probability value ($p \leq 0.05$).

Figure 2: Images of bean seeds treated with different carboxymethylcellulose concentrations. A) Control, no CMC; B) 1% CMC, C) 2% CMC, and C) 3% CMC.

The results of the physiological quality of bean seeds with and without CMC coating were subjected to analysis of variance (ANOVA) at the 5% probability level to verify the effects of CMC concentration on germination, vigor, seedling length, and seedling dry mass. The Tukey test was applied at a 5% probability level when the difference was significant.

TIBCO (2020) was used for statistical analyses and graph production.

Result and Discussion

The carboxymethylation process, considering the proportion of chloroacetic acid per unit of soybean hull pulp and the reaction time, resulted in the production of CMC with different degrees of substitution and apparent viscosity (Table 1). All the treatments were effective, with DS ranging from 0.95 to 1.56. Notably, these DS values are higher than those for commercial CMC, generally around 0.7 (Nóbrega; Amorim, 2015). This result indicates that CMC made from soybean hull cellulose can be obtained with the desired characteristics for specific applications.

Table 1: Degree of substitution and apparent viscosity of carboxymethyl cellulose obtained from soybean hull cellulose varying the proportion of chloroacetic acid and reaction time.

Treatment	Factors*		Degree of substitution	Apparent viscosity (cP)
	CP (g)	t (min)		
T_1	1.2	192	0.95±0.01	63.19±0.02
T_2	1.2	228	1.02±0.03	66.04±0.04
T_3	2.1	192	1.56±0.04	305.49±0.74
T_4	2.1	228	1.41±0.05	123.75±0.25

Data are mean ± standard deviation. *CP=chloroacetic acid/pulp ratio (g/g); t=reaction time (min).

The conditions used in this study made it possible to produce high-quality CMC, characterized by a greater number of substituted carboxymethyl groups in its structure, reflected in a high DS. T_3 was the treatment with the highest DS (1.56) and apparent viscosity (305.49 cP), while the lowest values were obtained in T_1 (Table 1).T_3 consisted of using 2.1 g of chloroacetic acid for 192 min at a constant temperature of 63 °C. Under these experimental conditions, molecular structural changes were observed in the cellulose from soybean hulls during the carboxymethylation process.

Initially, ground soybean hulls (Figure 3A) had a heterogeneous morphology, with more compact fibers and a rigid, smooth surface due to hemicellulose, lignin, and waxes. In Figure 3B, due to the chemical attack of the NaOH solution and chloroacetic acid on ground soybean hulls during the pulping and bleaching process, respectively, broke down the hull's surface, giving rise to small particles that formed lumps of cellulose.

Figure 3C shows that CMC particles have flattened, rounded shapes. In this image, no cellulose fibers were observed, which suggests the destruction of the plant cell (Cerrutti et al., 2017) due to the replacement of –OH groups by carboxymethyl groups in the cellulose structure (Cai et al., 2018).

Figure 4 confirms the loss of the main functional groups that characterize hemicellulose and lignin and the increase in cellulose functional groups during the pulping and bleaching processes of soybean hulls. Through the FTIR spectrum, we confirmed the presence of the main functional groups of CMC formed during the carboxymethylation process.

A gradual decrease in the peak located in the 3419 cm^{-1} band resulting from the asymmetric and symmetric stretching of the cellulose –OH group was observed after pulping and bleaching. This is due to the partial replacement of hydroxyl groups by carboxymethyl groups in the prepared CMC. A similar behavior was observed in the 2926 cm^{-1} peak corresponding to the stretching of the –CH group, which is related to the organic compounds of hemicellulose and lignin, reaffirming the degradation of non-cellulose compounds.

