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RESEARCH ARTICLE

Physiological and starch quality of potato tubers discarded from the potato chip industry in Mexico

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ABSTRACT

Agroindustrial potato (*Solanum tuberosum* L.) waste mainly includes peel and pulp; however, potatoes of low industrial quality are also discarded by industry. A viable alternative to give added value to these byproducts is the use of these as seeds, as well as the use of starch. The objective of this work was to evaluate the physiological quality (germination and vigor), starch content and relationship between these variables in discarded tubers from the industry through chemical-structural analyses to better understand their influence on starch composition. The physiological quality of the potatoes was evaluated through germination and vigor tests; likewise, starch was extracted, and the yield was determined. Physicochemical characterization of the starches was carried out through proximal and structural analysis using nuclear magnetic resonance (NMR). The results showed that the tubers had 100.00% germination and 98.66% vigor and were suitable for use as seeds. The starch yield did not significantly differ. However, characterization of the starch revealed significant differences ($p \leq 0.05$) in moisture, ash, carbohydrate and amylose contents, which were influenced by the storage temperature (4 °C) of the tubers subjected to a vigor test, which interferes with enzymatic activity. During germination, the NMR results showed type B crystallinity, which is typical of tubers, indicating that the viability tests did not promote changes in the type of crystallinity. The results obtained can help us understand the effect of germination on the composition of starch and, accordingly, choose the most appropriate applications for this starch, whether food or nonfood.

Key words: NMR, physiological quality, seed tuber, *Solanum tuberosum*, starch.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the third most important food crop globally after rice and wheat and is the main noncereal food crop (Nicolao et al., 2022). In 2021, its global production exceeded 376 million tons. Mexico has outstanding participation, as it is the main potato producer in Central America, with an annual production of just over 1.9 million tons (FAOSTAT, 2023); the states of Sinaloa, Sonora, Puebla, Veracruz, the State of Mexico, Nuevo León and Guanajuato are the main producers in the country (Vallejo Román and Moreno Andrade, 2021).

In Mexico, 56% of its national production is allocated for fresh consumption, 29% of which is demanded by the industry, and 15% is used as seed (Mejía and Castellanos, 2018). During its processing into French fries, crispy and frozen potatoes, large amounts of waste, an average of 90 kg t^{-1} (9%), which mainly includes

peels and pulp, have been reported (Pacifico et al., 2021); however, old potatoes, or those of size and dimensions not suitable for the industry and therefore of low quality, are also considered waste (Torres and Domínguez, 2020).

The desired quality characteristics in potato production depend on the type of industry to which the tuber is destined; however, the most important internal quality parameters include pulp coloration (from white to yellow), DM (18%-20% preferred for salads and canning, > 20% preferred for fried and dehydrated products), and starch content (minimum values of 15%), while external quality traits include size (30-110 mm diameter ideal for French fries, smaller sizes preferred for canning), shape (round/ovoid preferred for boiling and baking, elongated/long oval/round/ovoid for processing), and high resistance to mechanical stress during and after tuber harvest; thus, discarded potatoes could have acceptable physiological quality and be used as seed tubers (Torres and Dominguez, 2020).

Seed quality is important because it ensures high productivity and adequate cultivation under diverse edaphoclimatic conditions, and physiological quality includes germination and vigor (Khaeim et al., 2022). Germination is the most important developmental phase in the life cycle of seeds. This complex process occurs efficiently under appropriate environmental conditions, with temperature and water being the most influential factors. After harvest, seeds enter a state of dormancy, characterized by the absence of visible growth of meristems. In tubers, this dormancy is defined as endodormancy, as the cessation of bud growth is caused by endogenous factors and cannot be interrupted even under optimal environmental conditions for sprouting. Once dormancy is broken, the seed tubers enter a stage referred to as apical, during which the sprout from the apical eye suppresses the growth of lateral eyes. Planting tubers during this stage often results in plants with single stems and, consequently, reduced yields (Kammoun et al., 2020).

