Original Research Article

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Evaluation of packaging systems with O_2-absorbers on quality of minimally processed soybean sprouts

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Abstract

This study evaluated the influence of O_2-absorbing sachets into different packages polyethylene terephthalate (PET – E1), low density polyethylene (LDPE – E2), and PET/LDPE (E3) on the quality of minimally processed soybean sprouts (MP-sprouts). The MP-sprouts were stored up to 12 days and characterized for physicochemical, microbiological and sensory attributes. The O_2-absorbing sachet showed changes during exposure to the environment by the formation of amorphous iron hydroxide, which was observed by the analysis of functional groups and XRD. The packaging used in the study showed high transmittance and clarity, and low Haze, being a good feature for storing the MP product. All packaging systems showed an increase in the volume of absorbed oxygen (cm^3) up to 9 days of storage. The physic-chemical characteristics of the MP-sprouts stored for 12 days were preserved in the different packages, showing no difference regarding the use of the O_2-absorbing sachet. The O_2-absorbing sachet not influenced the quality of MP-sprouts during the stored (12 days). The best visual aspect was observed in PET package (with and without O_2-absorbing sachet) being classified as excellent, without color change. The MP-sprouts also fulfilled the microbiological quality standards and presented 75.11% acceptability and 72.40% purchase intention.

Keywords

Active packaging, consumer acceptance, microbiological quality, visual aspect

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INTRODUCTION

Health-conscious consumers are increasing in the last years, who prefer food health products, of easy preparation (Cantelli et al., 2020; Cantelli et al., 2017; Scherer et al., 2021a, 2021b). According to the Plant Based Foods Association of United States (2020), there is a market of $ 5 billion, which grew 11.4% more than in 2019. Among these type of foods, soy sprouts could be a potential alternative, since it is a natural food, good for human health, obtained from germination of grains, developing in a short period of time (5 days), without application of fertilizers or pesticides' (Cantelli et al., 2017). Soy sprouts have been recognized as functional food products and highly nutritious, rich in phytochemicals, vitamins, minerals, enzymes, and amino acids (Dahmer et al., 2018, 2020; Losado et al., 2018; Rigo et al., 2015).

The minimally processed products also called semi processed food ready to eat, are fresh vegetable obtained by the following processing steps: selection, washing, cutting, sanitization, drying, and packaging, among others (Cruz

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et al., 2019). However, for fresh soy sprouts minimally processed (MP-sprouts) there is a great challenge for their conservation, since they are highly perishable and susceptible to mechanical damage cells, which limits their shelf life. To maintain quality of minimally processed vegetables they can be stored for several days at adequate refrigeration, modified atmosphere or active packaging, which will aim extension of the shelf life. MP-sprouts to be marketed need specific packaging to ensure safety and maintenance of the nutritional and sensory quality (Costa Monção et al., 2021).

One of the factors that contribute for food deterioration is the presence of oxygen. High levels of oxygen in food packages should be avoided, as it promotes microbial growth, which can cause changes in color and losses in nutrition (Yildirim et al., 2018). Other way of mitigating this problem is the use of oxygen-absorbing sachets, which are able to decrease concentration of this gas, maintaining original quality of the product (Apicella and Incarnato, 2019; Dey and Neogi, 2019), and they can extend the shelf life from 3–4 to 14 days (Gaikwad and Lee, 2017). The efficiency of the absorbers depends on their composition, temperature and packing material and moisture. There are several types of oxygen absorbers, such as enzymes, photosensitive matrices, organic acids, polymers, powdered metals are used, among others (Yildirim et al., 2018).

Oxygen absorbers based on the iron oxidation principle, are found in the form of small sachets, containing reducing agents such as iron oxide, ferrous components or platinum, combined with various catalysts. The air-permeable sachets are placed inside the packaging where they react with water vapor from the food, forming active metal reducing agents, which capture oxygen inside the packaging and converts to more stable oxides (Cruz et al., 2008). These can also be applied under refrigeration and freezing conditions (Patel et al., 2019).

For greater effectiveness of absorbers, packaging or films with a low gas permeability rate should be used (Ribeiro-Santos et al., 2017), according to food characteristics and processing. PET package is commonly used in food industry due to its aesthetic aspect (brilliance and transparency), and to the composition that guarantees recyclability promoting lower impact to the environmental. Another type of package largely used is the LDPE which has excellent chemical resistance, is semi-rigid and translucent (Ahn et al., 2016; Gaikwad et al., 2018).

