Nano-clays from natural and modified montmorillonite with and without added blueberry extract for active and intelligent food nanopackaging materials

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Abstract

The aim of this study was to evaluate the potential of nano-clays as active and intelligent (A&I) food nanopackaging materials. Nanopackaging is a structured system that allows the storage of certain compounds in a stable form. Nano-clays were prepared from natural and modified montmorillonite (Mnt) with and without added blueberry extract, and characterized in terms of their: X-ray diffraction (XRD) patterns, thermogravimetric (TGA) properties, microstructure, moisture content, water activity (aw), infrared spectra (FTIR), Raman spectra, color parameters, response to pH changes, and antioxidant and antimicrobial activity. Mnt prepared with added blueberry extract showed antioxidant activity and intelligent behavior under different pH conditions. Modifying the Mnt increased the interlayer spacing, thus allowing more blueberry extract to be incorporated within the system. In conclusion, natural and modified Mnt are eco-friendly resources with potential applications for nano-packaging. The addition of blueberry extract imparted intelligent properties to the nano-clays as regards their responses to changes in pH.

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- Thermogravimetric analysis (TGA)
and accounts for nearly 70% of the clay market volume [1,2]. Clay is a naturally abundant, toxic-free mineral used in foods, medicines, cosmetics, and healthcare products [3] as well as being environmentally friendly and inexpensive.

Clays and clay minerals are technologically important materials mainly composed of hydrated aluminosilicate with neutral or negative charged layers [4]. Layered silicates typically have a stacked arrangement of silicate layers (platelets) held apart principally by electrostatic forces. Platelets are only 1 nm thick but have a huge surface area (over 750 m²/g) and aspect ratios ranging from 100 to 500 [1,5,6]. Each layer is characterized by a 2:1 structure or Mg²⁺ and accounts for nearly 70% of the clay market volume [1,2]. Clay is used especially in food and drug nanopackaging. These clay mineral-based systems, as mentioned above, allow the controlled delivery of drugs and prevent oxidative damage in foods, thus reducing food loss. Despite their importance, however, clay mineral systems have been poorly studied.

With this in mind, a nano-packaging system could be developed by incorporating blueberry extract in the silicate interlayer spaces of clay, i.e. between the silicate layers [6,28]. Blueberries contain anthocyanins which change color under different pH due to a shift in their molecular structure from a quinoidal to a flavilium form [29]. The addition of blueberry extract could thus transform these clays into active and intelligent nanocomposites that respond to changes in pH.

These active and intelligent materials derived from eco-friendly resources could be used to produce food nano-packaging that provides consumers with information about the quality and safety of food products. Changes in the coloration of the packaging could be associated with the fraudulent modification of foods, non-compliance of the cold chain, or simply alert consumers to the freshness of the food [30,31]. In this regard, the FAO [32] has estimated that approximately a third of the food produced annually for human consumption is wasted. This represents a loss of about 1.3 billion tons of food, valued at over USD 750 billion, which could feed the more than 842 million of people that still suffer from chronic hunger globally [33]. The incorporation of blueberry extract could, furthermore, enable the production of bioactive compounds that have a favorable impact on the prevention of diseases such as cancer [34] and kidney infections [35].

The goal of this research was to develop nano-clays with potential uses for active and intelligent food nanopackaging. In order to achieve this the physical and chemical properties of two nano-clays derived from Mnt, one natural, and the other modified with a quaternary ammonium salt (dimethyl dehydrogenated tallow ammonium), were analyzed. Nanocomposites prepared from the clays plus blueberry extract were also investigated.