Vigor refers to the ability of seeds to produce plants under unfavorable conditions; therefore, vigor can be evaluated through cold tests (development at suboptimal temperatures, generally at 4 °C), electrical conductivity (< 25 $\mu S\ cm^{-1}\ g^{-1}$ seed has a high vigor, 25-29 $\mu S\ cm^{-1}\ g^{-1}$ seed can be used for early sowing with risk in unfavorable conditions, 30-43 $\mu S\ cm^{-1}\ g^{-1}$ seed is not suitable for early sowing especially in unfavorable conditions and > 43 $\mu S\ cm^{-1}\ g^{-1}$ seed has a low vigor), growth rate of the radicle or accelerated aging (Torres et al., 2011).

On the other hand, potatoes discarded from industry could also be used to obtain starch, a polysaccharide composed of glucose units, which constitute the two main components of starch: Amylose and amylopectin. Amylose represents 20%-30% of potato starch and is a linear chain of glucose linked by α-1,4 glycosidic bonds; amylopectin comprises 70%-80% of starch granules and is a highly branched chain of glucose that has α-1,4 and α-1,6 glycosidic bonds (Bangar et al., 2022). The starch content and functional, structural and physicochemical properties vary depending on the botanical source of extraction, allowing a wide range of applications. In the food, textile, chemical, petrochemical and pharmaceutical industries, it is also used for the production of feed, paper, laundry finishes and bioethanol, as well as in the design of biodegradable materials (Maniglia et al., 2020).

In this sense, Torres et al. (2020) discarded potatoes from three cultivars (Agria, Kennebec and Neiker) due to their small size (average sizes, major × minor axis, ranging between 4×4.5 and 5×6 cm) or irregular shape to recover starch (with yields of 24.4%, 22.0%, and 18.5% for the 'Agria', 'Kennebec', and 'Neiker', respectively) and bioactive compounds (phenols) and developed functional hydrogels with attractive mechanical properties; the results indicated that these potatoes have great potential for food and nonfood applications. Liu et al. (2020) investigated the changes in the structural and physicochemical properties of mung bean starch during germination and reported no changes in the shape of the starch granules or in the type of crystallinity (C); however, these starches exhibited a low viscosity, which is why they suggest that sprouted mung bean starch may have potential applications in the production of foods that require low viscosity materials, such as cakes, cookies or biscuits. In this sense, the objective of the present investigation was to evaluate the effect of germination and vigor on residual potato starch through chemical-structural analyses to better understand its influence on starch composition.

MATERIALS AND METHODS

The potatoes (*Solanum tuberosum* L.) 'Fiana' were provided by a potato chip producing company located in the industrial zone of the State of Mexico (Mexico). These tubers did not meet the industrial quality standards established for processing.

Physiological quality of potatoes discarded by the industry

Germination test. Germination was determined by the storage method in bags in the warehouse according to Huraca et al. (2009). The experiment was carried out at 25 °C, 15% relative humidity, under a 12 h light/12 h dark cycle. After 10 wk and 5 d, weekly counts of the sprouts that emerged at weeks 8, 9 and 10 were carried out. The procedure was carried out in triplicate, with 25 experimental units per replicate. The germination percentage was determined with Equation 1.

$$\text{Germination (\%)} = (\sum\text{Germinated potatoes})/(\text{Total potatoes})\times 100 \ldots. (1)$$

Vigor test. The vigor of the potatoes was evaluated using the cold test according to Torres et al. (2011). The experiments were carried out in triplicate (25 experimental units per replicate) in a cold chamber (Icehaus, RV2PSSS01, Queretaro, Mexico) at 4 °C and 70% relative humidity under dark conditions for 4 wk. Subsequently, the tubers were subjected to germination as described above. The sprouts were counted at the eighth, ninth, and tenth weeks after the cold test started. Equation 2 was used to determine vigor.

$$\text{Vigor (\%)} = (\sum\text{Normal germinated potatoes after cold test})/(\text{Total potatoes})\times 100 \ldots. (2)$$

