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Replacing Hydrogenated Fat in Cookies with Oleogels based on *Butia odorata* Seed Oil and Beeswax

Running title: *Butia odorata* Seed Oil and Its Use in Oleogel

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SUMMARY

Research background. Hydrogenated fats are widely used to enhance texture, flavor, and shelf life in processed foods, but their excessive consumption contributes to cardiovascular diseases. While Butia seed oil (BSO) contains saturated fats, its potential as an alternative structuring lipid in food applications remains unexplored. This study investigates the formulation of BSO-based oleogels and their potential as a replacement for hydrogenated fats in cookies.

Experimental approach. This study aimed to develop oleogels based on BSO and beeswax (1, 3, and 5 %, *m/m*) and apply them to cookies as a substitute for hydrogenated fat. The chemical composition, thermal properties, and functional groups of the BSO and beeswax constituents were analyzed. The oleogels were characterized in terms of lipid stability, oil binding capacity, gel stability, thermal properties, and color parameters. Subsequently, the oleogels were applied to cookies as a

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substitute for hydrogenated vegetable fat. The cookies were evaluated for mass loss, color, expansion factor, specific volume, and texture properties.

Results and conclusions. BSO presented saturated fatty acids (22.87 mg/mL lauric and 22.45 mg/mL caprylic acid) and notably high levels of unsaturated fatty acids (oleic acid 33.21 mg/mL and linoleic acid 30.61 mg/mL). Oleogels containing 3 and 5 % beeswax remained stable for 90 days. Increasing the beeswax percentage resulted in greater oleogel hardness ($p<0.05$). Specifically, the oleogel with 5 % beeswax exhibited the highest oil binding capacity, reaching 99.9 %. Cookies formulated with oleogel showed lower hardness and mass loss, as well as a higher specific volume compared to the cookie control (without oleogel). Notably, the use of oleogels did not alter the cookies' visual characteristics, supporting their potential as a viable fat replacer in oven-baked products.

Novelty and scientific contribution. These findings suggest that BSO oleogels have the potential to replace hydrogenated vegetable fats in food products. This study demonstrates that BSO-based oleogels, particularly at 3–5 % beeswax concentrations, can effectively replace hydrogenated vegetable fats in cookie formulations. Unlike traditional structuring fats, these oleogels offer improved lipid profiles while maintaining desirable baking properties.

Keywords: beeswax; *Butia* seed oil; fatty acid profile; gas chromatography; texture profile

INTRODUCTION

Butia sp. (Arecaceae) is a genus of palm trees comprising 20 species found in South America, specifically in Paraguay, Uruguay, and Argentina (*1*). Brazil is the largest producer of these species (*1*). The fruit of *Butia* sp. is widely consumed in its natural form, used in artisanal cuisine, and employed in liqueur production (*1*). The seeds are often discarded, used for replanting, as natural fertilizer, or as food for local fauna (*2*). Studies emphasize the economic importance of fruit pulp, especially in the pharmaceutical and cosmetic industries (*2*). The seed of *Butia odorata*, often considered a by-product of fruit processing, yields almond oil rich in lipids. Its fatty acid composition consists of approximately 76 % saturated fatty acids (including caprylic, capric, lauric, myristic, palmitic, and stearic acids) and 24 % unsaturated fatty acids (such as oleic and linoleic acids) (*3*). Despite its rich lipid content, the processing of this oil has received limited attention in scientific research.

Fats found in foods generally consist of a combination of polyunsaturated, monounsaturated, and saturated fatty acids (*4*). Saturated fats are widely used in various food products, including bakery items, confectionery, sauces, fast foods, and others. These fats provide technological and functional properties to foods, such as flavor, crunchiness, and extended shelf life. However, excessive consumption of saturated fats is linked to an increased risk of cardiovascular diseases (*4*).