2. Experimental

2.1. Materials

The clays evaluated in this study were natural and modified montmorillonites (Mnt) supplied by Laviosa Chimica Mineraria S.p.A. (Livorno, Italy), and were used as received. According to the manufacturer’s instructions, the modified Mnt is a nanoclay derived from a naturally occurring Mnt, purified and modified with a quaternary ammonium salt (dimethyl dehydrogenated tallow ammonium). This modification was selected based on the fact that it has been well known in the literature for years, and the resulting modified clay is nontoxic with no risk to human health [36–38]. The cation exchange capacity (CEC) of natural Mnt assessed by the methylene blue method, gave a CEC of 105 meq/100 g clay. Blueberry (Vaccinium corymbosum) extract was obtained according to the methodology proposed by Dai et al. [39] using ethanol as a solvent, since it maintains the properties of blueberries. According to Dai et al. [39] the chemical composition of blueberry extract is 100% anthocyanin. Ripe fruits were purchased from a local market in Mar del Plata, Buenos Aires, Argentina. The fruits were selected, discarding any diseased or stained specimens, before weighing out 170 g, crushing and filtering. The residue obtained, mainly the fruit skin, was then washed with 100 mL of ethanol (Aldrich: product code: 34923). Interestingly, this type of waste is normally produced by manufacturing processes during the preparation of filtered blueberry juices. The blueberry extract was prepared the same day the clays were developed, and maintained refrigerated at 5 °C in a dark container until further processing in order to avoid oxidative damages.

2.2. Formation of the nano-clays

The nano-clays were prepared by mixing 2 g of clay and 40 mL of blueberry extract. The mixture was then frozen at −20 °C for 48 h after which it was lyophilized at 100 mTorr and −70 °C for 72 h using a Gland type Vacuum Freeze Dryer, Columbia International, Model FD-1B-50 (Shaan Xi, China) in order to obtain a free flowing product. Lyophilization also preserves the active compounds of the blueberry extract and ensures a size of clay particle in the nanometer range. The resultant clays were conditioned in containers with a saturated solution of NaBr (aq) (0.575 at 25 °C) for seven days prior to each test. During this period the clays were protected from light in a dark room to avoid photodegradation of the antioxidant compounds and pigments. Samples used to determine the water activity (aw) of the clays were not conditioned. Four types of clay samples were prepared as follows: natural montmorillonite (NMnt), natural montmorillonite with added blueberry extract (NMnt-BE), modified montmorillonite (MMnt) and modified montmorillonite with added blueberry extract (MMnt+BE).

2.3. Characterization of clays

2.3.1. X-ray diffraction (XRD)

The X-ray diffraction patterns of the clay powders were
determined using an X-Pert Pro diffractometer (Netherlands) operating at 40 kV and 40 mA, with Cu Kα radiation (λ = 1.5406 Å). Samples of finely ground clay powder were placed in horizontal glass holders. Diffractograms were recorded at a scanning speed of 0.5° per min in an angular range of 2θ = 2°–8°. The distances between the planes of the crystals d (Å) were calculated from the diffraction angles (°) measured from the X-ray patterns, according to Bragg’s law:

\[ d = \frac{n\lambda}{2\sin\theta} \]  

(1)

where \( \lambda \) is the wavelength of radiation Cu Kα, and \( n \) is the order of reflection. For the calculations, \( n \) was taken as 1. The differences between the interplanar distances (\( \Delta d_d \)) of the samples tested were calculated taking as a reference the interplanar distance of the natural montmorillonite (\( d_{NM} \)).

\[ \Delta d_d = d_c - d_{NM} \]  

(2)

where \( d_c \) is the interplanar distance of each clay sample.

2.3.2. Thermogravimetric analysis (TGA)

Thermogravimetric analysis was carried out with a thermal analyzer (TA Instruments) Model TGA Q500 (Hüllhorst, Germany) at a heating rate of 10 °C/min from room temperature to 900 °C under nitrogen atmosphere. The clay mass was in the range of 7–15 mg. The mole fraction of blueberry extract (\( X_{be} \)) incorporated into the clays was calculated as follows:

\[ X_{be} = \frac{R_{Wn+be} - R_{Wn}}{R_{Wbe} - R_{Wn}} \]  

(3)

where \( R_{Wn+be} \) is residual mass of clay plus blueberry extract, \( R_{Wn} \) is the residual mass of clay without blueberry extract, and \( R_{Wbe} \) is the residual mass of blueberry extract. Residual mass values were taken at 800 °C where the decomposition of the blueberry extract was about 0.7%. Analyses were performed in triplicate to ensure repeatability and data were reported as mean values ± SD.