Starch extraction. Starch was extracted from the germinated potatoes and subjected to a vigor test (discarding the damaged potatoes) according to the method of Vargas et al. (2016); the potatoes were subsequently washed and peeled (without removing the sprouts), after which their size was reduced with a custom-made grater. The mass obtained was macerated for 30 min with constant stirring at 50 rpm on a magnetic stirrer (Daigger model 22407 A) (100 g potato 200 mL^{-1} water), after which the supernatant was subsequently recovered. After filtering through Whatman N°1 filter paper with a pore size of 11 µm, this process was repeated three times (50 mL water 100 g^{-1} potato). The mass was discarded, filtrate was left to rest for 1 h, and the sedimented starch was recovered by decantation. The starch obtained was washed twice with distilled water. Finally, the decanted sample was dried for 1 h at 60 °C and stored in hermetically sealed plastic bags until later use. The yields are expressed as percentages and were obtained with Equation 3:

$$\text{Yield (\%)} = (\text{Final weight (g)})\times 100/\text{Initial weight (g)} \ldots. (3)$$

Proximal composition analysis of starch

Humidity content. The moisture content was determined in triplicate according to Pardo et al. (2013) using a moisture analyzer (OHAUS MB35, Parsippany, New Jersey, USA) and a 1 g sample.

Ash content. The ash content was obtained according to the total ash method (Ismail, 2017). Briefly, the constant weight of the crucibles was determined at 600 °C for 2 h; subsequently, a 1 g sample was deposited in each crucible and placed in a muffle furnace (FE-360, Felisa, Jalisco, Mexico) at 550 °C for 8 h until homogeneous white-grayish ashes were obtained. The test was carried out in triplicate to determine the percentage of ashes, as shown in Equation 4:

$$\text{Ash content (\%)} = (\text{Crucible weight at constant weight with ash (g)} - \text{Constant crucible weight (g)})/(\text{Sample weight (g)})\times 100 \ldots. (4)$$

Protein content. The protein content was determined by Kjeldahl according to Nielsen (2017) with some modifications. For the digestion stage, 0.1 g sample was added to the Kjeldahl tubes, 4 mL concentrated H_2SO_4 and 0.4 g digester mixture (selenium reactive mixture) were added, and the tubes were placed in a digester (MBC-6 TS, RAYPA, Barcelona, Spain) at 360 °C until no black dots were perceived in the tubes

and a light blue color was observed. The distillation stage was carried out by adding 40 mL distilled water and 14 mL 40% NaOH to previously cooled Kjeldahl tubes. Then, the tubes were connected to a distillation system, to which a glass of water was first placed at the coolant outlet and precipitated with 10 mL 5% boric acid and a few drops of the Shiro Tashiro indicator. The solution was distilled until the color changed to emerald green, and approximately 40 mL distillate were obtained. In the last titration stage, the distillate was titrated with 0.1 N sulfuric acid, and the experiment was carried out in triplicate. The protein present in the sample, expressed as a percentage, was calculated using Equation 5:

$$\text{Protein content (\%)} = (V \times N \times 0.014 \times 100)/m \times 6.25 \ldots. (5)$$

where V is the volume of sulfuric acid used in the titration (mL), N is the normality of sulfuric acid, 0.014 is the milliequivalent of N, m is the mass of the sample (g), and 6.25 is the conversion factor (Khalid et al., 2018).

Lipid content. The lipid content was determined using the Soxhlet method (Nielsen and Carpenter, 2017). The flasks were held at constant weight at 100 °C for 2 h. Afterwards, 2 g sample were added to a cellulose cartridge, which was placed in the extractor and covered with cotton. Hexane was added, and the coolant was coupled. The mixture was heated to boiling for 3 h. Once the lipids were extracted, the flasks were placed in an oven at 60 °C for 1 h to evaporate the residual solvent. Three replicates of each sample were performed. The percentage of lipids was determined by Equation 6:

$$\text{Lipid content (\%)} = ((\text{Weight of flask with sample (g)} - \text{Weight of flask (g)}) \times 100)/(\text{Weight of sample}). (6)$$

Amylose content. The amylose content was determined using the methodology of Hoover and Ratnayake (2001). First, 25 mg of previously extracted starch in 10 mL 90% dimethyl sulfoxide (DMSO) was added, and the mixture was stirred for 20 min and then subjected to a water bath at 85 °C for 15 min. The mixture was cooled and brought to 25 mL with distilled water. An aliquot of 1 mL was taken and diluted with 5 mL iodine solution (0.0025 mol L^{-1}) in potassium iodide (0.0065 mol L^{-1}) (I_2 KI^{-1}) and made up to 50 mL with distilled water. The absorbance was read at 600 nm using a spectrophotometer (Multiskan GO, Thermo Scientific, Waltham, Massachusetts, USA), and mixtures of DMSO and iodine solution in potassium iodide were used as blanks. The amylose content of the starch samples was determined using a standard curve prepared from mixtures of potato amylose and amylopectin (Sigma Aldrich, Mexico), which contained 0%-100%, 10%-90%, 20%-80%, 30%-70%, 40%-60%, 50%-50%, 60%-40%, 70%-30%, 80%-20%, 90%-10% and 100%-0% starch (amylose-amylopectin) treated in the same way as the extracted starch sample. All the determinations were analyzed in triplicate.