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Modifications of liquid vegetable oils to convert into solid fats, such as hydrogenation, interesterification, and fractionation, have been employed for application in the food industry. According to Manzoor *et al*. (*5*), the hydrogenation process involves introducing hydrogen atoms into unsaturated fats with a cis configuration, converting them into more saturated fats. This conversion results in solid or semi-solid fats with improved properties, such as a higher melting point, increased stability, longer shelf life, and greater resistance to oxidation (*5*). However, the partial hydrogenation process leads to the formation of harmful trans fats. In 2018, the United States Food and Drug Administration (FDA) announced a ban on partially hydrogenated vegetable oil to eliminate the consumption of foods containing trans fats (*6*). The elimination or replacement of trans and saturated fatty acids in solid fats has been a challenge for the food industry, due to the importance of fats in texture and flavor (*6*). Consequently, there has been an increased exploration of innovative technologies in this field, such as gelation.

The gelation of liquid oil using structurants to produce oleogel is considered a viable method for replacing saturated fats (*7*). Oleogels are three-dimensional network structures formed through non-covalent interactions, which restrict the movement of liquid oil and form a solid fat (*6*). Oil gelation with structuring agents involves various interactions, such as hydrogen bonds, electrostatic forces, and van der Waals forces (*5*). Some oils, including canola (*8,9*), corn, soybean, sunflower, olive oils (*10,11*), palm oil (*12*), and walnut oil (*13*), have been used in oleogel production. These oils can also serve as a base to act as gelling agents in food systems, such as biscuits and other products, replacing other fats.

Cookies are popular and widely consumed products among various consumer groups. These products typically contain high amounts of fat, ranging from 20 to 30 % based on the mass of the flour (*14*), with saturated fat being commonly used. Kim *et al*. (*13*) developed oleogels using oil extracted from an edible insect (*Tenebrio molitor*) along with oleogelators (candelilla wax, carnauba wax, and beeswax). They applied them as a substitute for solid fat in cookies. Giacomozzi *et al*. (*14*) formulated muffins using sunflower oil monoglyceride oleogel and compared them to muffins made with commercial margarine. Gorghi *et al*. (*15*) used grape seed oil to produce oleogel with beeswax as the oleogelator for use in chocolate. However, to our knowledge, no studies have produced oleogel using almond oil from *Butia* seeds or applied this oleogel in food.

This study aimed to extract and characterize fatty acids from BSO and assess their application in oleogel production. We also evaluated cookies made with oleogel from *Butia* seed oil and beeswax as a substitute for saturated fat, analyzing their texture, color, expansion factor, specific volume, and mass loss.

MATERIALS AND METHODS

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Material

Butia odorata was harvested in 2022 in Rio Grande, RS, Brazil (S 32°10.097' W052°24.595'). The beeswax used to prepare the oleogels was purchased from a local market in the city of Pelotas, RS, Brazil. The reagents used were analytical grade.

Oil extraction was performed in a Soxhlet extractor using 20 g of *Butia odorata* seed and 250 mL hexane P.A. solvent. The solvent was evaporated on a rotary evaporator (Buchi, Rotavapor RII, Merck, Darmstadt, Germany). The oil extraction yield was calculated gravimetrically based on the initial mass of seed.

Fatty acid profile of BSO

The fatty acid profile was determined using the gas chromatography (GC) method; the equipment is coupled with a CG flame ionization detector (FID) (Clarus 500, PerkinElmer®, Shelton, Washington, USA) equipped with a capillary column (30 m × 0.25 mm, 0.25 mm Elite-1, PerkinElmer, Waltham, MA, USA). An aliquot of 0.1 mL of BSO was added to 2 mL of hexane and 2 mL of potassium hydroxide. The sample was in an ultrasonic bath for 5 min and then centrifuged (2 min, 4000×*g*) (Kasvi K14-4000, Paraná, Brazil). The supernatant was collected for analysis (*16*). The operating conditions were as follows: the carrier gas was nitrogen with a constant flow rate of 1.5 mL/min. The column was heated at 120 °C for 1 min, then at a heating rate of 15 °C/min to 160 °C for 3 min, and finally at a heating rate of 3 °C/min to 230 °C and held for 10 min.