2.3.3. Field emission scanning electron microscopy (FESEM)

FESEM micrographs of the clays were taken, and their average size calculated using a FESEM Supra55, Zeiss (Oberkochen, Germany) at an acceleration voltage of 5 kV. The average size of the clay particles was determined using the well-known image processing software ImageJ by randomly choosing at least 5 FESEM images. All samples were sputter coated with an Ar⁺ ion beam at an energy level of 3 kV and sputter rate 0.67 nm/min with a thin layer of gold for 35 s to ensure electrical conduction and to reduce surface charging during the analysis. The sputter rate was determined using a Ni/Cr multilayer standard.

2.3.4. Moisture content

The moisture contents of the different clays were determined using a Moisture Analyzer, Model MA150 (Goettingen, Germany). Samples (~0.5 g) were dried at 105 °C until constant mass was reached. Measurements were conducted in triplicate for each clay and the results were reported as % of average moisture ± SD.

2.3.5. Water activity (\( a_w \))

The water activity of the different clays was determined in order to evaluate their susceptibility to microbiological growth: a limiting factor for their use as a food additive. Water activity was calculated using a psychrometric \( a_w \) meter Aqualab Cx-2 (Decagon Devices, Pullman, USA) previously calibrated with water at 25 °C. The average value of three measurements per clay type ± SD was reported.

2.3.6. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR/FTIR)

The infrared spectra of the clay samples were recorded on a Nicolet 8700 (Thermo Scientific Instrument Co., Madison, Wisconsin, USA) equipped with a diamond ATR probe at an incident angle of 45°, over the range 4000–600 cm⁻¹, from 32 co-added scans at 4 cm⁻¹ resolution. About 10 mg of each of the finely ground clay samples were placed on the sample holder. Each sample was scanned three times, observing good reproducibility.

2.3.7. Raman spectroscopy

Raman spectra of the clays were obtained using an Invia Reflex confocal Raman microscope (Renishaw, U.K.) with an argon laser. The laser power level was set at 3 mW and the Raman spectra were acquired at 785 nm. The microscope was operated with a 50× objective lens to focus the beam onto the sample. Integration time was 0.5 s, and the number of accumulations 200. spectral resolution and repeatability were better than 1 cm⁻¹ and 0.2 cm⁻¹, respectively. The Raman spectrum for each sample was measured as an evenly-distributed number of points. No thermal effects were observed on the samples during these measurements.

2.3.8. Color

The color parameters of the clays were determined with a Macbeth® colorimeter (Color-Eye 2445 model, illuminant D65 and 10° observer) standardized with a white reference plate (\( L^* = 93.52, a^* = -0.81, b^* = 1.58 \)). Hunter scale values were expressed as \( L^* \) (0 black to \( L^* = 100 \) white), \(-a^*\) (greenness) to \(+a^*\) (redness), \(-b^*\) (blueness) to \(+b^*\) (yellowness) [40]. Differences in color (\( \Delta E^* \)) were calculated according to Eq. (4) described by Gennadios et al. [41]:

\[ \Delta E = \sqrt{\Delta a^2 + \Delta b^2 + \Delta L^2} \]  

(4)

where \( \Delta L \), \( \Delta a \) and \( \Delta b \) represent variations between the color parameters of the samples and the white standard.