Structural analysis

NMR Spectra. ^{13}C nuclear magnetic resonance (NMR) analyses were performed using a Avance III spectrometer (Bruker, Billerica, Massachusetts, USA) operating at 500 MHz using CDCl3. The NMR spectra were referenced using the residual CHCl3 signal at 77.26 ppm (chemical shifts) (Gómez and González-García, 2018).

Statistical analysis

The results are presented as the average of triplicate measurements ± standard deviation. The data obtained in percentage form were transformed by arcsine to optimize the normality of the data. The ANOVA was carried out with a completely randomized design, and the Tukey test was applied ($p \leq 0.05$) to determine differences between means using SAS statistical software (version 9.3, SAS Institute, Cary, North Carolina, USA).

RESULTS AND DISCUSSION

Physiological quality of potato seed tubers

The potatoes discarded from industry showed 100% germination, and the germination percentage after 10 wk of submission to the vigor test was 98%, with an average of 5.62 and 5.78 sprouts per potato, respectively. It has been reported that low temperatures (4 °C) induce early sprouting of simultaneous shoots

and increase the number of stems when tubers sprout (Kammoun et al., 2020). Melo et al. (2021) reported germination percentages of 65%-94% and an average number of sprouts per potato of 3-11 depending on the genotype used over a period of 4 wk. On the other hand, Siregar et al. (2021) obtained 91.67% germination, with an average number of sprouts per potato of 1.79 at 5 wk.

In the present investigation, sprouts began to emerge after week 8; that is, a latency period was observed, unlike what has been reported by other authors (Melo et al., 2021; Siregar et al., 2021). Torres et al. (2011) state that this period lasts between 2 and 14 wk. This period extends from harvest until the moment the tubers begin to sprout and depends on several factors, such as variety, size, damage caused to the tuber, ripening conditions at harvest, temperature during the vegetative growth stage and storage conditions, such as temperature, humidity and light, since they significantly affect the biological and biochemical enzymatic activities in the germination and growth stages of seedlings (Khaeim et al., 2022).

Starch yield

The starch yields from ungerminated, germinated, and tubers subjected to the vigor test were 4.14 ± 0.92%, 4.40 ± 0.49%, and 4.04 ± 0.13%, respectively, and were not significantly different. These values were lower to those reported by Peña Carrasco (2017), who obtained potato starch yields ranging from 6.58%-10.19%. Vargas et al. (2016) and García-Mogollón et al. (2018) reported yields of 16.5% and 17.8%, respectively. These variations are attributed to various reasons, the extraction conditions (centrifugation, filtering, grinding, etc.) being the most important. The germination process could play an important role in determining yield compared to starches from ungerminated sources since enzymes such as α-amylase, β-amylase, and α-glucosidase, which are responsible for starch degradation, are produced (Li et al., 2017).

Proximal composition analysis of starch

Humidity. Table 1 shows the results of the proximal starch analysis of germinated and sprouted potatoes subjected to the vigor test. Pardo et al. (2013) reported values similar to those obtained in this study with 5.8%-6.9% humidity; on the other hand, Acosta et al. (2018) reported higher humidity (16.9%-20.8%) for potato starch. Humidity levels for dry starches typically range from 6% to 16%. The storage temperature and drying method employed are responsible for this variance; however, the humidity should not exceed 13%, as greater humidity levels might harm products by causing microorganism growth and a decline in quality (Ziegler et al., 2023). The starch moisture content of tubers subjected to vigor tests significantly decreased ($p \leq 0.05$) because these potatoes were subjected to low temperatures, which caused a loss of water, as cooling has been reported to promote water removal from food (Duque et al., 2011).

