

Article

Characterization of Beeswax, Candelilla Wax and Paraffin Wax for Coating Cheeses

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Abstract: A study on the physical and mechanical properties of beeswax (BW), candelilla wax (CW), paraffin wax (PW) and blends was carried out with the aim to evaluate their usefulness as coatings for cheeses. Waxes were analyzed by X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), differential scanning calorimetry (DSC), permeability, viscosity, flexural and tensile tests and scanning electron microscopy. Cheeses were coated with the waxes and stored for 5 weeks at 30 °C. Measured parameters were weight, moisture, occurrence and degree of fractures, and dimensional changes. The crystal phases identified by XRD for the three waxes allowed them to determine the length of alkanes and the nonlinear compounds in crystallizable forms in waxes. FTIR spectra showed absorption bands between 1800 and 800 cm⁻¹ related to carbonyls in BW and CW. In DSC, the onset of melting temperature was 45.5 °C for BW, and >54 °C for CW and PW. Cheeses coated with BW did not show cracks after storage. Cheeses coated with CW and PW showed microcracks, and lost weight, moisture and shrunk. In the flexural and tensile tests, BW was ductile; CW and PW were brittle. BW blends with CW or PW displays a semi ductile behavior. Cheeses coated with BW blends lost less than 5% weight during storage. The best waxes were BW and the blends.

Keywords: cheese; waxes; X-ray diffraction; Fourier Transform Infrared Spectroscopy (FTIR); thermal analysis; image analysis; viscosity; coating materials; mechanical properties; water permeance



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1. Introduction

Waxes are a heterogeneous class of lipids containing hydrocarbons and other non-polar substances. Natural waxes can be of vegetable, animal, or mineral origin. Waxes from animals and vegetable sources are mixtures of long-chain fatty acids, esters of aliphatic alcohols and hydrocarbons; those from mineral origins are composed mainly of paraffinic hydrocarbons [1]. Waxes have many industrial uses. Some waxes are used as primary coatings for some hard or semi hard cheese varieties [2]. Waxes for cheese coatings are used in the following way: they are heated to become molten, and then cheeses are dipped in the molten waxes and then are cooled after withdrawal. Waxes protect cheeses from water evaporation and weight loss and also prevent mold growth during storage and transportation [2–4]. Although some other materials are also used for cheese packaging, many traditional cheese manufacturers use waxes [5,6], mainly paraffin wax (PW) and mixtures, as cheese coatings [6–13]. Its use is very convenient and environmentally friendly at small-scale use, and preserves the nostalgic image of many traditional brands of cheese that have used waxes for cheese coating for ages.

Waxes for coating cheeses ideally should have at least the following characteristics: low propensity to fracture when subjected to external forces [3,14] and low water vapor permeance. Moreover, they should have the Generally Recognized as Safe (GRAS) Status and a low impact on the environment. In order to prevent defects in cheese coatings, conventional waxes of mineral origin can be replaced with microcrystalline waxes, triglycerides, or fatty acids [3]. PW blends with some organic clays also improve both strength and ductility [14]. Donhowe and Fennema suggested that some food-grade waxes with low water permeability, such as beeswax (BW) and candelilla wax, obtained from the shrub (*Euphorbia antisiphilitica*) (CW) may be suitable materials for use in foods [15].

However, it is important to measure the resistance to fracture of the wax films [16]. There is a study that shows evidence that beeswax can be used as a good coating for cheese, but the mechanisms are not described [10]. Cheese coated with 100% BW and stored for 30 days at 4 °C keeps its moisture as high as vacuum-packaged cheese. Sometimes BW is adulterated with PW or microcrystalline wax to reduce the cost of the raw materials. As an example, it can be mentioned the waxes used for bee production systems [17]. Some researchers stated that CW could replace BW in different food systems [18]; however, for the coating of cheeses or foods, no available information was found.

BW is generally used in combination with other polymers to make coatings. The main purpose of this component applied in fruits and legumes is to reduce water loss while maintaining a certain level of oxygen and carbon dioxide exchange [15]. Because respiration is kept at a low level, the shelf life is extended in fruits and legumes. BW is generally mixed with other compounds, such as polysaccharides (cellulose, starch, chitosan and their derivatives) and proteins (gelatin and soy protein) [19]. Examples of foods coated with beeswax as ingredients are various fruits and vegetables [20,21]. In paper, BW has a role in reducing the water vapor permeance and filling pores [22].

Candelilla wax is generally used in combination with other materials to confer a hydrophobic layer in coatings for fruits protection [23]. It is also used in vegan formulations [24]. The use of candelilla as a coating in mixtures prevents weight loss, impart gloss and prolong shelf-life in fruits and vegetables [25]. Candelilla wax is extracted from the wild shrub, *E. antisiphilitica*. Wax production by this plant occurs mainly during the dry season as an adaptive mechanism to reduce excessive water loss on the stem surface and to avoid plant desiccation [26]. Candelilla is hard and brittle, its surface can reach high brightness levels, which is one of the most valued properties for various applications such as coating of chocolates and fruits [23].

The objective of this research was to carry out a comparative study on some physical, chemical, mechanical and technological properties of BW, CW and mixtures of PW and BW with CW or PW as coatings for Poro cheese.

2. Materials and Methods

2.1. Waxes

Three kind of waxes were used: BW was supplied by Beekeepers Association of Tabasco, Mexico; CW by Ceras Naturales Mexicanas, S.A. de C.V., Saltillo, Coahuila, Mexico; and PW, melting point 64–66 °C by Petrowax S.A. de C.V, Mexico which is currently used by the local cheese makers and which is commonly used to make candles. Two binary mixtures, $CWx/BW(1 - x)$ and $PWx/BW(1 - x)$ ($x = 0.25, 0.5, 0.75$ and 1), were prepared as melting waxes in a Teflon pan at 100 °C. Four replicates of each binary mixture were assessed for the experimental characterization of the waxes.

2.2. Cheese

For the experiments, we choose a semi-dry cheese to use experimental waxes for coatings [27]. The selected cheese was Poro cheese. This cheese is characterized by a water activity (a_w) <0.95, a low pH value (~4.5); humidity (29% ± 0.2%) and 3–4% NaCl [28,29]. Storage lasts up to 5 weeks at room temperature [29]. Cheeses were purchased from Quesos

de Poro Genuino de Balancán, Tabasco SPR, México. The cheeses have been made from raw milk and are manufactured following traditional techniques [27].