The yellowness index (\( YI \)), which as its name suggests determines the degree of yellowness of a substance, was calculated according to ASTM D-1925 [42] and MacFarlane et al. [43] using the CIE L’‘a’‘b’’ scale:

\[ YI = \frac{100 (1277X - 1067Z)}{Y} \]  

(5)

Chromaticity (\( C’ \)) and hue angle (\( \phi \)) were calculated using the following equations [44]:

\[ C’ = \sqrt{(a’)^2 + (b’)^2} \]  

(6)

and

\[ h(o) = 180 + \tan^{-1}\left(\frac{b’}{a’}\right) \]  

for \( a’ > 0 \) and \( b’ < 0 \), and

\[ h(o) = 180 - \tan^{-1}\left(\frac{b’}{a’}\right) \]  

for \( a’ < 0 \) and \( b’ > 0 \)  

(7)

Measurements were taken in triplicate for each type of clay.

2.3.9. Response to pH changes

In order to evaluate the responses of the clays to changes in pH, samples of each system (0.1 g of clay) were placed in 4 mL solutions of pH equal to 1, 7, and 13, prepared from NaOH (0.1 M) and HCl (0.1 M). Changes in clay color were then assessed from images.
taken with an 8.1 mega pixel Cyber-shot Sony camera; model DSC-H3 (Tokyo, Japan).

2.3.10. DPPH antioxidant activity

The total antioxidant activity of each clay type was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical method described by Molyneux [45]. Briefly, 1.97 mg of DPPH radical was dissolved in 50 mL of pure methanol and adjusted to 1.0 absorbance units at 517 nm. Then, 60 µL of each clay sample (0.01 mg/mL) and 840 µL of DPPH radical adjusted solution were mixed in individual tubes and incubated for 60 min at room temperature in the dark. The absorbance of samples was read at 517 nm using a UV-visible spectrometer UV-1601 PC (Shimadzu Corporation, Kioto, Japan). The antioxidant activity of the samples tested was expressed as percent inhibition of DPPH, calculated according to the following equation:

\[ \% \text{Inhibition} = \left( \frac{A_0 - A_{60}}{A_0} \right) \times 100 \]  

where \( A_0 \) and \( A_{60} \) are the absorbance values of the blank sample and the radical plus sample, respectively. Assays were carried out in triplicate.

2.3.11. Antimicrobial activity of clays – disc-diffusion assay

The antimicrobial activity of the clays was evaluated by the agar diffusion method according to Ponce et al. [46]. The zone of inhibition on solid media assay was used for determining the antimicrobial effects of the clays against two typical pathogens: a Gram-negative bacteria, Escherichia coli O157:H7 (32158, American Type Culture Collection), and a Gram-positive bacteria, Listeria monocytogenes innocua, provided by CERELA (Centro de Referencia de Lactobacilos, Tucumán, Argentina). For this, 30 µL of each clay were hydrated with 1 mL of sterile water. The resulting solutions were then poured into 5 mm diameter wells on Mueller Hinton (Merck, Darmstadt, Germany) agar plates, previously seeded with 0.1 mL of inoculum containing approximately 10⁵ CFU/mL of the test bacteria. Plates were incubated for 24 h at 37°C, and then examined to study the inhibitory effect. The total area was used to evaluate the antimicrobial potential of the clays by exactly measuring the diameter of the inhibitory zones surrounding the wells, as well as the area in contact with the agar surface. The antimicrobial effect was then classified using the diameter of each inhibition halo as: not sensitive for diameters less than 8 mm; sensitive for diameters of 9–14 mm; very sensitive for diameters of 15–19 mm and extremely sensitive for diameters larger than 20 mm [46]. Each assay was performed in triplicate on two separate experimental runs.

2.4. Statistical analysis

OriginPro 8 (Version 8.5, Northampton, USA) was used to analyze the resulting data of the properties of the clays. Data were initially evaluated by analysis of variance (ANOVA) and significant results further analyzed using Duncan’s multiple range tests \((p < 0.05)\) to compare mean values.

3. Results and discussion

3.1. X-ray diffraction (XRD)

The X-ray diffraction patterns of the developed clays (Fig. 1) showed that the NMnt exhibited a strong reflection at 2θ = 4.78° corresponding to a basal spacing of 18.6 Å. Similar results have been reported by Merino et al. [47] for natural bentonite clay (13.2 Å). The interlayer spacing of the MMnt (modified) clay \((d\)-value \(= 26.6 \text{ Å})\) was significantly wider than that of the NMnt, representing an increase of around 8.0 Å (Table 1). This is consistent with results reported by de Azeredo [5] where inorganic cations in the clays tested were replaced by other organics by means of exchange reactions.