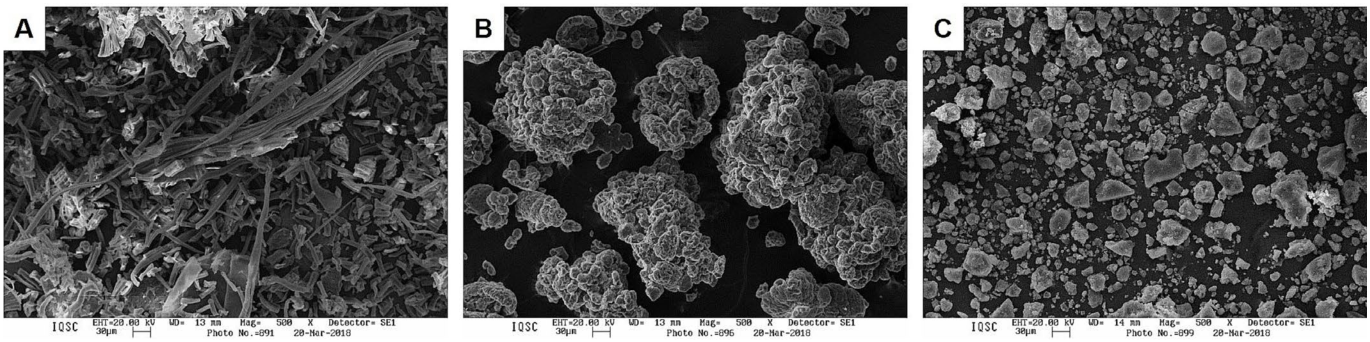

Figure 3: Scanning electron microscopy at 500x magnification. A) Ground soybean hulls, B) bleached pulp, and C) carboxymethyl cellulose obtained under conditions of 2.1 g chloroacetic acid/bleached pulp, reaction time of 192 min, and temperature of 63 °C.

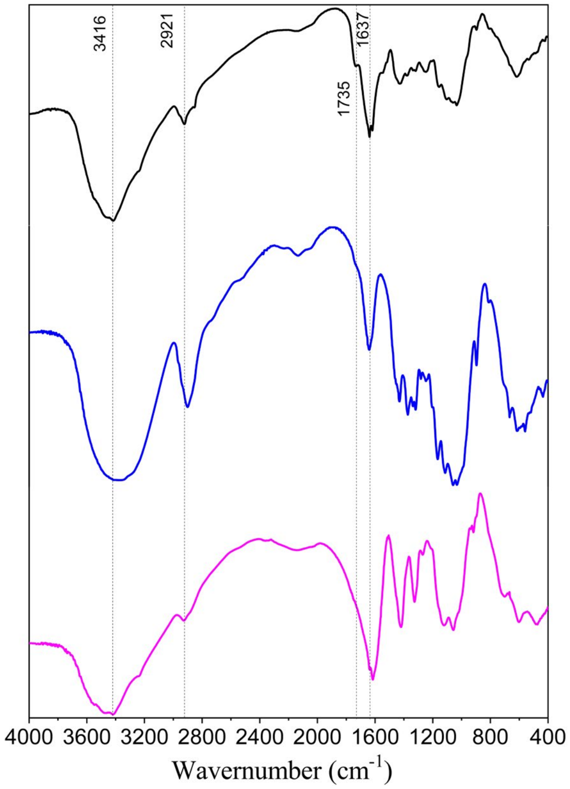

Figure 4: FTIR spectrum of ground dry soybean hulls (——), bleached pulp (——), and carboxymethyl cellulose (——) obtained with a ratio of 2.1 g chloroacetic acid/bleached pulp, a reaction time of 192 min, and temperature of 63 °C.

The band located in the 1730 cm^{-1} region is attributed to the C=O stretching of the ester group of the hemicellulose structures or the ester of the carboxylic group that make up lignin. The reduction in amorphous content is suggested by the decrease in the intensity of the spectrum in the raw and bleached pulp, indicating the removal of most of the hemicellulose and lignin from the soybean hulls. This peak was not observed in the CMC spectrum, indicating that the bleaching of the cellulose pulp was suitable for synthesizing CMC. The most intense peak at 1617 cm^{-1} is attributed to the C=O stretching of the COO^- group, which confirms the presence of the carboxymethyl substituent (Oun & Rhim, 2015). The intensity of this peak increased during the process, confirming the CMC obtained during the process.

The physiological quality of beans coated with a 2% CMC solution (E_2) was superior to the other treatments. On the other hand, the physiological quality of beans treated with a 3% CMC solution (E_3) was inferior to all the treatments studied (Table 2). This discrepancy amongthe treatments was revealed by analysis of variance (ANOVA), as shown in Table 3.

Seed coating creates an environment conducive to germination and initial plant establishment, resulting in faster and more uniform emergence, as well as protection against adverse conditions and soil pathogens. Together, the rate of germination and establishment is improved contributing to an increase in crop yield. The results in Table 2 indicate that the coating treatments had a positive effect on the physiological quality of bean seeds when compared to untreated seeds. Figure 5 shows representative seedlings from each treatment.

Table 2: Variation in the physiological quality of bean seeds from the Pérola cultivar according to the concentration of carboxymethyl cellulose applied as a coating agent.