2.3. Characterization of Waxes

X-ray Diffraction (XRD)

Samples of waxes were characterized by XRD to identify the crystalline phases. Wide-angle XRD analysis was performed on a D8 Advance diffractometer (Bruker AXS, Billerica, MA, USA) operating at conditions of 40 KV and 30 mA, with a $\text{CuK}\alpha$ radiation. The diffracted intensities corresponding to the 2θ angle were collected from 4 to 90° by means of a lynx-eye detector. The diffractograms for PW, CW and BW allowed identifying crystalline phases by consulting the International Centre for Diffraction Data database (ICDD) [30].

2.4. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of the waxes were determined in Attenuated Total Reflectance (ATR) mode using a FTIR spectrometer (Perkin Elmer 100, Perkin Elmer, Waltham, MA, USA), in the 650 to 4000 cm^{-1} zone, using 16 scans. No specific preparation was required for the analysis of the wafer-shaped samples [31].

2.5. Raw Waxes Characterization by Thermal Analysis

Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) were carried out in a Thermal Analysis System TA Instruments SDT Q600 (TA Instruments, Castle, MA, USA). The heating rate was $10\text{ }^\circ\text{C}/\text{min}$ in an air atmosphere. The sample amount was between 5 and 10 mg. An additional DSC was carried out at a heating rate of $5\text{ }^\circ\text{C}/\text{min}$ from room temperature to $100\text{ }^\circ\text{C}$ to obtain well-defined peaks for the melting point. Waxes were analyzed by three characteristics: (a) recording the solid-liquid transition temperatures by DSC [32]; (b) by looking at the shape of DSC; and (c) by determination of melting enthalpies [33].

2.6. Water Vapor Permeance (WVP) and Water Vapor Transmission Rate (WVTR)

WVP and WVTR were measured using a variation of the “dry and wet cup method” [34]. BW, CW, PW, as well as the binary mixtures including BW, as one ingredient were melted and poured in a silicone mold of 2 cm diameter and 0.5 mm thickness. Samples were preconditioned at $30 \pm 2\text{ }^\circ\text{C}$ for 24 h. The thickness at five points for each wafer sample was measured with a digital micrometer (Mitutoyo, Kanagawa, Japan). Samples were assessed with 80%/43% of relative humidity (RH) (dry cup method) and 43%/80% RH (wet cup method) kept at $30 \pm 2\text{ }^\circ\text{C}$. A saturated solution of $(\text{NH}_4)_2\text{SO}_4$ was used to generate 80% RH; and a saturated K_2CO_3 solution to generate a 40% RH. The weight change of the glass cup was recorded until the sample reached a constant weight. Calculations were made according to the standard [34]. At least three replicates of each film type were tested for WVP and WVTR.

2.7. Viscosity

An Anton Paar rheometer MCR 101/301/501 (Anton Paar, Graz, Austria) was used with continuous strain in the shear rate range from 2 to 100 s^{-1} . The measuring cell of the device represents a system of coaxial cylinders consisting of the outer fixed cylinder with a cylindrical rotor immersed into it. Waxes were placed in the annular gap between the cylinders and were heated with the Peltier system. The device was controlled with the Rheo-plus/32 V3.40 program at 70 – $100\text{ }^\circ\text{C}$, the temperature of waxes for cheese waxing [35].

2.8. Density of Waxes

The density of waxes at $25\text{ }^\circ\text{C}$ were determined with the American Society for Testing Materials (ASTM [36] method. The Density of waxes at $100\text{ }^\circ\text{C}$ were determined by weighing molten waxes into a pycnometer of known volume kept in a stove at $100\text{ }^\circ\text{C}$ [37]. A glass thermometer was calibrated at $0\text{ }^\circ\text{C}$ [38].

2.9. Mechanical Properties of Waxes

A three-point bending test and tensile test were performed for each specimen in a Shimadzu, Autograph AG-100 KNG, Universal Testing Machine (Shimadzu Scientific Instruments, Kyoto, Japan), with a crosshead speed of 0.5 mm/min. Specimens for the three-point-bending test consisted of prisms with a rectangular cross-section ($5 \times 3 \text{ mm}^2$) width and thickness respectively, and 38 mm length. The distance between the two support points was 30 mm. Samples for the tensile test consisted of “I” shaped specimens with a rectangular cross-section ($5 \times 3 \text{ mm}^2$) width and thickness respectively, and 31 mm length. Stress–strain curves were plotted for each specimen.

2.10. Scanning Electron Microscopy (SEM)

Samples of fractured surfaces were examined using a scanning electron microscope (JSM-5600 LV JEOL, Tokyo, Japan) operated at an accelerating voltage of 5–20 kV. The micrographs were obtained from samples as pieces that were fixed by means of a carbon tape were placed on an aluminum cylindrical sample-holder and covered with a thin film of gold.

2.11. Cheese Coatings

Four cheese blocks of approximately $15 \times 7 \times 6 \text{ cm}^3$ were dipped in molten waxes of each mixture at 100°C for 10 s; afterward, they were placed in a waxed paper at room temperature. The weight of coating materials was calculated by subtracting the weight of cheeses before and after coating. Thickness of coatings were measured after removing coatings. The cheeses were stored for 5 weeks at 30°C and 40% RH. Evaporative weight loss of cheeses was calculated weekly by measuring the weight difference [3]. The dry matter of cheeses was measured by Association of Official Agricultural Chemists (AOAC) method 926.08 [39]. Water activity (a_w) was measured at $25 \pm 1^\circ\text{C}$ with Aqualab model CX2, Decagon Devices, Pullman, WA, USA.

2.12. Degree of Cracking of Coatings during Cheese Storage

Images of cheese coatings were recorded with an HP Scanjet G4050 (Hewlett-Packard Inc., Palo Alto, CA, USA) with a resolution of 2400 dpi. A millimetric paper was placed over the scanner's glass plate, beside the cheese's area to be used as reference to measure total cheese area; and dimensions of the cracks. The degree of cracking was calculated by measuring peeled areas in the wax coatings of cheeses. The Digitizelt ver 2.5.3 software (Braunschweig, Germany) was used to digitize scanned images. Standards [40,41] were used as guides to examine wax coatings of cheeses for five weeks. A failure was called a crack only if the underlying surface of cheese was visible in some of the stages of cheese storage. The report consisted of (i) the length of cracks, and (ii) the area peeled by cracks (degree of cracking) Area reduction was measured examining the digitalized images of cheese before and after cheese storage, calculating the percentage of change.

2.13. Statistical Analysis

Statistical analysis was done using SPSS 15 software (SPSS Inc., Chicago, IL, USA). For each variable, a comparison of different means between waxes, a one-way analysis of variance (ANOVA), and Tukey's multiple range test ($P < 0.05$) was performed. Samples with non-homogeneous variances were assessed by the Kruskal–Wallis test, which uses ranks for variance analysis. Significance between the samples at the end of the testing period (5 week) were analyzed by the Mann–Whitney test ($P < 0.05$).