Fatty acid quantification was performed using F.A.M.E. with a six- point standard curve using mixture C37 (18919-1AMP, Supelco, Bellefonte, PA, USA), and compounds were identified by comparing elution patterns and retention times with the reference mixture.

Polar and nonpolar metabolomics analysis of beeswax

For the extraction of polar metabolomics, 0.1 mg of beeswax was placed in a 2 mL bottle, then 1 mL of hexane was added and homogenized using ultrasound for 5 min, and then centrifuged at 2000×*g* for 5 min. A 0.1 mL aliquot was then placed in a vial, and derivatization was performed by adding 0.1 mL of N-metil-N-(trimethylsilyl) trifluoroacetamide. The vial was then placed in a bath at 60 ºC for 40 min. Analytes were quantified and identified using a GC system (Shimadzu QP2010 UltraPlus, Shimadzu, Kyoto, Japan) equipped with a mass detector mass spectrometer (MS) (Shimadzu Corporation, Kyoto, Japan) and Rxi-1MS capillary column (30 m×0.32 mm×0.25 µm, Restek). The ramp temperature was maintained at 70 °C for 2 min, increased to 180 °C at 2.5 °C/min, and to 230 °C at 10 °C/min and maintained under isothermal conditions for 2 min. MS was operated in scan mode (mass range m/z=35–450).

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For the extraction of nonpolar metabolomics, 0.1 mg of beeswax was placed in a 2 mL bottle, then 1 mL of hexane was added and homogenized using ultrasound for 5 min, and finally, centrifuged at 2000×*g* for 5 min. Analytes were quantified and identified using a GC system equipped with a mass detector mass spectrometer and an Rxi-1MS capillary column (30 m×0.32 mm×0.25 μm, Restek). The ramp temperature was maintained at 60 °C for 1 min, increased to 180 °C at 5 °C/min, and then to 280 °C at 40 °C/min and maintained under isothermal conditions for 15 min (*17*).

Determination of volatile compounds of BSO and beeswax

BSO and beeswax were prepared by the headspace solid-phase microextraction method with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (50/30 μm×20 mm; Supelco, Sigma-Aldrich, Bellefonte, PA, USA) preconditioned following the manufacturer's protocol. For the extraction of volatile organic compounds (VOCs), 0.1 mL of sample was placed in a 20 mL bottle, then 1 g of sodium chloride and 10 μL of standard benzophenone solution (2 μg) were added. The sealed flasks containing the extract were submerged in a water bath at 40 °C for 15 min, then the fiber was exposed to headspace for 15 min under constant agitation. VOCs were quantified and identified by a GC system (QP2010 UltraPlus) equipped with a mass detector mass spectrometer (Shimadzu, Kyoto, Japan), a Rxi-1MS capillary column (30 m×0.32 mm×0.25 μm, Restek), and LabSolution (GCMS solution v. 4.11 SU2, Shimadzu). MS was operated in full scan mode (*m*/*z*=30–450). GC-MS data were analyzed by VOCs were identified by comparing similarity indices and mass spectrum with the NIST11 system database (*18*). Retention index, and retention index calculated from a homologous series of C8-C40 hydrocarbons, and the quantitative analysis was determined by internal standardization with benzophenone (*17*).