Due to the high perishability of MP soybean sprouts, studies on packaging that improve shelf life, customer acceptability, and food security must be explored. This work shows as novelty the use of packaging systems with and without O_2-absorbers on quality of minimally processed soybean sprouts. The literature is still little explored on minimally processed soybean sprouts. Thus, there is a need to study the storage and shelf life of this product. In addition, to date, no studies have been found on the effect of using oxygen-absorbing sacks in packages containing minimally processed soybean sprouts. In this sense, this work aimed to evaluate the influence of O_2-absorbing sachet in different packages (PET, LDPE, and PET/LDPE) for storage of MP-sprouts and evaluated during the storage and characterized for physicochemical, microbiological and sensory attributes.

MATERIALS AND METHODS

Materials

The commercial O_2-absorbing sachets with absorption capacity of 100 mL O_2 were purchased from PackFresh company (USA). The active compound was iron carbonate ($FeCO_3$), which is activated by humidity to promptly react with atmospheric O_2. The total sachet filler mass was 5.0 g.

The packages used in this study were a rigid polyethylene terephthalate (PET) bowl (12.3 cm × 3.7 cm) purchased from Nova Pack Packages (Brazil), and a flexible low density polyethylene (LDPE) (5.5 cm x 7.7 cm) purchased from Atacadão das Embalagens (Brazil), and a stand up pouch PET/LDPE (30%/70%, 15 cm x 10 cm) purchased from CBC Embalagens (Brazil).

Characterizations

The structural properties and molecular composition changes of the O_2-absorbing sachets were characterized before and after prolongated exposure (12 days) to the environment by X-ray diffraction (XRD) and Fourier-transform infrared spectroscopy (FTIR). The XRD measurements were performed on an XRD 6000 diffractometer (Shimadzu, Japan) operating with monochromatized Cu Kα radiation ($\lambda = 1.5405$ Å) with a Ni filter. The XRD patterns were recorded at room temperature over the 2θ range 5–80° using a scanning speed of $2° \text{ min}^{-1}$. FTIR was carried out with a VERTEX spectrometer (Bruker, Germany) using the attenuated total reflectance (ATR) mode.

The optical properties luminous transmittance, haze and clarity of the packages (PET, LDPE, and PET/LDPE) were evaluated according to ASTM D 1003-07 (Committee, 2013). The determinations were performed in quadruplicate on a BYK Gardner meter (Haze-Gard Plus, Germany) at 25°C.

Minimally processed soybean sprouts (Mp-sprouts)

The soybean sprouts were produced according to (Cantelli et al., 2020). The soybean seeds used in this work were from the cultivar BRS 216 (Season 2017/2018) provided by the Embrapa Trigo, Passo Fundo/RS. Briefly, the soybean seeds (80 g) were placed in a germination chamber previously cleaned with 10% sodium hypochlorite. The germination was carried out using a soaking time of 6 h, with the seeds being irrigated every 12 h with 20 mL of chlorinated (2 mg.L^{-1}) potable water. The sprouts were harvested manually after 120 h of germination when they were 10 cm tall. The roots were taken out, and the sprouts were washed in running potable water followed by centrifugation (Dynasty) for 2 min to remove the water excess. At the next step, 40 g of MP-sprouts were packed in each type of package (PET (E1), LDPE (E2), and PET/LDPE (E3)) (supplemental Fig. S1) without or with the O_2-absorbing sachets. The packages were sealed and stored under refrigeration (5°C) and relative humidity (RH) of approximately 90%. The sachets and sprouts were analyzed after 3, 6, 9 and 12 days of storage.

Determination of O_2 volume reacted with the O_2-absorbing sachets

The volume of O_2 reacted with the sachets was estimated by thermogravimetry (TG) using a Q50 thermal analyzer (TA Instruments) previously calibrated with a zinc standard. The fillers of the sachets (30–50 mg) were placed in a Pt crucible and immediately heated up to 800°C using a heating rate of 20°C.min^{-1} and dynamic N_2 atmosphere flowing at 60 mL.min^{-1}. Thus, the water mass relative to the total sample mass was determined. The reacted O_2 volume was estimated considering the oxidation of Fe^{2+} ions to $Fe(OH)_3$ and its further thermal decomposition, according to reactions 1 and 2 (Cichello, 2015).

$$4Fe + 3O_2 + 6H_2O \leftrightarrows 4Fe(OH)_3 \quad (1)$$

$$2Fe(OH)_3 \leftrightarrows Fe_2O_3 + 3H_2O \quad (2)$$

The total reacted O_2 volume was estimated from the total $Fe(OH)_3$ mass found in the sachets after storing. The total $Fe(OH)_3$ mass was calculated from the water mass determined from the TG curves. The experiments were carried out with one repetition per sample.