![Fig. 1. X-ray diffraction pattern of the different clays studied: (a) natural montmorillonite (NMnt), (b) natural montmorillonite containing blueberry extract (NMnt+BE), (c) modified montmorillonite (MMnt) and (d) modified montmorillonite containing blueberry extract (MMnt+BE).](image)

Table 1

| Parameter               | NMnt          | NMnt+BE       | MMnt          | MMnt+BE       |
|-------------------------|---------------|---------------|---------------|---------------|
| \( \Delta d \) (Å)      | –             | 1.9 ± 0.1a    | 8.0 ± 0.1b    | 10.8 ± 0.1c   |
| \( X_{aw} \)            | –             | 0.21 ± 0.001a | 2.1 ± 0.1a    | 2.5 ± 0.1a    |
| Moisture (%)            | 1.9 ± 0.1a    | 2.2 ± 0.1b    | 2.1 ± 0.1a    | 2.5 ± 0.1a    |
| \( a_w \)               | 0.314 ± 0.006a| 0.526 ± 0.004b| 0.347 ± 0.009b| 0.571 ± 0.004b|
| \( a^t \)               | 93.13 ± 0.01a | 53.21 ± 0.01b | 91.19 ± 0.01 | 40.54 ± 0.01c |
| \( b^t \)               | −0.73 ± 0.01a | 0.70 ± 0.002b | −0.51 ± 0.01b | 4.80 ± 0.02c  |
| \( b' \)                | 11.52 ± 0.01 | −2.58 ± 0.03b | −13.24 ± 0.06d| −9.95 ± 0.01a |
| Color difference (DE)   | 9.95 ± 0.01a | 40.57 ± 0.01c | 11.90 ± 0.01c | 54.53 ± 0.01d |
| Whiteness Index (WI)    | 86.57 ± 0.01a | 53.13 ± 0.01b | 84.09 ± 0.01  | 39.52 ± 0.01c |
| Yellow Index (YI)       | 20.74 ± 0.02  | −7.30 ± 0.08b | 24.27 ± 0.01d | −34.33 ± 0.07a|
| \( C^* \)               | 11.54 ± 0.01 | 2.68 ± 0.03b  | 13.2497 ± 0.0002d | 11.049 ± 0.0009b |
| \( h^* \)               | 176.36 ± 0.03  | 344.8 ± 0.3d  | 177.81 ± 0.02b | 334.2 ± 0.1e |

Equal letters in the same row indicate no statistically significant differences \((p \leq 0.05)\).
incorporated into the clays was obtained for the MMnt+BE clay as calculated from the TGA curves (Table 1). This high organic matter content can be explained by the fact that the modified Mnt had the widest interlayer spacing [53], thus making more room available for the packaged blueberry extract.

### 3.3. Field emission scanning electron microscopy (FESEM)

The FESEM micrographs of the clays (Fig. 3) revealed that the clay particles were slightly transformed from an irregular (NMnt clay; Fig. 3a) to a spherical morphology (MMnt clay; Fig. 3c), although the irregular clay remained predominant. The average size of the nanoclay particles also increased from approximately 20 - 37 μm (NMnt clay) to 40 - 57 μm (MMnt clay). These results agree with the increase in the interlayer spacing observed from the XRD patterns. Similar results have been reported by Oztop, & Shahwan [54] for Mnt modified using an alkaline hydrothermal treatment.

An increase in the average size of the clay particles was also observed for the clays nano-packaged with blueberry extract. This is again in line with the XRD results. According to Ruiz et al. [55], one of the factors affecting the morphology of this type of material is that it undergoes a zeolitization process characteristic of the medium, in which ions, such as Na⁺ and K⁺, act as templates around the aluminosilicate units.