Table 1. Proximal starch composition of germinated potatoes, and tubers subjected to vigor. Values in each line with different letters are significantly different (Tukey, $p \leq 0.05$). The average value of three replicates ± standard deviation. **Carbohydrates were determined by weight difference.

	Starch	
Component (%)	Germinated potatoes	Potatoes subjected to vigor
Humidity	7.58 ± 0.08[a]	4.39 ± 0.110[b]
Ash	0.03 ± 0.00[b]	0.08 ± 0.00[a]
Lipids	0.33 ± 0.04[a]	0.33 ± 0.05[a]
Protein	0.76 ± 0.08[a]	0.81 ± 0.02[a]
Carbohydrates**	91.27 ± 0.14[b]	94.36 ± 0.13[a]
Amylose	20.60 ± 0.89[a]	14.16 ± 0.58[b]

Ash. The ash content in the starches was lower than that reported by other authors; Neeraj et al. (2021) reported an ash content of 0.2% to 0.48%. Vargas et al. (2016) and Pardo et al. (2013) reported 0.43% and between 0.23% and 0.44%, respectively (without sprouting). It is known that ashes are made up of minerals such as P, which is necessary in the germination process for obtaining energy for the growth and development of plants, which can explain the low values found in this study.

A significant difference ($p \leq 0.05$) was detected in the ash content; the highest value (0.08%) was for potato starch subjected to the vigor test, and these potatoes were subjected to low temperatures (4 °C) during the vigor tests. Cold stress directly affects the rate of nutrient and water absorption, which may explain the greater amount of ash produced due to low nutrient mobilization (Hussain et al., 2018).

Protein content. The protein content in potato starch observed in the present investigation was similar to that reported by other authors for ungerminated potato starch; Alvis et al. (2008) reported a value of 0.62% for 'Ica Nariño' potato starch. Pardo et al. (2013) reported values of 0.28% to 0.33% for potato starch from various cultivars, while Vera Bravo and Chavarria Chavarria (2020) reported a value of 0.35% for 'Leona Blanca' potato starch. Germination leads to a decrease in proteins, since there is an increase in hydrolytic enzymes, which breakdown main compounds such as starch and proteins; the hydrolysis of proteins produces small peptides and amino acids, which can be used by developing plants for the synthesis of new proteins or to supply energy through the oxidation of their C skeleton (Yang et al., 2021).

Lipid content. The lipid content in starch (0.33%) was similar to that reported by Alvis et al. (2008), with 0.35% from potato starch from the 'Ica Nariño'. Vargas et al. (2016) obtained 0.30% lipids from 'Unica' potato starch. Vera Bravo and Chavarria Chavarria (2020) reported a lower lipid content (0.12%) in potato starch from the 'Leona Blanca'. Several reports indicate that lipids tend to increase during germination since they are related to the synthesis of new lipids (Xu et al., 2019), such as fatty acids and glycerol from triacylglycerol, to subsequently produce energy. Furthermore, lipid compounds are degraded after the radicle emerges, and they are subsequently converted into starch or soluble carbohydrates (Ramírez-Pimentel et al., 2015).

Carbohydrate content. The percentage of carbohydrates reported in the present study is greater than that reported by other authors (for ungerminated potato starch); Vera Bravo and Chavarria Chavarria (2020) reported a value of 85.87% for 'Leona Blanca' potato starch. Chuiza-Rojas et al. (2021) obtained potato starch with a lower carbohydrate content (71.46%). The increase in carbohydrate content after germination may be due to what was previously mentioned by Ramírez-Pimentel et al. (2015). During this process, lipid compounds, which are converted into starch or soluble carbohydrates, are degraded after the radicle emerges.

The carbohydrate content was greater in the potato starch subjected to the vigor test. During germination, the activity of certain enzymes, such as hydrolytic enzymes, increases; however, factors such as temperature determine the activation time. At low temperatures, enzymatic activation is generally slower, so a higher carbohydrate content could be observed in the starch of potatoes subjected to vigor since there was slower enzymatic degradation due to the lower temperatures to which the potatoes were subjected. This could also be reflected in the protein values obtained, although these did not present differences; moreover, the protein content was greater for the starch of potatoes subjected to vigor (Guzmán-Ortiz et al., 2019).