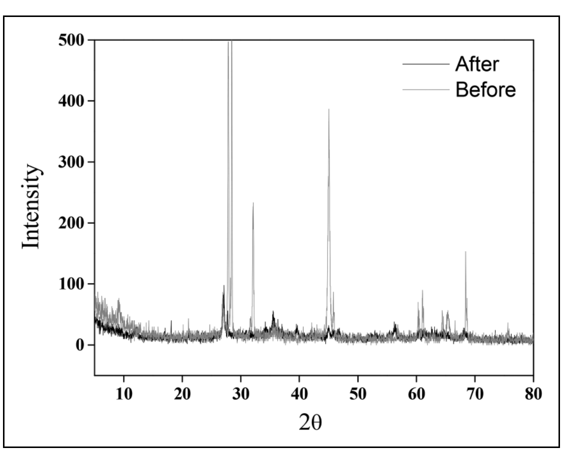

Figure 1. X-ray diffraction patterns of O_2-absorbing sachet filler before and after exposure to regular atmosphere at 23°C for 12 days.

Characterization of MP-sprouts

The microbiological analyses of the minimally processed sprouts consisted in determining the most probable number (MPN) of coliforms at 45°C, counting of positive coagulase *Staphylococcus* and *Salmonella sp* (Brasil, 2001). These tests were performed on the MP-sprouts stored with and without the O_2-absorbing sachets for 12 days. The quality of the potable water used in the soybean sprout germination was evaluated in relation to heterotrophic bacteria counting, total coliforms, and thermotolerant coliforms (Brasil, 2001).

The visual aspect of the MP-sprouts was evaluated by a quality index, according to a scale 1 (excellent quality) to 5 (putrefied appearance) (Table S1). Visual quality index is an important factor for food quality and can be used in studies of preservation or storage (Pathare et al., 2013).

The MP-sprouts were analyzed for moisture, protein, ash, mineral components, lipids, and Kunitz trypsin inhibitor activity (KTI), urea activity, and phytic acid. The sprouts were frozen (Indrel @IUT 355D), lyophilized (Edwards® Modulyo) for 48 h, and ground (Cuisinart® DCG-20BKN). The samples were stored in plastic flasks at approximately 8°C prior to use.

The moisture was determined in an air recirculating oven at 105°C for 4 h (AOAC, 2011). The total nitrogen was obtained by Kjeldahl method (IAL, 2008) and multiplied by a factor of 6.25. Ash was obtained by a gravimetric method, after calcination at 550°C for 6 h (IAL, 2008). Mineral components: Mn, K, Zn, Mg, Cu, Fe, and Ca, were quantified by flame atomic absorption spectrometry (Varian, Spectron AA 5) (AOAC, 2011). The lipid content was performed by Soxhlet method (IAL, 2008). The KTI was determined according to (Hamerstrand et al., 1981). This method is based on the trypsin inhibition degree, and the benzoyl-DL-arginine p-nitroanilide hydrochloride substrate (BAPA) hydrolysis. Urease activity was determined according to (AOAC, 2005), where 0.2 g of sample was incubated in 10 mL of phosphate buffered urea solution at 30°C for 30 min, after which the increase in pH units (ΔpH) from pH 7.00 was recorded. The phytic acid was determined spectrophotometry (Latta and Eskin, 1980) with modifications (Ellis and Morris, 1986).

The sensory evaluation of the MP-sprouts was carried out using a consumer acceptance test - a structured 9-point hedonic scale (9 = like it a lot and 1 = dislike a lot) (Queiroz and Treptow, 2006). The tests were performed with the aid of 50 untrained panelists, of both genders and ages from 18 to 50 years. Each panelist received 3 unit of each MP-sprout distributed in containers and encoded with three random digits, accompanied by water. The sprouts attributes evaluated were global acceptance and purchase intention. The experiment was approved by the Research Ethics Committee (URI-Erechim) and registered at the Plataforma Brazil, CAAE number 98627918.4.0000.5351.