Treatments	AA (%)	FC (%)	G (%)	SL (cm)	SDM (g)
Control	41.5±0.7ab	85.5±1.1b	87.0±0.8b	26.2±0.4b	0.03±0.01b
E_1 (CMC 1%)	39.0±0.8b	85.5±0.7b	86.8±0.9b	30.1±0.6b	0.03±0.02b
E_2 (CMC 2%)	43.5±0.5a	93.0±0.8a	94.5±0.5a	34.2±0.6a	0.05±0.01a
E_3 (CMC 3%)	29.8±0.9c	83.0±0.5b	83.8±0.6c	28.6±0.8c	0.02±0.01b

Data are mean ± standard deviations (n = 4). Significant differences (Tukey test, P<0.05) among treatments are shown by letters. AA= accelerated aging; FC= first count of germinated seeds; G= germination; SL= seedling length; SDM= seedling dry mass.

Table 3: Summary of the analysis of variance applied to the physiological quality values of bean seeds from the Pérola cultivar with and without carboxymethyl cellulose coating.

Factor	DF	AA		FC		G		SL		SDM	
		SS	F	SS	F	SS	F	SS	F	SS	F
Treatment	3	443.19	23.7*	225	19.6*	251.5	49.1*	133.57	109.8*	0.0028	3.5*
Residue	12	74.75	-	46	-	20.5	-	4.86	-	0.0032	-
Total	15	517.94	-	271	-	272	-	138.43	-	0.0061	-

Significant differences are shown by *. DF= degrees of freedom; SS= sum of squares; F= F test; AA= accelerated aging; FC= first count of germinated seeds; G= germination; SL= seedling length; SDM= seedling dry mass.

Figure 5: Photographic images of bean seedlings from the Pérola cultivar. Seeds were treated with different concentrations of carboxymethyl cellulose as a coating agent.

According to TeKrony (2005), the accelerated aging test exposes seeds to high temperature and high relative humidity. These conditions induce rapid deterioration of the seeds. High-vigor seeds resist these stress conditions, deteriorating more slowly and exhibiting high germination after aging, compared to low-vigor seeds (TeKrony, 2005).

Our accelerated aging test at 42 °C/48 h/100% relative humidity revealed that bean seeds from the Peróla cultivar coated with 2% CMC solution, showed superior preservation compared to the other treatments. Although the Tukey test showed no significant differences between this treatment and the control, E_2 had more than 43% of germinated seeds, a difference of 2% compared to the control treatment. On the other hand, the higher concentration of CMC (E_3) resulted in less than 30% of seeds germinating after the accelerated aging procedure. Statistically, germination of seeds in treatment E_1 was similar to the control (39.0%).

Nakagawa (1999) stated that the first count of germinated seeds effectively determines seed vigor. Table 2 showed that coating bean seeds with 2% CMC resulted in a better response in the first count of germinated seeds (93%), further confirmed by the germination test.

After 8 days of germination, the number of normal seedlings in treatments E_1 and E_3 was 86.8% and 83.8%, respectively, with no significant differences compared to control seeds (Table 3).

Seedling growth can be quantified using the seedling length and dry mass, indicators of seedling performance, which depend on the physiological quality of the seeds (Nakagawa, 1999). Seedlings from treatment E_2 had the highest length (average of 34.2 cm) (Table 2). We also observed longer primary and secondary roots with greater density in seedlings from this treatment. On average, the control seeds were 26.2 cm long, with a better formed root system than the other treatments. However, the length of the shoot (hypocotyl) was reduced. Seedlings from E_1, (30.1 cm), and E_3 (28.6 cm) were longer than the control, but the root system was less vigorous and the hypocotyl length was similar to the control seeds without coating. Thus, E_1 and E_3 coatings positively affected seedling length, although to a lesser extent than E_2 (Figure 6).

Figure 6: Seedling length from seeds coated with different concentrations of carboxymethyl cellulose.

The highest seedling dry mass was also, achieved in treatment E_2 (0.05 g/seedling on average). The other treatments showed no significant differences, with values from 0.02 to 0.03 g/seedling (Table 2).

CMC coating acts as a physical barrier around the seed (Keawkham, Siri, & Hynes, 2014). Our results revealed that 1% CMC was insufficient to promote the seeds' physiological potential, probably resulting in insufficient adhesive capacity. In contrast, 2% CMC increased this capacity and probably improved the passage of water and oxygen for seedling development (Schoeninger & Bischoff, 2014).

However, the 3% CMC resulted in lower proportion of normal seedlings compared to the 2% treatment, indicating that an even more pronounced adhesive capacity and excess water permeability can compromise cell turgidity, leading to cell wall rupture and seed death (Madsen et al., 2016). Scarsi et al. (2020) highlight that higher polymer concentrations reduce seedling emergence, and highly hydrophilic polymers also reduce seed germination and vigor.