3. Results and Discussion

Although the wax compositions were not thoroughly analyzed in this study, information from X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR) were compared to the literature regarding their composition. It is important to under-

stand the main components involved in their physical properties and interactions during crystallization and coating formation.

3.1. DRX

The diffractograms for BW, CW and PW show the assignments of identified crystalline phases to the Bragg peaks by consulting the ICDD database (Figure 1a,c). They are listed in Table 1 for a comparative purpose. As a general observation, BW contained the structure of the crystalline phase of n-alkanes and fatty acids; CW contained alkanes and other crystalizable groups and PW contained solely straight-chain alkanes. For BW (Figure 1a), the phases that reasonably match with the Bragg peaks were n-nonacosane, $C_{29}H_{60}$ (Powder Diffraction File, PDF 40-1997); with n-heneicosane, $C_{21}H_{44}$ (31-1705); n-tricosane-n-pentacosane, $C_{25}H_{52}$ (53-1798); n-tricosane, $C_{23}H_{48}$ (43-1854); n-pentacosane, $C_{25}H_{52}$ (53-1793) and particularly oleic acid, $C_{18}H_{34}O_2$ (37-1810). In the same way, for CW (Figure 1b), the identified phases were also n-nonacosane, $C_{29}H_{60}$ (40-1997) with the very clear presence of n-heneicosane, $C_{21}H_{44}$ (31-1705); accompanied with tritriacontane, $C_{33}H_{68}$ (54-1717); triacontanoic acid, $C_{30}H_{60}O_2$ (34-1796) and sitosterol, $C_{29}H_{50}O$ (10-0545). For the case of PW (Figure 1c) the phases were: n-nonacosane, $C_{29}H_{60}$ (40-1997); n-heneicosane, $C_{21}H_{44}$ (31-1705); n-tricosane-n-pentacosane, $C_{23}H_{48}$, $C_{25}H_{52}$ (53-1798); n-tricosane, $C_{23}H_{48}$ (43-1854); and n-pentacosane, $C_{25}H_{52}$ (53-1793). They have been previously reported for BW [42–44]; CW [45–47]; and PW [1,42]. Hentriacontane, $C_{31}H_{64}$, has been reported as the major constituent for CW [47,48]; but diffractometer data for this phase was not found in the database. Oleic acid was clearly detected as a minor compound in BW, and could be present as a trace compound for CW. The diffracted intensities were considerably stronger as follows $PW > BW > CW$. Higher diffracted intensities in PW were related to the presence of straight alkanes (C_nH_{2n+2} [48]). Linear alkanes show more crystallinity than nonlinear alkanes [49]. A high degree of crystallinity generally requires greater energy for solid-liquid transitions [enthalpy of fusion]; as it was observed in PW with different crystallinities [1]. All the samples were measured under the same experimental conditions in the DRX.

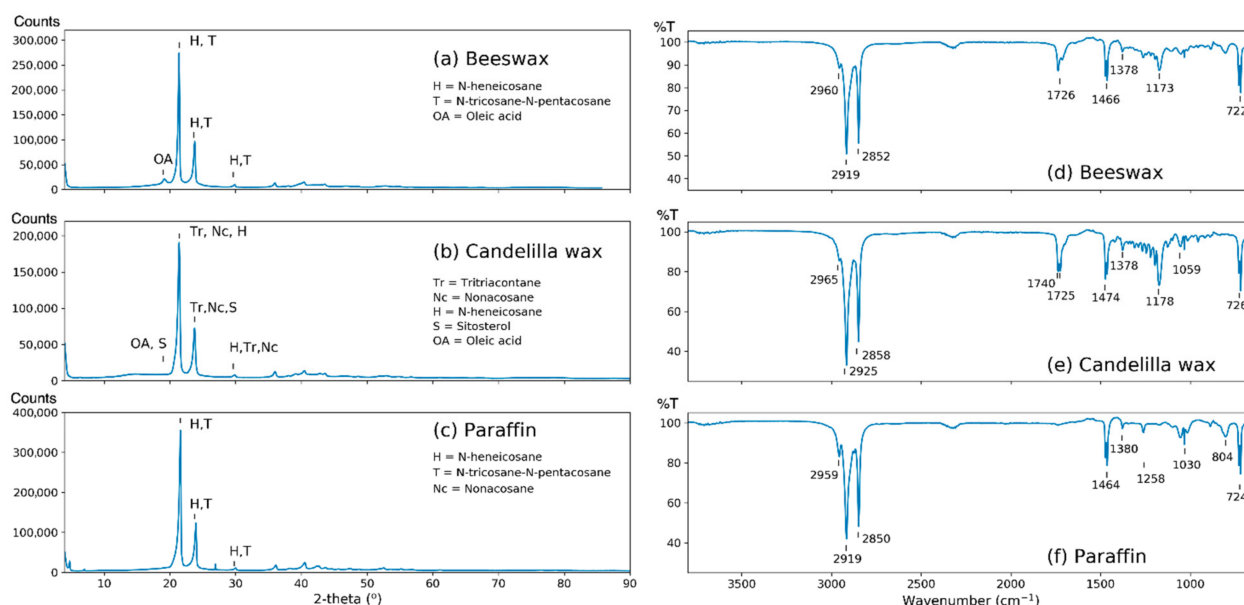


Figure 1. Left: X-ray diffractograms for (a) beeswax, BW; (b) candelilla wax, CW; (c) paraffin wax, PW. Right: Fourier Transform Infrared spectra (FTIR) for (d) BW; (e) CW, and (f) PW.

Table 1. Characteristics of waxes.

Characteristics of Waxes	BW	CW	PW
Crystal phases identified by X-ray Diffraction *	-	-	-
C ₂₉ H ₆₀ (straight-chain alkane), <i>n</i> -nonacosane (40-1997)	X	X	X
C ₂₁ H ₄₄ (straight-chain alkane), <i>n</i> -heneicosane (31-1705)	X	X	X
C ₂₃ H ₄₈ , C ₂₅ H ₅₂ (straight-chain alkanes), <i>n</i> -tricosane, <i>n</i> -pentacosane (53-1798)	X	-	X
C ₂₃ H ₄₈ (straight-chain alkane), <i>n</i> -tricosane (43-1854)	X	-	X
C ₂₅ H ₅₂ (straight-chain alkane) <i>n</i> -pentacosane (53-1793)	m	-	X
C ₃₃ H ₆₈ (straight-chain alkane), <i>n</i> -tritriacontane (54-1717)	-	X	-
C ₃₀ H ₆₀ O ₂ (straight-chain, saturated fatty acid), triacontanoic acid (34-1796)	-	X	-
C ₁₈ H ₃₄ O ₂ (nonlinear, saturated fatty acid), oleic acid (34-1810)	m	tr	-
C ₂₉ H ₅₀ O (terpene), β -sitosterol (10-0545)	-	X	-
Other Physical Characteristics			
Peak melting temperature (°C)	70	76	66
Enthalpy (J/g)	192	177.5	199.0
Lower temperature limit for melting (°C)	45.5	54.5	56
Upper temperature limit for melting (°C)	88.4	91.4	81.9
Density 100 °C (g/cm ³)	0.82	0.90	0.78
Density 25 °C (g/cm ³)	0.95	0.98	0.87
Kinematic viscosity (centistokes) 100 °C	8.9	16.2	4.6
Kinematic Viscosity (mPa·s) at Various Temperatures			
100 °C	4.7	8.30	1.9
95 °C	5.3	10.1	2.1
90 °C	6.3	10.9	2.4
85 °C	7.0	13.1	2.8
80 °C	8.2	15.7	3.2