Amylose content. The amylose content allows starches to be categorized as waxy (< 15%), normal (20%-35%) or high (> 40%) (Gonçalves et al., 2020). In the present study, the amylose content ranged from 14.1% to 20.6%. Torres et al. (2020) obtained similar values of amylose (15.6%-29.3%), while Solarte-Montúfar et al. (2019) obtained values of 24.8%-26.2% in potato starch from various varieties; moreover, 19.28% amylose was reported for Chinese potato (*Colocasia esculenta* (L.) Schott) starch (Chuiza-Rojas et al., 2021).

The germination process reportedly causes a decrease in the amylose content. In this sense, Li et al. (2017) reported a decrease in amylose content after germination in rice and millet starch of 20%-19% and 3.3%-2.9%, respectively, attributed to the production of enzymes responsible for starch degradation.

In this study, a significant decrease ($p \leq 0.05$) in amylose content was observed in the starch of potatoes subjected to the vigor test. At a lower temperature, a higher amylose content was expected due to slow enzymatic degradation; however, Neoh et al. (2020) reported, for example, that, for wheat during the grain filling period, the synthesis of α-amylase (the enzyme that degrades amylose) occurs when the seed experiences a thermal shock of cold temperature, thus increasing the amount of this enzyme, which may explain the decrease in amylose in the starch of potatoes subjected to vigor test.

Structural analysis

NMR spectra. The ^{13}C NMR spectra for starch from germinated and vigor test potatoes are shown in Figures 1 and 2, respectively. In both spectra, five peaks were detected between 60 and 105 ppm; the peak detected at approximately 60 ppm was due to C6 of glucose; the broad peak observed between 70 and 80 ppm was related to C2, C3 and C5; and at 80 ppm, C4 was detected, while C1 showed two peaks at approximately 100 ppm.

The C1 resonance provides additional information about the double helical symmetry, conformation, and crystallinity of the starch molecule. For type A crystallinity, the resonance of C1 displays three peaks at 102, 101 and 100 ppm; for type B crystallinity, which is typical of tubers, it presents two peaks at 101 and 100 ppm. While a broad peak at around 103-104 ppm is characteristic of individual amorphous helices and the crystalline V-type phase, suggesting a higher proportion of short amylopectin chains attributed to their degradation (Pardo et al., 2013; Li et al., 2017; Liu et al., 2020).

The spectra of this study reflected two peaks for the C1 resonance, indicating type B crystallinity. Germination is known to produce changes in crystallinity, causing differences in the chemical structure and composition of starches, as well as damage to amylose and amylopectin; however, it was observed that the germination process failed to damage the structure of glucose (a starch monomer), which suggests that additional germination time is required to achieve a change or reduction in crystallinity (Li et al., 2017).

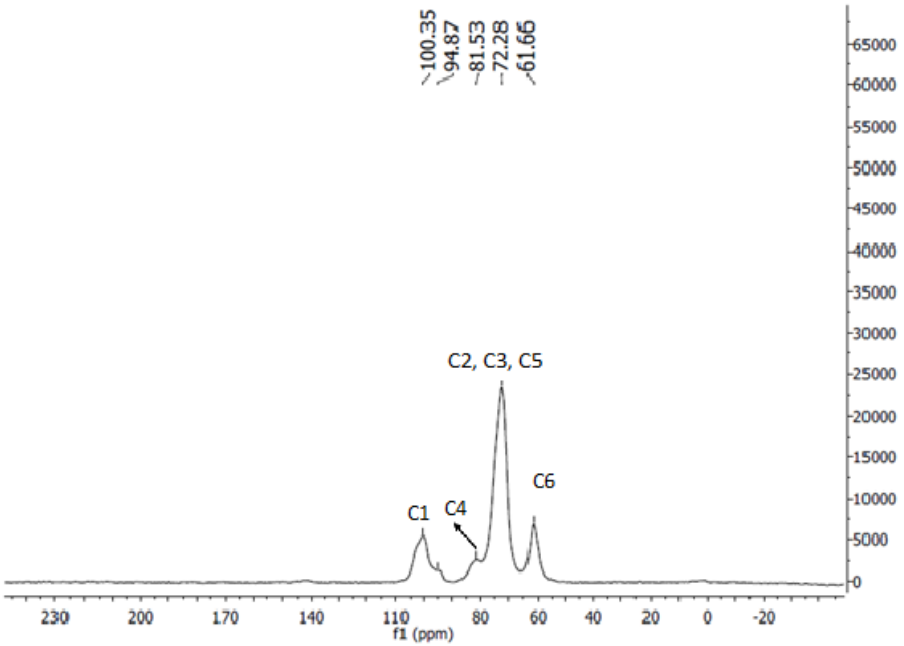

Figure 1. Nuclear magnetic resonance (NMR) spectra of germinated potato starch.