All experiments were performed in triplicate and the results were subjected to analysis of variance (ANOVA). The mean values were compared by the Tukey test at the 95% confidence level using the Statistica 5.0 software.

RESULTS AND DISCUSSION

Characterization of O_2-absorbing sachets

The O_2-absorbing sachets were characterized before and after prolongated exposure (12 days) to regular atmosphere at 23°C to describe the chemical changes in the filler due to reaction with atmospheric O_2. For the sachet before exposure to the environment (Figure 1), the XRD pattern shows main reflections at 27.8°, 28.4°, 38.1°, 45.0° and 68.4° of 2θ. The reflection at 45.0° and 68.4° of 2θ is attributed to the main phase of iron carbonate ($FeCO_3$). The reflections at 27.8° and 28.4° of 2θ are indicative of the presence of quartz, since the sachet is composed of $FeCO_3$ (O_2 scavenger) and silica (moisture absorber). The XRD pattern of the exposed filler showed no reflections at 28.42 ° of 2θ while the other decreased in intensity. This is related to the formation of amorphous iron hydroxide (Fe_2O_3) (Cerantola et al., 2017). Metallic iron is the active component of O_2-absorbers due to its high reactivity with O_2 in the presence of moisture. In addition, this component is cheap, safe, and approved as a GRAS (Generally Recognized as Safe) by the FDA (Food and Drug Administration).

The spectrum of the oxygen-absorbing sachets without exposure to the environment (Figure 2), shows a band at 3373 cm^{-1} characteristic of hydroxyl groups stretching, 1637 cm^{-1} characteristic of C = O groups complexed with iron oxide, 1542 cm^{-1} associated with CH_2 vibrations, 1405 cm^{-1} referring to nitrate ions, 746 and 516 cm^{-1} linked to iron oxide (Gaikwad et al., 2017). Nassar et al. (2016) found bands at 1392, 1109, 859 and 735 cm^{-1} for synthesized $FeCO_3$. The authors describe that the absorbance of pure iron carbonate and these stretching vibrations are fingerprints of D3 h symmetry, which in turn is evidence for the presence of carbonate anions in iron carbonate products. The bands at 3140 cm^{-1} and 1660 cm^{-1} can be attributed to the stretching and bending vibrations, respectively, of the adsorbed water molecules interacting with the carbonate anions (CO_3^{-2}) of $FeCO_3$ (Nassar, 2013). After exposure to the environment, the spectrum still displays the bands at 3365 cm^{-1} and 1405 cm^{-1}. The band at 1631 cm^{-1} characteristic of the C = O groups, indicates the complexation with iron oxide (Hwang et al., 2014), and the band at 509 cm^{-1} refers to silicon. The differences in the bands occurred because the reactions with the atmospheric O_2 (Cruz et al., 2008).

Optical characterization of packaging materials

All packages showed high clarity (C), and luminous transmittance (T), and low haze (H) values (Figure 3), which relates to the packaging materials with high optical quality. Thus, the PET bowl (E1) showed the highest C value. PET has high clarity, chemical stability, biocompatibility, good mechanical and gas barrier properties, it can also be recycled, being a suitable material for transparent food packaging applications (Cheng et al., 2022; Palit et al., 2020). LDPE has satisfactory properties such as mechanical resistance and low water vapor permeability (Hong and Rhim, 2012). PET/LDPE packaging has large flexibility and present optical characteristics to be applied in food.

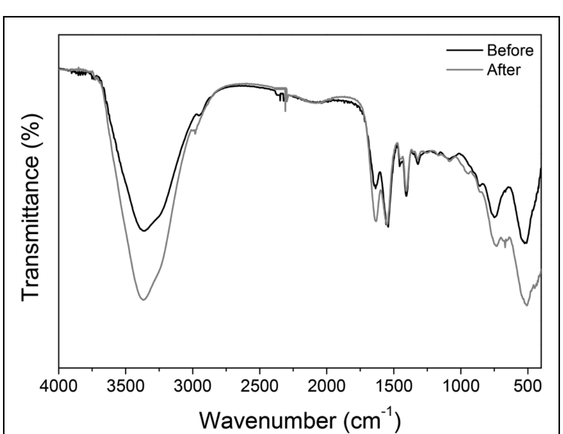

Figure 2. ATR-FTIR spectra of O_2-absorbing sachet filler (a) before and (b) after exposure to regular atmosphere at 23°C for 12 days.