* Note: X = major phase; m = minor phase; tr = trace compound. After the name, the code for PDF (powder diffraction file) of the ICDD database (ICDD, 2007), has been included.

3.2. Wax Characterization by Fourier Transform Infrared Spectroscopy

Figure 1 shows the FTIR spectra of the three kinds of waxes. The absorption bands related to alkanes were similar for the three waxes; the following bands can be assigned: C–H stretching (at 2950 and 2850 cm^{−1}); C–H bend or scissoring (1466 cm^{−1}); and C–H rock, methyl, seen only in long-chain alkanes (725 cm^{−1}). In previous studies, similar spectral bands to those described in this study were reported for BW [50–52]; CW [53]; and for PW [52,54]. Spectral region between 1800 and 800 cm^{−1} has been considered as a fingerprint region for BW because of a high number of absorption bands. The sharp band between 1700 and 1750 cm^{−1} arises from the carbonyls in the ester linkages between the fatty acids and glycerol backbone [55,56]. Such absorption is attributed to the beeswax monoesters, which are the major ester component (~40%) of beeswax [45]. BW and CW were similar in many functional groups; However, band 1378 cm^{−1} was reported as a characteristic band in BW [57]. In our study, it was also present in CW.

3.2.1. DSC and TGA

Thermal analysis is shown in Figure 2; some key points of DSC are summarized in Table 1. It can be seen that DSC curves for BW were broader than for CW and PW. The lowest melting temperature for BW was 45.5 °C, approximately 10 °C lower than the values for CW and PW. That means that during cooling, BW has a longer time to spread on the surfaces before waxes get solidified, and it reduces the probability of the occurrence of failures.

The peak melting temperature and enthalpy for BW was 70 °C and 192 J/g, respectively (Figure 2a). The lower and upper temperature limits associated with a complete melting of BW are listed in Table 1. The TGA indicates that the sample lost weight starting at around 209 °C and then changed its slope in a complicated way according to the complex composition of BW, particularly around 10% wt. of long-chain molecules which decompose at higher temperatures (Figure 2g).

The results of DSC are in general agreement with other studies [58,59] where melting temperature had similar values (69.6 °C for BW). Other authors have recorded lower peak melting temperatures (≈63 °C) and enthalpies (between 182–186 J/g). It has been

suggested that waxes obtained from honeybee's hives built-in warmer places, could be of higher peak melting temperatures than waxes from cold places [58]. It has also been demonstrated that organisms respond to high temperatures producing waxes with high peak melting temperatures [60].

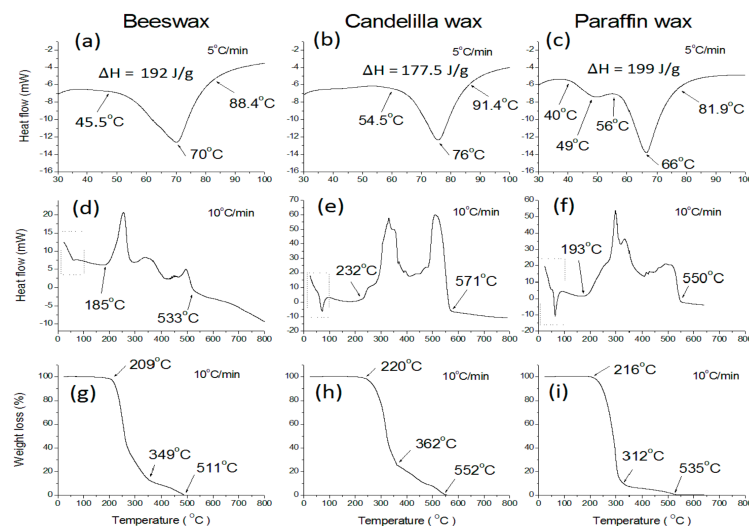


Figure 2. Above: DSC plots in a range temperature from room temperature to 100 °C for (a) beeswax, BW, (b) candelilla wax, CW, (c) paraffin wax, PW, heat rate at 5 °C/min. Middle: DSC plots in a range temperature from room temperature to 800 °C for (d) beeswax, BW, (e) candelilla wax, CW, (f) paraffin wax, PW, heat rate at 10 °C/min. Bottom: TGA plots in a range temperature from room temperature to 800 °C for (g) beeswax, BW, (h) candelilla wax, CW, (i) paraffin wax, PW, heat rate at 10 °C/min.

The peak melting temperature and enthalpy for CW were 76 °C and 177.5 J/g, respectively (Figure 2b). Both values were very close to 77 °C and 116–161 J/g [61]. Other authors [46] have also recorded lower melting enthalpies (149.75 g^{-1}) than that obtained in this study for CW. In this case, it is also feasible that CW samples contained a little amount of non-crystalline wax. This is in agreement with the diffuse scattering component observed in the X-ray diffractogram for CW (Figure 1b).

Peak melting temperature and enthalpy for PW was 66 °C and 199 J/g, respectively; the lower and upper-temperature limits associated with the melting transition peak of PW (Figure 2c,f) were closer to those values described previously for intermediate crystalline PW [1]. The enthalpy of 199 J/g is slightly higher than that obtained for intermediate crystalline wax (173 J/g) [1]. As higher enthalpies are inherent of amorphous waxes, then it is feasible that a PW sample contains a small amount of non-crystalline wax. This is in agreement with the diffuse scattering component observed in the X-ray diffractogram for PW (Figure 1c). Concerning TGA results, around 90 wt.% of the PW sample (Figure 2) was lost by combustion starting at 216 °C, and the remaining 10% was slowly lost in a relatively long range of temperature from 312 to 535 °C (Figure 2i), likely related to the presence of long-chain molecules in PW.