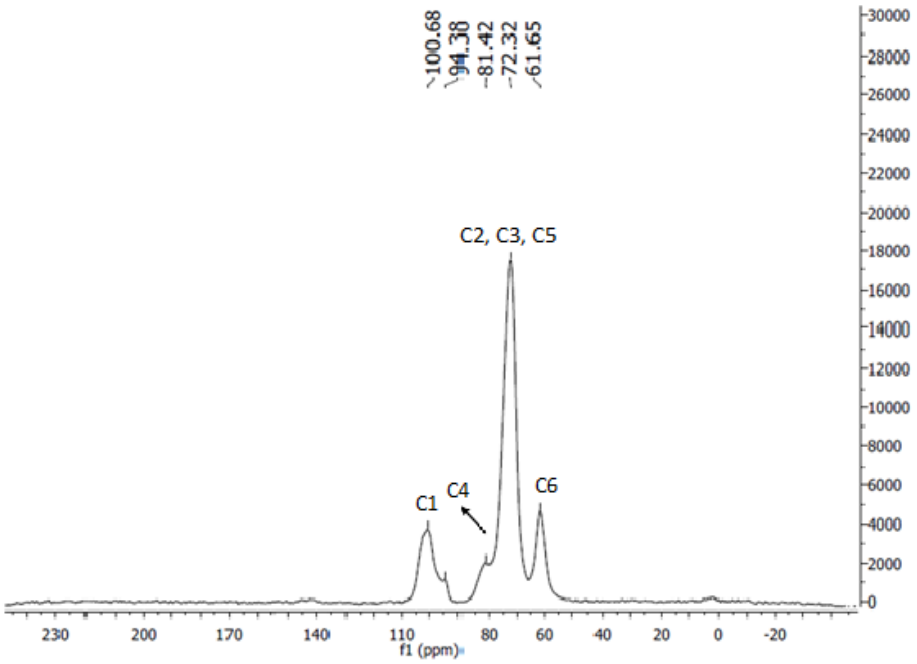

Figure 2. Nuclear magnetic resonance (NMR) spectra of potato starch subjected to a vigor test.

CONCLUSIONS

In this study, the physiological quality of residual potatoes that did not meet the quality requirements for industrial use was evaluated. The viability tests indicated that these tubers are suitable for use as seeds, providing an alternative to solve the generation of this agroindustrial waste. The starch yield was low, and no differences were observed in the starch content after the germination and vigor tests. The nuclear magnetic resonance (NMR) spectra of the extracted starch revealed type B crystallinity, and germination and vigor tests also failed to affect the structure of the glucose. In addition, changes in the proximal composition of the starch due to the storage temperature (4 °C) during the vigor test were detected, which influences the enzymatic behavior and quality of the starch obtained. The results of this study allow us to understand the influence of germination and vigor tests on the composition of starch and therefore choose appropriate applications for this starch in the food or nonfood industry according to its composition.

Author contribution

Conceptualization: D.G.M-A., C.L.A-M., J.R.R-N. Methodology: C.L.A-M., J.R.R-N., T.J.M-S. Software: T.J.M-S. Validation: J.C-P., G.G-G., G.M.G-B. Formal analysis: D.G.M-A. Investigation: D.G.M-A., C.L.A-M., J.R.R-N., T.J.M-S. Resources: C.L.A-M., J.R.R-N., T.J.M-S. Data curation: J.C-P. Writing-original draft: D.G.M-A. Writing-review & editing: C.L.A-M. Visualization: G.M.G-B. Supervision: C.L.A-M., J.R.R-N. Project administration: J.R.R-N. Funding acquisition: J.R.R-N. All co-authors reviewed the final version and approved the manuscript before submission.

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