Preparation of the oleogels

The preparation of the oleogels was made according to Lim *et al*. (*19*), with some modifications. Beeswax was added to the BSO at concentrations of 1, 3, and 5 % (*m*/*m*), and BSO was used as a control. Initially, the BSO was heated in a thermostatic bath until (90±2) ºC to ensure complete melting (Fisatom, São Paulo, Brazil), with stirring in a digital mechanical stirrer 1107×*g* (IKA, RW20, Hamburg, Germany). After reaching the temperature, the beeswax was added and stirred for 2 min for complete dissolution, then they were placed in an acrylic plate of 32.8 mm (Kasvi, Parana, Brazil). After this period, the oil-wax constituents formed an oleogel. Finally, the oleogels were cooled to room temperature ((22±2) ºC), and the plates were turned off until subsequent analyses.

Oil binding capacity, visual appearance, and storage gel stability of the oleogels

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The oil binding capacity (OBC) of the oleogels was determined according to the methodology of Zheng *et al*. (*20*), with some modifications. About ~1.5 g of sample was centrifuged (Eppendorf 5430 R, Hamburg, Germany) at 7870×*g* for 20 min. Afterwards, the overrun BSO oleogel was drained onto a soft paper towel for 2 min, and the tube mass was weighed together with the solid oil. The empty tube was weighed previously. The values were calculated according to Eq. 1.

$$\text{OBC}\ (\%) = \frac{Mass\ after\ draining}{Total\ mass\ of\ sample} \times 100 \qquad /1/$$

For storage gel stability, the oleogels based on BSO and beeswax (~4mL) were placed in clear glass vials and placed upright up and down. Then, the oleogels were stored at 5 °C/24 h. After this period, the oleogels were maintained within the temperature-controlled room with an air conditioner at (22±2) °C, including phase separation and liquid oil exudation on the surface in the 90 days of analysis. The classification was based on the scale in which a totally liquid [1] system would be defined as oil-like, with high fluidity and phase separation; a weak [2] system would be characterized by high viscosity, slow flow, and minor liquid oil exudation; a medium [3] system exhibited a visual gel property and could easily flow under gravitational force; a firm [4] system possessed a visual gel property and could flow slowly under gravitational force; and totally firm [5] system possessed a visual gel property and could not flow under external force (*21*).

Thermal analyses

The thermal properties of BSO, beeswax, and oleogel were analyzed by differential scanning calorimetry (DSC) (Q20, Instrument TA, Delaware, USA). The oleogel (~5 mg) was heated in an aluminum pan at a rate of 10 °C/min. The thermal behavior was evaluated over a temperature range of –20–300 °C. Nitrogen gas with a flow of 25 mL/min was used as the vehicle.

Fourier Transform Infrared (FTIR) analyses

The samples BSO, beeswax, and oleogels were evaluated by FTIR analysis was performed in conjunction with attenuated total reflection (ATR), using a spectrophotometer model SPIRIT (Shimadzu, Kyoto, Japan) scanned from 4000 cm^{-1} to 400 cm^{-1}, resolution of 4 cm^{-1}, and 100 scan readings.

Oxidative and hydrolytic stabilities of the BSO and oleogels

The oxidative and hydrolytic stabilities of samples were evaluated by peroxide index and acidity index, according to the method of AOCS (*22*). The BSO or oleogel (5 g) was dissolved in the acetic acid and chloroform (3:2 *V*/*V*). Starch (1 g/100 g) was used as a starch indicator on the

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dispersion. The oleogel and BSO were titrated with sodium thiosulfate (0.01 mol/L) The peroxide value was expressed as milliequivalents of oxygen per kilogram of sample (mmol/kg). For acidity index analysis, first, 4 g of the sample was weighed and added to 25 mL of ether-alcohol (2:1, *V*/*V*). Titration was performed with 0.1 mol/L sodium hydroxide solution. The acidity index was expressed as mg of KOH per gram of sample (mg KOH/g).