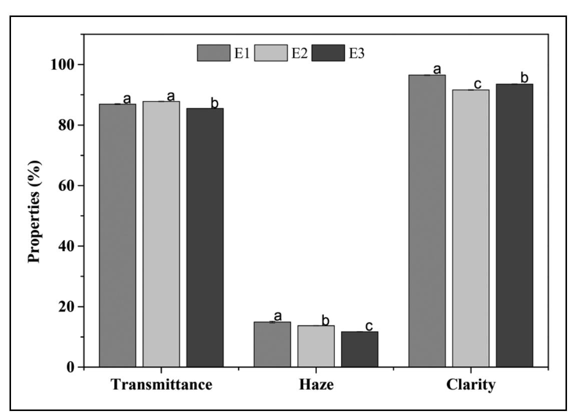

Figure 3. Optical properties of the packages PET (E1), LDPE (E2), and PET/LDPE (E3). Mean ± standard deviation (n = 3) followed by the same lowercase letters are not statistically different at 5% (Tukey's test).

O_2 volume scavenged by the sachets during MP-sprout storage

The TG curves of the O_2-absorbing sachets for different storage times of the MP-sprouts (3, 6, 9, and 12 days) in the different packages are showed in supplementary material (supplemental Fig. S2, S3 and S4). Up to 200°C there was a rapid mass loss, followed by a gradual loss up to 400°C in all the TG/DTG curves. The first mass loss can be attributed to elimination of sachet moisture, while the second loss is ascribed to the decomposition of iron hydroxide ($Fe(OH)_3$) formed from the reaction of the $FeCO_3$ with atmospheric O_2 during MP-sprout storage. $FeCO_3$ is activated with the moisture and automatically starts to absorb the oxygen in the headspace of the package, thus being oxidized to a ferric state, hydrated iron (III) (Cichello, 2015).

In the Figure 4 was verified an increase in the scavenged O_2 volume up to 9 days. However, at 9 and 12 days a drop in the O_2 volume was observed, which may be related to the irreversible reactions of iron with oxygen, which was in excess. Another fact is the formation of a barrier by iron hydroxide particles closer to the sachet walls, which difficult the diffusion of O_2 molecules inside the sachet (Cruz et al., 2008).

In general, both packages showed the same behavior of the volume O_2 absorbed by the sachets. The scavenging efficiency of O_2-absorbing sachet is influenced by the type, size, and capacity of the sachet (Cichello, 2015), as well as the food water activity and initial oxygen level in the packaging headspace.

MP-sprouts

For the MP-sprouts, the microbiological analyses showed absence of *Salmonella sp.*, coagulase-positive *staphylococci* (<10 CFU.g^{-1}) and coliforms at 45°C (<3 MPN.g^{-1}), in accordance with the current legislation (RDC n°. 12 of 2 January 2001), which establishes absence of *Salmonella sp.*, for coagulase-positive

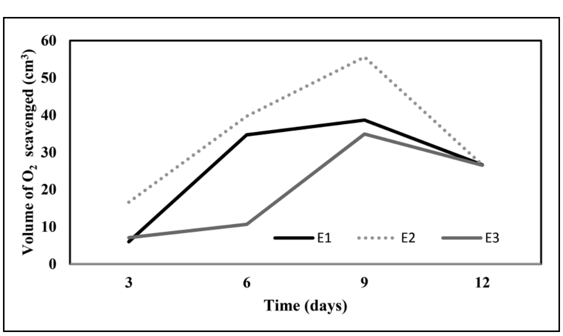

Figure 4. Volume of O_2 (cm^3) scavenged by O_2-absorbing sachets during storage of MP-sprouts in the different packaging systems PET (E1), LDPE (E2), and PET/LDPE (E3).

Staphylococci a maximum of 5×10^2 CFU.g^{-1}, and coliforms at 45°C a maximum of 1.0×10^2 MPN.g^{-1} (Brasil, 2001). Several extrinsic hygiene conditions could cause microbial contamination in soybean sprouts, as water (Banerjee et al., 2016). The MP-sprouts stored for 12 days in the different packaging systems, with and without O_2-absorbing sachet did not present microbial contamination. The oxygen scavenger has the purpose to create a low oxygen atmosphere in sealed packs, preventing the growth of microorganisms (Gomes et al., 2009), as observed in the present study.