3.2.2. Viscosity

Viscosity values of the three waxes at 100 °C allowed to coat cheeses thoroughly during dipping time (Figure 3a,d). However, during cooling at 70 °C, PW flowed better than the other waxes. At 70 °C, CW stabilized quicker than BW. BW had less viscosity than CW (Table 1). Because viscosity can be measured only at temperatures near above melting point peak; DSC can be helpful to describe the temperature of the liquid–solid transition. In the rheometer reading, the viscosity measurements indicated that the molten waxes change the fluid's viscosity significantly at different temperatures shown in Figure 3a,d.

The viscosity from highest to lowest was CW > BW > PW. A Lower viscosity in PW than the other waxes is due to a more uniform laminar flow related to the presence of low density linear short hydrocarbon chains (Table 1) (alkanes) [1]. The highest viscosity in CW could be related to the longest hydrocarbon chains, some of them nonlinear. BW's high viscosity can be resultant of long esters mixed with some hydrocarbon chains.

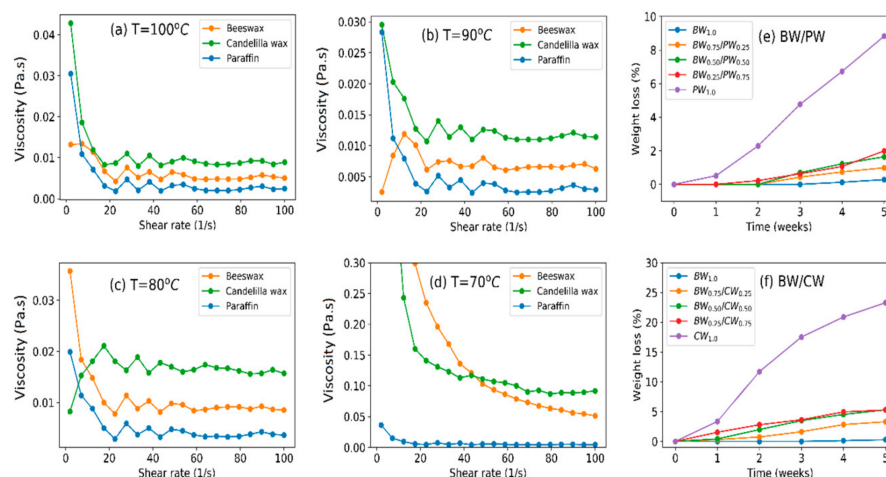


Figure 3. Viscosity of waxes at (a) 100 °C, (b) 90 °C, (c) 80 °C, (d) 70 °C; and weight loss of cheese coated with (e) PW x /BW $1-x$ blend ($x = 1.0, 0.75, 0.50, 0.25, 0$) and (f) CW x /BW $1-x$ blend ($x = 1.0, 0.75, 0.50, 0.25, 0$).

3.2.3. Density

The three waxes showed higher density at 25 °C than at 100 °C (Table 1); thus, specific volume is reduced after the waxes are cooled and solidified. In this study, the density at 25 °C from highest to lowest was CW > BW > PW. It can be assumed that after cheese is dipped in molten wax, and cooled at room temperature; the specific volume of waxes will be reduced (shrunk), generating contraction forces in the waxes. Because the different compounds of waxes have different melting points and densities, it could be expected that they will produce irregularly shaped layers after waxes are cooled [62] in the cheeses.

3.2.4. Water Vapor Permeance and Water Vapor Transmission Rate

The WVP of PW, CW and BW has as low permeability values as many “plastics” used for packaging, such as low-density polyethylene or polyvinyl chloride [63–65]. In the dry cup method (80/43% RH) CW and BW exhibited higher WVP than PW. PW displayed the smallest WVP of the waxes tested ($0.89 \times 10^{-5} \text{ g}/[\text{m}^2\text{hPa}]$). The same situation can be observed for the wet method (Table 2). The absence of polar compounds and the composition of alkanes in PW are likely responsible for its excellent water vapor barrier properties. The different degrees of higher permeability to water vapor in CW, BW and blends are probably attributable to the specific larger concentration of fatty acids, fatty alcohols and esters in the specific wax or specific blend [15].

Table 2. Water Vapor Permeance (WVP) and water vapor transmission rate (WVTR) at 30 ± 2 °C.

Conditions	80/43% RH * (Dry Cup Method)			43/80% RH * (Wet Cup Method)		
Wax	Thickness Mm	WVP g/(m ² hPa) × 10 ⁻⁵	WVTR g/(m ² day)	Thickness mm	WVP g/(m ² hPa) × 10 ⁻⁵	WVTR g/(m ² day)
BW	0.63	3.22	19.42	0.46	1.24	7.00
CW	0.78	4.18	34.70	0.80	6.63	35.01
PW	0.58	0.89	5.73	0.55	1.17	7.96
BW ₂₅ /CW ₇₅	0.81	1.31	8.91	0.68	2.91	19.74
BW ₅₀ /CW ₅₀	0.74	1.78	12.73	0.50	1.77	13.37
BW ₇₅ /CW ₂₅	0.76	2.03	15.28	0.87	2.42	20.05

* RHs are inside and outside the glass vial.

BW, CW and PW are very good barriers to moisture transfer due to their hydrophobic nature [63–65]. Weight changes in the cups coated in the experiments performed in this study for the three waxes were not higher than 1% of the original weight after 20 days of measurements. That information shows that there was a low permeability to water vapor in the three materials.

Brittleness of waxes is the main constraint to their use as coatings. It is very difficult to avoid the appearance of cracks during application of these lipids to foods and during subsequent handling of the foods [54,63]; therefore, some authors recommend that some waxes should be combined with hydrocolloids to form more resistant films [65]. Differences in permeability among the wax films are attributed mainly to differences in chemical composition and crystal type as determined by X-ray diffraction [15].

3.2.5. Weight Loss of Cheese

The best waxes to coat cheeses were BW and blends. The worst were CW and PW (Figure 3e,f). In the first experiment, the cheese coated with BW_{1.0}, at five weeks showed the least weight loss (BW_{1.0}, $P < 0.05$) (Figure 3e,f) in relation to cheeses coated with the other waxes and their mixtures. The greatest weight loss was recorded in both CW_{1.0} and PW_{1.0}. It was previously reported little changes in weight in the cheeses coated with BW_{1.0} and stored in refrigeration for several weeks [10]. The mixtures with BW_{0.25}/CW_{0.75} and BW_{0.25}/PW_{0.75} and the other mixtures were effective coatings, because weight reduction was less than 5%. A value acceptable for cheese coatings [3].

In the second experiment (Table 3), moisture content and water activity (a_w) for the cheese coated with BW were significantly higher ($P < 0.05$) than the values for cheeses coated with CW_{1.0} and PW_{1.0}.