### 3.4. Moisture content

The moisture content data for the different clays studied (Table 1) reveals that the moisture contents of the NMnt and MMnt clays were not statistically significant (p > 0.05). The addition of blueberry extract did, however, significantly increase (p < 0.05) clay moisture content (NMnt +BE and MMnt+BE). This suggests that the incorporation of blueberry extract increases the susceptibility of clays to the adsorption of water from the environment. The MMnt+BE clay was the wettest system, possibly because of the higher Xbe that was incorporated into this clay.

### 3.5. Water activity (aw)

The water activity (aw) values of the clays used (Table 1) shows a direct relationship between aw and moisture content. The highest aw value (~0.571) was obtained for samples of blueberry extract-containing modified Mnt (MMnt+BE) which contained the highest mole fraction of the blueberry extract within the clay. Water activity values between 0.6 and 0.8 allow the growth of molds and yeasts, whereas for values between 0.8 and 1.0 bacterial growth is more likely. The aw values obtained here thus suggest that the developed clays are not susceptible to microbiological growth [56].

### 3.6. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR/FTIR)

The FTIR spectra for the developed clays over the entire absorption range (Fig. 4A) show absorption peaks at about 3334 cm⁻¹, corresponding to the stretching and bending vibrations for the hydroxyl (O–H) groups of the water molecules present in the clay samples [47,57]. The increase in intensity of the peak at 3334 cm⁻¹ due to O–H stretching vibrations suggests that the available cations are replaced by protons of polar groups during the different treatments, thus increasing the number of available O–H groups [57]. Specifically, in the region between 3600 and 3000 cm⁻¹ the intensity of the absorption band associated with the O–H groups and free water increases, which can be directly related to the moisture content of the clays (Table 1). In other words, higher moisture contents led to stronger absorption band associated with the O–H

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**Fig. 2.** TGA curves of the different systems studied: (a) natural montmorillonite (NMnt), (b) natural montmorillonite containing blueberry extract (NMnt+BE), (c) modified montmorillonite (MMnt), (d) modified montmorillonite containing blueberry extract (MMnt+BE) and (e) blueberry extract (BE).
groups and free water. The bands located at 2918 cm\(^{-1}\), 2850 cm\(^{-1}\), 2361 cm\(^{-1}\) and 1469 cm\(^{-1}\) correspond to the CO\(_2\) environment. The absorption band centered at around 999 cm\(^{-1}\) is attributed to the Si-O group in plane vibration. Al\(_{3}\)Al\(_{2}\)OH, Al\(_{3}\)Fe\(_{2}\)OH and Al\(_{3}\)Mg\(_{2}\)OH bending vibrations show absorption bands at 916, 880 and 800 cm\(^{-1}\), respectively. A diminution in the intensity of the bands at 999 cm\(^{-1}\) (Si-O stretching) and 814 cm\(^{-1}\) (O-Si-O asymmetric stretching) due to changes in the Si environment can also be observed.

The FTIR spectrum for the blueberry extract (mainly anthocyanins) also shows an absorption peak at about 3334 cm\(^{-1}\) (Fig. 4B) assigned to the stretching and bending vibrations of the O-H groups. In addition, a strong absorption band with a maximum at 1021 cm\(^{-1}\) assigned to aromatic ring C-H deformation, as well as bands at 1638 and 1414 cm\(^{-1}\) corresponding to the stretching vibration of the C-C aromatic ring, can also be seen. The absorption band with a maximum at 1257 cm\(^{-1}\) is assigned to the stretching of pyran rings, typical of flavonoid compounds. Finally, the band appearing at 1344 cm\(^{-1}\) corresponds to C-O angular deformations of phenols.