It was possible to keep the microbiological characteristics of the MP-sprouts due to the processing and adequate storage at 5°C. The MP-sprout shelf life was 12 days for the different packaging systems. The labels of commercial soybean sprouts describe a shelf life up to seven days. Results obtained in the present work showed that with adequate processing and packaging, there was a considerably increasing in the durability of the sprouts. Once, the minimum-processing approaches to which soybean sprouts are submitted render it highly perishable product. The PET (E1), LDPE (E2), and PET/LDPE (E3) provide a very effective packaging system to ensure a reasonable shelf life of the soybean sprouts.

The shelf life of the sprouts is widely evaluated by their visual quality. The most important parameter is the color of the outer surface and hypocotyl of the sprouts. Discoloration of any part and hypocotyl darkening reduce sprout quality and consumer appeal. The MP-sprouts stored in the packaging system PET (E1) (12 days of storage) showed the best visual appearances, without formation of water droplets inside the container, being classified with quality index 1, which means (excellent, without color change) (Figure 5 and Supplemental Table S1). Appearance is the parameter that consumers mostly consider when buying fruits and vegetables (Schifferstein et al., 2019). The MP-sprouts stored in the PET/LDPE (E3) package were the ones with the worst visual aspect, where in 12 days storage showed a darkening color in the hypocotyls. Comparing the visual aspect of the MP-sprouts, the samples packed with O_2-absorbing sachet (12 days of storage) showed a slightly darker surface. This fact may be due to the reduction of the O_2 level inside the container promoted by the sachet, leading to an anaerobic condition, causing darkening in the MP-sprout. This color change was also observed by Silva et al. (2003) when using O_2-absorbing sachets to preserve minimally processed cabbage leaves.

Packaging with and without O_2-absorbing sachet did not presented differences in physic-chemical properties of the product (results not presented). This can be state that the use packaging O_2-absorbing sachet maintained the quality of MP-sprouts. The moisture of the lyophilized MP-sprouts after 12 days of storage were standardized at 5.97% (94.03% dry extract) for the analysis of proteins,

Figure 5. The visual aspect of the MP-sprouts stored (1 and 12 days) in the different packaging systems PET (E1), LDPE (E2), and PET/LDPE (E3) with and without O_2-absorbing sachet.

lipids, ashes, KTI, phytic acid and minerals. There was no significant difference ($p > 0.05$) in the moisture, protein, ash, lipids, KTI, urea activity, phytic acid and mineral components of MP-sprouts, stored for 12 days in the different packaging systems PET (E1), LDPE (E2), and PET/LDPE (E3) (Supplemental Table S2).

According to the results, the general acceptance of the MP-sprouts showed an average of 6.76 "I like it slightly" (Supplemental Fig. S5). The acceptability index of the MP-sprouts was 75.11%. A minimal acceptability index of 70% is necessary to consider the food product acceptable by consumers. Thus, the MP-sprouts showed good acceptance for commercial purposes. The acceptance is a parameter associate with sensory profile of consumption by taster. In addition, soybean sprout consumer acceptability is associated with beany flavor, which is not well accepted, in general. Regarding the purchase intention, 72.40% of the panelists affirmed that they would buy the MP-sprouts.

Conclusions

The O_2 volume scavenged by the sachets in the packaging systems was proportional to the storage time up to 9th day. There was a significant increase in the moisture of the MP-sprouts in the different packaging systems compared to the freshly processed, while the other physical-chemical characteristics were preserved. The MP-sprouts stored for 12 days in both packages met the microbiological quality standards. The E1 package with and without O_2-absorbing sachet stood out in relation to visual characteristics. In this way, the package system E1 was sufficient to ensure the shelf life, color, visual aspect, and good acceptability of the MP-sprouts without addition of the O_2-absorbing sachet.

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Statements

The data that support the findings are available from the corresponding author.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request

Compliance with ethical standards

Ethical guidelines

Ethics approval was not required for this research.

Human and animal right

This article does not contain any studies with animal subjects and has humans' studies of sensorial analysis approved by the CAAE number 98627918.4.0000.5351.

Declaration of Conflicting Interests

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Supplemental material

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