Our observations indicate that treatment E_2, containing 2% CMC, has a lower capacity to inhibit the speed of seed germination (Moeljani, Priyadarshini, & Wuryandari, 2023). In other words, this treatment accelerates the growth of seedlings by providing adequate water supply, resulting in normal seedlings with a higher vigor index. This finding is supported by the fact that carboxymethyl cellulose (CMC) also acts as a regulator of the soaking process (Palupi et al., 2012).

Conclusions

Our study explores a novel coating derived from soybean hulls, offering an eco-friendly alternative to synthetic coating compounds. Utilizing cellulose pulp bleached with an acetate clarifying solution, CMC of exceptional quality was produced, with a degree of substitution of 1.56 and a viscosity of 305.49 cP. This water-soluble polymer, synthesized with chloroacetic acid, promoted bean seed germination and seedling growth at a concentration of 2% of CMC, underscoring the potential of soybean hulls for sustainable agricultural applications.

Author Contribution

Conceptual idea: Ascheri, D. P. R.; Barros, P. J. R.; Methodology design: Ascheri, D. P. R.; Barros, P. J. R.; Data collection: Ascheri, D. P. R.; Barros, P. J. R.; Data analysis and interpretation: Ascheri, D. P. R.; Barros, P. J. R.; Ascheri, J.L.R.; Signini, R.; Writing and editing: Ascheri, D. P. R.; Barros, P. J. R.; Ascheri, J.L.R.; Signini, R.

Acknowledgments

To the State University of Goiás for financial support through the Institutional Platform for Research and Innovation in Bioinputs and Pro-Laboratories. To the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for granting scholarships. To the Embrapa Food Technology and the UFSCar for their support with the analyses.

References

Abeywardana, L. et al. (2023). Advanced coating on *Zea mays* seeds using modified hydroxyapatite nanoparticles as a plant nutrient delivery system for enhanced plant growth. *Current Science*, *124*(5):599-606.

Ascheri, D. P. R. et al. (2014). Characterization of starch extracted from the roots of *Cissus simsiana Roem*. & Schult. *Semina: Ciências Agrárias*, *35*(2):787-800.

American Society for Testing and Materials - ASTM. (2003). *Standard test methods for sodium carboxymethylcellulose*. (ASTM:D1439-03), ASTM Internationa: United States.9p.

Barros, P. J. R. et al. (2020). Soybean hulls: Optimization of the pulping and bleaching processes and carboxymethyl cellulose synthesis. *International Journal of Biological Macromolecules*, *144*:208-218.

Bertoldo, J. G. et al. (2010). Emprego de polímeros na qualidade tecnológica de grãos de feijão sob condições de armazenamento. *Semina: Ciências Agrárias*, *31*(4):975-984.

Bouri, M. et al. (2022). Efficacy of a newly developed seed coating formulation for the control of seed-borne diseases. *Turkish Journal of Agriculture and Forestry*, *46*:280-291.

Brasil. (2009). Ministério da Agricultura, Pecuária e Abastecimento. *Regras para análise de sementes*. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Brasília, DF: Mapa/ACS, 395p.

Cai, Z. et al. (2018). Impact of distribution of carboxymethyl substituents in the stabilizer of carboxymethyl cellulose on the stability of acidified milk drinks. *Food Hydrocolloids*, *76*:150-157.

Camargo, F. R. T. et al. (2017). Physiological quality of soybean seeds treated with carboxymethyl cellulose and fungicide. *American Journal of Plant Sciences*, *8*:2748-2757.

Cerrutti, B. M. et al. (2017). Synthesis of carboxymethylcelluloses with different degrees of substitution and their performance as renewable stabilizing agents for aqueous ceramic suspensions. *Industrial Crops and Products*, *107*:54-62.

Chin, J. M., Lim, Y. Y., & Yien, A. S. (2021). Ting biopolymers for biopriming of brassica rapa seeds: A study on coating efficacy, bioagent viability and seed germination. *Journal of the Saudi Society of Agricultural Sciences*, *20*(3):198-207.

Companhia Nacional de Abastecimento - CONAB. (2024). *Acompanhamento da safra brasileira de grãos.* Sétimo levantamento - Safra 2023/24. Conab, v. 11, n. 7, p. 1-117. Available in: <https://www.conab.gov.br/info-agro/safras/graos/boletim-da-safra-de-graos>.

Debiagi, F., Faria-Tischer, P. C. S., & Mali, S. (2020). Nanofibrillated cellulose obtained from soybean hull using simple and eco-friendly processes based on reactive extrusion. *Cellulose*, *27*:1975-1988.