3.7. Raman spectroscopy

Raman spectroscopy was used in an attempt to investigate the structure of the developed nano-clays a little further. Fig. 5 shows the Raman spectra of the clay samples obtained. This technique is more sensitive in the short range order than XRD, as different crystal modifications of Mnt produce different sets of characteristic Raman bands. Obvious differences in the Raman spectra of the NMnt and MMnt samples were observed, confirming the modification of the clay. However, the samples containing blueberry extract (NMnt+BE and MMnt+BE) did not show clear bands. This suggests that blueberry extract produces a fluorescence phenomenon in clay samples, thus limiting the detection of the spectra.

3.8. Color

The results of the color parameters of the clays studied (Table 1) show that the highest \(L^*\) value was obtained for the NMnt clay, followed by the MMnt, NMnt+BE and MMnt+BE clays, in that order. This indicates that both clay modification and the addition of blueberry extract led to lighter clays. The MMnt+BE clay was the darkest of the four types, possibly due to the greater molar fraction of blueberry extract packaged in this system. The incorporation of the blueberry extract in the clays also increased the \(a^*\) (redness) values probably due to the blueberry extract pigment (anthocyanins). The reddest clay was the MMnt+BE clay, which would be in line with that established above, i.e. the MMnt+BE clay was the system with the greatest capacity to package the blueberry extract. In contrast, the clays without blueberry extract tended towards a green color, with the most negative

![Fig. 3. FESEM micrographs of the different systems studied: (a) natural montmorillonite (NMnt), (b) natural montmorillonite containing blueberry extract (NMnt+BE), (c) modified montmorillonite (MMnt) and (d) modified montmorillonite containing blueberry extract (MMnt+BE).](image)
The $a^*$ value registered for the NMnt clay, i.e. NMnt is the system with the greatest tendency towards green.

The MMnt clay showed the highest positive $b^*$ value, showing that this was the most yellow system. Unsurprisingly, a direct relationship between the $b^*$ values and yellowness index ($YI$) was obtained.

The combination of all the changes observed in each of the $L^*$, $a^*$ and $b^*$ chromatographic parameters produced lower color differences ($\Delta E$) between the clays without blueberry extract. This is consistent with the $WI$ indexes obtained, since the $\Delta E$ values were calculated taking a standard white plate as a reference. According to Obón et al. [67], $\Delta E$'s from 0 to 1.5 can be considered small and virtually identical to the human eye, from 1.5 to 5 color differences can be distinguished, and are obvious for $\Delta E$'s higher than 5. The color differences between the clay samples and the standard white reference plate was thus evident as detected by visual observation. Similarly, the clay samples with added blueberry extract (NMnt+BE and MMnt+BE) were clearly different in color to the samples without the extract (NMnt and MMnt).

A decrease in the chromaticity ($C^*$) values was also observed when the blueberry extract was incorporated into the clay systems. The NMnt+BE clay showed the lowest chromaticity value, meaning that clay had a less vivid color.

The hue angles ($^\circ h$) of the clay systems were correctly located in the quadrants of the CIE $L^*a^*b^*$ color chart.

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Fig. 4. Panel A- FTIR spectra of the different clays studied in all the range absorption: (a) natural montmorillonite (NMnt), (b) natural montmorillonite containing blueberry extract (NMnt+BE), (c) modified montmorillonite (MMnt) and (d) modified montmorillonite containing blueberry extract (MMnt+BE). Panel B- FTIR spectrum in all the range absorption of the blueberry extract.
3.9. Response to pH changes

The images of the responses of the clays to different pH (Fig. 6) reveal that the clays with added blueberry extract (NMnt+BE and MMnt+BE) changed color in both the acid and alkaline mediums. Both these clays showed a red coloration at the acid pH due to the formation of the flavylum cation (red color) of anthocyanins contained in the blueberry extract. In contrast, at alkaline pH the clays with blueberry extract (NMnt+BE and MMnt+BE) changed to a green color as a result of the quinoidal structure of anthocyanins that forms at pH > 8 [68]. At pH = 7 the blueberry extract-containing clays had a purple coloration.