Table 3. Parameters measured in cheeses and wax coatings during storage at 30 °C, 40% RH.

Variables	Statistical Test *	Waxes		
		BW	CW	PW
Wax weight/cheese weight (% w/w)	ANOVA	6.1	6.13	5.4
Thickness of coatings (mm)	ANOVA	1.0	1.0	0.98
Weight change (%) of cheese after 5 weeks of storage	ANOVA	3.2 ^A	11.6 ^B	11.2 ^B
Moisture (%) (at 0 weeks of storage)	ANOVA	34.9	34.9	34.9
Moisture (%) (at 5 weeks of storage)	ANOVA	33.8 ^C	24 ^A	25.7 ^B
a_w (at 0 weeks of storage)	ANOVA	0.95	0.95	0.95
a_w (at 5 weeks of storage)	ANOVA	0.94 ^C	0.87 ^A	0.91 ^B
Cracks (average of the length of cracks)	KW	0.11 ^A	132.8 ^B	122.5 ^B
Area peeled by cracks (% in cheese area)	KW	0.006 ^A	7.29 ^B	7.19 ^B
Area reduction (%)	KW	0.3 ^A	5.58 ^B	4.84 ^B

* KW = Kruskal–Wallis test; a_w = Water activity. Water activity is defined as follows: $a_w = P/P_0$; A,B,C Values within rows with different superscripts differ ($P < 0.05$) Values without superscripts do not differ.

3.2.6. Image Analysis of Coatings of Cheeses

Image analyses allowed to make a rough and fine assessment of how fractures developed in cheese coated by CW and PW. Fractures were scarcely visible in BW during the whole experiment. In coatings of CW and PW some failures were detected at the beginning of the experiments; in CW they showed up as deep cracks failures. In PW defects showed as pinholes (Figure 4). Initial failures might lead to further damage in coatings. Cracking and breaking of a cheese coating results in a shrinkage caused by the loss of moisture through evaporation from cheese [66]. A defective coating is: “a brittle coating of PW that breaks and peels off in the form of scales or flakes” [9]. Initial defects in the barrier film recorded in CW and PW might explain a significant moisture loss [$P \leq 0.05$] through a cracked section leading to significant loss of weight of cheeses [$P \leq 0.05$], and significant shrinkage of cheeses [$P \leq 0.05$]. In CW, cracks appeared as irregular linear cracks, as shown in Figure 4 and Table 3.

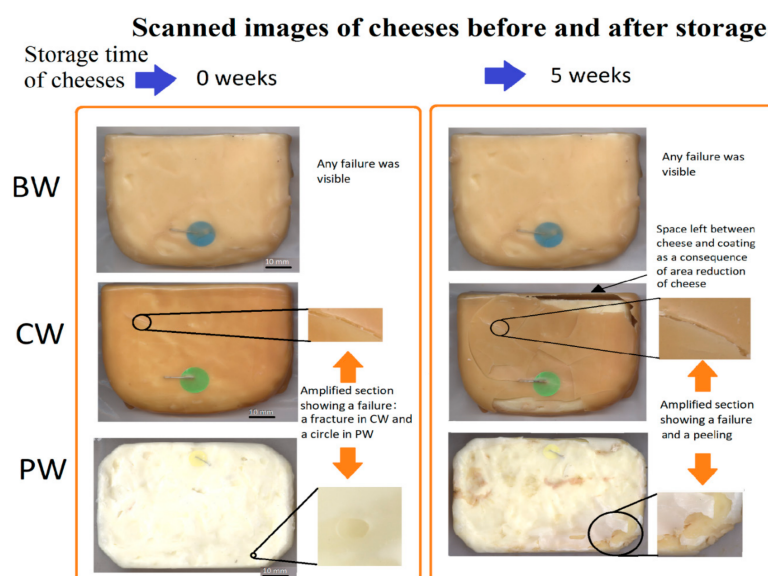


Figure 4. Scanned images of cheeses before and after storage. Cheese coating at the beginning of the study and after 5 weeks of storage: Beeswax (BW), candelilla wax (CW), and paraffin wax (PW).

There were significant differences in the cracks, area peeled by cracks, and area reduction in cheese coated with BW, CW and PW according to multiple comparison tests across [Kruskal–Wallis test; $\chi^2 = 8.11$, degrees of freedom (d.f.) = 2, $P = 0.017$]. The differences between BW in comparison of either CW and PW were highly significant [Mann–Whitney U-test; $z = -2.36$, $P = 0.018$, two-sided], suggesting that CW and PW had more likelihood to cracking than BW [Table 2], suggesting that cheese coated with CW and PW shrunk more than cheese coated with BW. It is feasible that shrinkage of cheese releases empty spaces between coatings and cheese surfaces, weakening coatings support and they become more susceptible to cracking.

Microcracks might appear in brittle materials, such as CW, which do not resist deformation after liquid–solid transition. BW, however, is viscoelastic, and it is more easily deformed after liquid–solid transition [62]. The greater viscoelasticity of the BW than PW or CW make it more flexible and less susceptible to crack after shrinkage [62]. BW contains a small number of unsaturated hydrocarbons with low melting point such as oleic acid which are responsible for its flexibility [16,43]. They also have been related to resilience and stiffness in BW [58,67]. On this respect, oleic acid has been identified clearly as a minor component in BW and as a trace compound in CW (Figure 1 and Table 1). In Figure 5 major differences in the acid oleic content between BW and CW in the X-ray diffractogram can be observed in a logarithmic scale.

3.2.7. Mechanical Properties and Microstructure of Waxes

Food packaging materials must support handling, shipping and storage to preserve the integrity of the food product. Waxes applied as coatings in cheeses should protect them during storage. Waxes should be resistant to fractures and produce ductile fractures. Mechanical testing showed that BW had a ductile behavior after a stress applied (i.e., capable of deformation without fracture). The other waxes were more resistant to fracture but yield brittle fractures. In the flexural test, specimens of BW_{1.00} exhibit the highest physical plasticity [see picture of BW_{1.0} in the bottom of Figure 6, and bent after flexural test, as a result of a great displacement of materials. CW_{1.00} and PW_{1.00} exhibited a brittle behavior with increasing loads (Figure 6a,b). Specimens made of wax blends showed stress–strain curves with some plastic deformation because BW was present in the blend, in equal or higher proportion than CW or PW. Samples of PW and CW showed cracks and fractures perpendicular to the applied normal stress (see picture PW_{1.0} at the bottom of Figure 6). Micrographs of CW in a fractured surface specimen exhibited spherical pores (~20 µm) (see

picture of $CW_{1.0}$ in the bottom of Figure 6). Holes subjected to a uniform stress generally trigger a nucleation and a successive crack formation and propagation [68]. Reassembled pieces are shown in the lower part of Figure 6.