Jahangiri, F., Mohanty, A. K., & Misra, M. (2024). Sustainable biodegradable coatings for food packaging: Challenges and opportunities. *Green Chemistry*, *26*:4934.

Keawkham, T., Siri, B., & Hynes, R. K. (2014). Effect of polymer seed coating and seed dressing with pesticides on seed quality and storability of hybrid cucumber. *Australian Journal of Crop Science*, *8*(10):1415-1420.

Krzyzanowski, F. C., França-Neto, J. B., & Henning, A. A. (1991). Relato dos testes de vigor disponíveis para grandes culturas. *Informativo ABRATES*, *1*(2):15-50.

Ludwig, E. J. et al. (2020). Polymer coating in soybean seed treatment and their relation to leaching of chemicals. *Revista Ambiente & Água*, *15*(6):e2602.

Madsen, M. D. et al. (2016). Emerging seed enhancement technologies for overcoming barriers to restoration. *Restoration Ecology*, *24*(2):77-84.

Moeljani, I. R., Priyadarshini, R., & Wuryandari, Y. (2023). Seed coating application on the quality of rice seeds, shallot TSS seeds, red chili seeds, and cucumber seeds. In R. F. Setiawan. et al. *Proceedings of the 3rd International Conference on Agriculture (ICA 2022)*. Dordrecht, The Netherlands: Atlantis Press, v. 33, (pp.74-82).

Mondal, M. I., Yeasmin, M., & Rahman, M. (2015). Preparation of food grade carboxymethyl cellulose from corn husk agrowaste. *International Journal of Biological Macromolecules*, *79*:144-150.

Nakagawa, J. (1999). Testes de vigor baseados no desempenho das plântulas. In F. C. Krzyzanowski., R. D. Vieira., & J. B. França Neto. (Ed.). *Vigor de sementes: Conceitos e testes*. Londrina, Paraná: ABRATES, (pp.19-50).

Neto, B., Scarminio, I. S., & Bruns, R. E. (2010). *Como fazer experimentos: Pesquisa e desenvolvimento na ciência e na indústria*. 4. ed., Porto Alegre, Rio Grande do Sul, Brasil: bookmam, 414p.

Nóbrega, K. C. et al. (2019). Influence of the chemical structure of different carboxymethylcelluloses on the performance of clay dispersions. *Cerâmica*, *65*(373):28-34.

Nóbrega, K. C., & Amorim, L. V. (2015). Influence of the molar mass of CMC in the rheological behavior and of filtration of clay suspensions. *Cerâmica*, *61*(360):399-408.

Oun, A. A., & Rhim, J-W. (2015). Preparation and characterization of sodium carboxymethyl cellulose/cotton linter cellulose nanofibril composite films. *Carbohydrate Polymers*, *127*:101-109.

Palupi, T. et al. (2012). Pengaruh formula coating terhadap viabilitas dan vigor serta daya simpan benih padi (*Oryza sativa* L.). *Journal of Agronomi Indonesia*, *40*(1):21-28.

Paravar, A. et al. (2023). Microbial seed coating: An attractive tool for sustainable agriculture. *Biotechnology Reports*, *37*(3):e007813.

Pirzada, T. et al. (2020). Recent advances in biodegradable matrices for active ingredient release in crop protection: Towards attaining sustainability in agriculture. *Current Opinion in Colloid & Interface Science*, *48*:121-136.

Rocha, I. et al. (2019). Seed coating: A tool for delivering beneficial microbes to agricultural crops. *Frontiers in Plant Science*, *10*:1357.

Santos, D. M. et al. (2015). Microwave-assisted carboxymethylation of cellulose extracted frombrewer's spent grain. *Carbohydrate Polymers*, *131*:125-133.

Scarsi, M. et al. (2020). Desempenho germinativo de sementes de soja revestidas com polímeros hidrofílicos. *Colloquium Agrariae*, *16*(3):48-59.

Schoeninger, V., & Bischoff, Z. T. (2014). Tratamento de sementes. *Journal of Agronomic Sciences*, *3*:63-73, 2014.

TeKrony, D. M. (2005). Accelerated aging test: Principles and procedures. *Seed Technology*, *27*(1):135-146.

TIBCO Software Inc. (2020). TIBCO Statistica™ (Versão 14.0.0) *Browse Documentation by Product Category*. Available in: <https://docs.tibco.com/products/tibco-statistica-14-0-0>.

Zhang, K. et al. (2022). Biochar coating is a sustainable and economical approach to promote seed coating technology, seed germination, plant performance, and soil health. *Plants*, *11*:2864.