These results suggest that incorporating blueberry extract into nanoclays imparts them with intelligent behavior as it enables them to respond to pH changes in the surrounding environment. These intelligent nanocomposites could be applied in polymeric matrices in contact with fishery products since the production and accumulation of amines, mainly ammonia, trimethylamine, and histamine, as a result of microbial growth would produce an alkaline medium. This would cause blueberry extract-containing clays to change in color, indicating a loss of quality in these types of foods. Similarly, these nanocomposites could be applied in slightly acidic food polymeric matrices, such as meats and fruits [31].

Interestingly, not only the color of the blueberry extract-containing clays changed, but also, slightly, the solution they were in. This confirms the migration of anthocyanin molecules toward the aqueous medium. Anthocyanins can then act as tracers of events occurring within the clay. This suggests that the anthocyanins packaged within the interlayer spaces of the clays generate van der Waals-type interactions between clay and pigment. Otherwise the chromophore groups of the anthocyanins would not allow color changes in the clays under different pH conditions.

3.10. DPPH antioxidant activity

The results of antioxidant capacity of the clays developed (Fig. 7) show that the native and modified Mnt (NMnt and MMnt) had an almost negligible antioxidant activity (~1.5%).

In contrast, the blueberry extract (BE) demonstrated a potent antioxidant effect (approx. 90% inhibition of the DPPH radical),
giving it a potential functional effect. This is consistent with results reported in the literature [34].

A direct link between the mole fraction of blueberry extract (XBE) contained within the clays and the antioxidant activity of these systems (NMnt+BE and MMnt+BE) was also found. The antioxidant effect of these clays can, thus, be attributed to the nano-packaged blueberry extract contained within them. The blueberry extract-containing modified clay (MMnt+BE) showed an antioxidant activity ~1.66 times greater than that of the NMnt+BE clay. This could be because the former has a larger interlayer spacing allowing for a greater packaging capacity. Modifying the Mnt may also ensure that the antioxidant properties of the blueberry extract are better preserved.

Finally, the measurement of the antioxidant activity in these systems allows us to confirm the migration of the nano-packed compounds from the clays to the aqueous medium.

3.11. Antimicrobial activity of the clays – disc-diffusion assay

The antimicrobial capacity of the clays was evaluated in order to establish their potential as active nanocomposites. Neither the clays nor the blueberry extract, however, showed antimicrobial activity against the two pathogenic microorganisms tested (Escherichia coli O157:H7 and Listeria monocytogenes innocua). Similar results were obtained by de Azeredo [5] and Abreu et al. [69] for unmodified Mnt. Other studies, nevertheless, have reported antimicrobial activity from two commercial modified Mnt clays: Cloisite 20A and Cloisite 30B [5,69]. Finally, although migration of the blueberry extract towards the surrounding environment was demonstrated, it did not show significant antimicrobial activity.

Despite this, no microbial growth was registered on the clays, thus agreeing with the water activity results.

4. Conclusions

The nanoclays developed have potential as nano-packaging materials for the development of bio-nanocomposites applicable to the cosmetic, biomedical, and food industries, among others: the antioxidant activity of the blueberry extract was maintained in these systems, and the blueberry extract-containing clays showed intelligent behavior when tested under different pH conditions. Unfortunately, none of the clays showed antimicrobial activity so that the hypothesis of the active effects of the systems obtained had to be discarded. Despite this, the food nanopackaging developed could be used as a potential reinforcement material in thermoplastic composites. Modifying the Mnt increased the interlayer spacing, thus allowing more blueberry extract to be packaged. Finally, the nanoclays obtained show promise as nancontainers for converting substances initially found in a liquid state into solid-state powders, thereby facilitating their manipulation and transport.

Conflicts of interest

The authors declare no conflict of interest.

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Fig. 7. DPPH scavenging activity of the clays and the extract evaluated: natural montmorillonite (NMnt), natural montmorillonite containing blueberry extract (NMnt+BE), modified montmorillonite (MMnt), modified montmorillonite containing blueberry extract (MMnt+BE) and blueberry extract (BE).