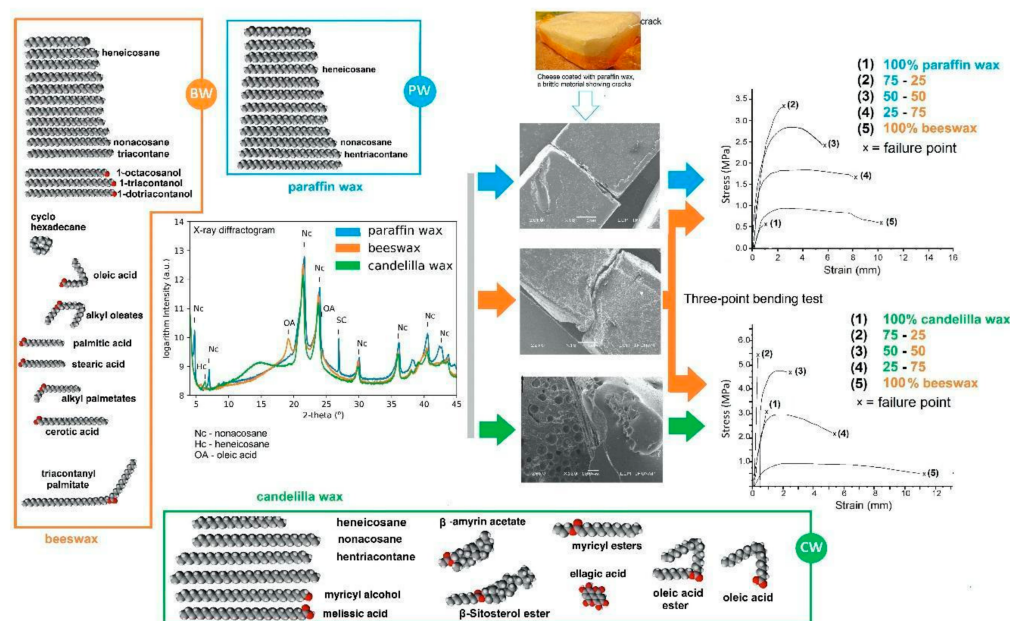


Figure 5. Composition linked to mechanical properties of beeswax (associated with orange box and arrows), paraffin wax (associated to blue) and candelilla wax (associated with green).

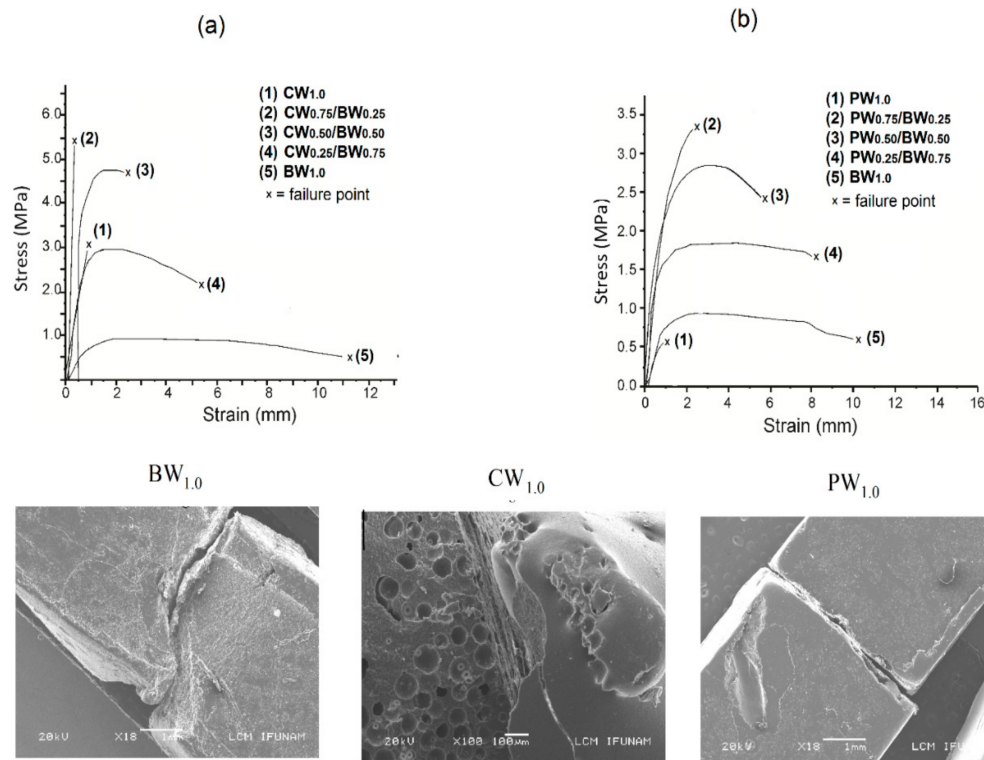


Figure 6. Flexural stress–strain curves of specimens made of two binary mixtures: (a) CW_x/BW_{1-x} , candelilla wax blended with increasing content of beeswax, (b) PW_x/BW_{1-x} , paraffin wax blended with increasing content of beeswax. SEM images showing fractures generated during flexural test of a specimen made of $BW_{1.0}$, beeswax showing plastic deformation, $CW_{1.0}$, candelilla wax showing elliptical holes and $PW_{1.0}$, paraffin showing a typical brittle failure.

Wax blends containing either $BW_{0.25}/CW_{0.75}$ or $BW_{0.25}/PW_{0.75}$ exhibited the highest flexural strength. The results are somewhat opposite to those previously observed [60], who found the highest flexural strength in samples of $PW_{0.25}/BW_{0.75}$. However, in that study; the speed of the crosshead in the Universal Testing Machine was 10 mm/min, 20 times faster than in our study.

Elongation tests were carried out to obtain additional information of the mechanical properties of waxes (Figure 7) [69]. Micrographs of BW showed rearrangements of materials flowing aligned in the direction of the axial shear forces (Figure 7c,f). A principal effect of plasticity is to truncate the elastic stress distribution by limiting the shear stress to the flow stress [59]. Specimens of $PW_{1.00}$ and $CW_{1.00}$ after elongation test behave as brittle and highly susceptible materials to cracking. Elongation test was more sensitive to stress than the tensile test [~ 10 times]. The stress–strain curves of specimens made of $BW_{0.5}/PW_{0.5}$ or $BW_{0.5}/CW_{0.5}$ behave as brittle materials (Figure 7a,b); they got fractured after deforming elastically. Brittle materials generally exhibit failure with stress as low as 5% (0.05 mm/mm) of axial load.

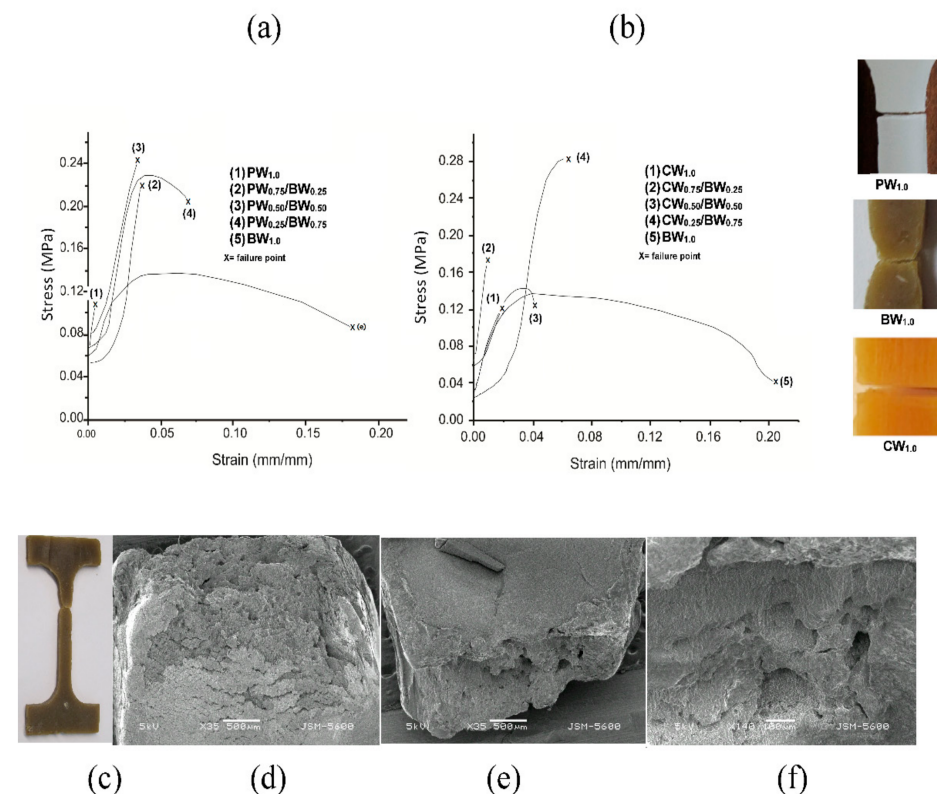


Figure 7. Elongation stress–strain curves of specimens made of (a) PW, paraffin wax blended with increasing content of BW, beeswax; (b) CW, candelilla wax blended with increasing content of BW. Pictures of some fractured specimens of pure waxes (left); (c) Specimen made of BW, showing a failure after elongation test; SEM images of BW showing a: (d) convex region; (e) concave region; (f) amplified concave region.

Wax blends containing either $BW_{0.5}/CW_{0.5}$ or $BW_{0.5}/PW_{0.5}$ were within this category. Specimens containing higher amounts of BW such as $BW_{0.75}/CW_{0.25}$ or $BW_{1.0}$ behave as ductile materials. Samples of $BW_{1.00}$ exhibited the highest plastic deformation with an elongation of approximately 10%; after reaching its maximum capacity, they gradually got deformed forming a neck and failed in a cup-and-cone fracture, which is indicative of plastic deformation (Figure 7c,f). The conical tip and the cracked bands on the sides of the conical tip are evidence of highly plastic materials [70]; (Figure 7d,f). Cracked bands are formed when samples are being stretched, until they finally break. Chemical and physical properties of waxes depend on the length of alkanes, the ramifications, the unsaturation

and the content of other compounds such as alcohols and fatty acids. An experimental study demonstrated that fatty acids confer ductile properties to BW [58], because the experimental removal of the fatty acids from BW diminished yield stress, resilience and stiffness [58]. Fatty acids such as oleic acid have lower melting point, density and viscosity than the other components. In this study, oleic acid was identified in both BW and CW, although the XRD signal for CW could barely be seen.

Materials in contact with foods or medicines or biological matrices should fulfil various characteristics according to the functionality that they will have [62].

Like other natural materials with the characteristics of flexibility and strength for optimal performance, BW and mixtures are the preferred materials used in cheese protection due to their flexibility and strength. As with other natural material, it can be re-used or composted after use [71].

4. Conclusions

The present study describes some chemical and physical characteristics of BW, CW, PW and blends related to their behavior as cheese coatings. Single BW consists of a heterogeneous mixture of alkanes with esters, free fatty acids, and carboxylic acids. It has the broadest melting temperature curve; unimodal and without shoulders. The onset of melting temperature was 45.5 °C, a closer value to the room temperature than the other waxes. It has a very high viscosity at the peak of melting temperature. Specimens made of BW exhibited a ductile behavior (plastic deformation) under increasing flexural loads. As a coating for cheese, BW did not show the appearance of fractures during cheese storage. CW had the longest alkanes of the three waxes; they were either straight or nonlinear. CW also has esters, free fatty acids, and carboxylic acids (Figure 5). CW exhibited the highest melting temperature with the sharpest peaks, it begins to melt at 55 °C, a very high temperature above the room temperature; it also has the highest viscosity at temperatures at the peak of the melting temperature. Specimens made of CW resist the highest flexural load, which caused brittle fractures. Inner images of specimens made of CW fractured shows bubbles. As a coating for cheese, CW shows the appearance of fractures during cheese storage. PW had short straight alkanes. PW exhibited the highest enthalpy of fusion. It has the lowest viscosity at the peak of melting temperature. Specimens made of PW resisted the lowest flexural load, which caused a brittle fracture. As a coating for cheese, it shows the appearance of fractures during cheese storage. Specimens made of mixtures of either CW or PW with BW behave differently from single waxes.

The more the BW in the mixture the higher the ductility. Blends 0.5/0.5 were stronger and became partly ductile. Evidence of plasticity or ductility for single BW and wax blends was observed as a flow of materials by Scanning Electron microscopy. Under flexural static loadings, the stress–strain curves of wax blends gradually change behavior from brittle to plastic as the BW content in the blend increases. Cheeses coated with BW or blends containing BW with either CW or PW during storage for 5 weeks' loss less than 5% of the original cheese weight. Cheeses with single CW and PW lose more than 10% of cheese weight after five weeks. The occurrence of microcracks in CW and PW, likely allowed moisture migration from cheese to the environment, causing the loss of weight, aw and shrinkage of volume of cheeses. Blends containing BW with CW and PW are easy to use, environmentally friendly and provide good mechanical and barrier properties to contribute to a longer shelf life for cheese.

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