Preparation of the cookies

The cookies were prepared according to Barragán-Martínez *et al*. (*8*), with modifications, using wheat flour (100 g: composition the proteins 14g, moisture 13g, ash 0.8 %) refined sugar (44 g), hydrogenated vegetable fat Primor (30 g) or BSO (30 g) or oleogel (30 g), baking powder (2.2 g; ingredients: Corn starch, chemical leavening agents sodium acid pyrophosphate, sodium bicarbonate and monocalcium phosphate), salt (0.9 g), and water (18 g). The oleogel chosen for the cookie formulation was the one with the highest OBC, textural, and thermal characteristics (BSO oleogel with 5 % beeswax). Cookies with the addition of pure BSO and cookies with the addition of hydrogenated vegetable fat (control) were also produced. Hydrogenated fats are chemically formed by two saturated fatty acids, stearic acid and palmitic acid (*23*). The dough was prepared in an electric planetary mixer (Kitchen Aid, Troy, Ohio, USA). The dry ingredients, part of the flour, and hydrogenated vegetable fat were mixed for three minutes at low speed, followed by the addition of water and mixing the dough for one minute at low speed and one minute at medium speed. After adding all the flour, the dough was mixed for two minutes at low speed, rolled to a thickness of 5 mm, and cut into a die of 30 mm in diameter. The samples were baked at 180 °C for 9 min in an automatic electric oven (VP 80649, Pratica, Pouso Alegre, Minas Gerais, Brazil). After 2 h of cooling, the physical analyses of the cookies were performed.

Mass loss, expansion volume, specific volume of the cookies

Mass loss of the cookies was obtained by weighing before and after out of the oven (expressed in grams), according to the Eq. 2. The expansion factor was calculated by the ratio between the cookie's diameter and thickness values as per the AACC 10-50D method (*24*). A caliper (Stainless Hardened, Digital caliper, MTX, Moscow, Russia) was used to measure the thickness and diameter of cookies, using a millimeter scale, 1 h after removal from the oven. The calculation of the expansion factor was by the ratio between the cookie volume (cm^3), measured by the 'millet' seed displacement method, and the cookie mass after baking. The analysis was performed in triplicate.

$$Mass\ loss\ = cookie\ mass\ before\ oven\ (g)\ -\ cookie\ mass\ after\ baking\ (g) \qquad /2/$$

Texture profile and color parameters

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The texture profile of the samples was measured using a texturometer (TA.XTplus, StableMicro Systems, Austria, Vienna). The oleogel samples were placed inside plastic Petri dishes measuring 90×15 mm in diameter and filled to the brim. For oleogels, a cylindrical probe (20 mm in diameter and 56 mm in length) was used to compress the samples at 15 mm/s to a depth of 10 mm (*25*), and the probe was positioned at a fixed distance of 20 mm from the base of the equipment. The samples were compressed to 50 % of their initial height by the movement of the probe. The parameters were hardness (g), springiness, cohesiveness, chewiness, and cohesiveness. The cookies were evaluated by hardness (g) and fracturability on at least six cookies from each treatment.

The color parameters of the BSO, oleogel, and cookies were evaluated by the CIE *L**, *a**, and *b** method with a colorimeter (Minolta CR-300, Minolta, Osaka, Japan) and illuminator (D65, 10°). The color parameters determined were: *L** (dark 0 to light 100), *a** (green − to red +), and *b** (blue − to yellow +). The chroma is the ratio of the values of *a** and *b**, according to Eq. 3. For the analysis, five readings were performed on 5 different positions for each sample.

$$Chroma = \sqrt{(a^2 + b^2)} \qquad /3/$$

Statistical analysis

The determinations were performed in triplicate, and the results, expressed as means ± standard deviation, were analyzed using Statistica v 7 software (*26*). ANOVA based on factorial design and Tukey's test ($p \leq 0.05$) were used for data analysis.

RESULTS AND DISCUSSION

Oil extraction yield and determination of fatty acids

The BSO presented an extraction yield of (38.4±3.1) % (*m*/*m*) in basis seed. Hoffmann *et al*. (*1*) reported a range of 29 to 56 % for *Butia odorata* seed oil extraction. In contrast, Pereira *et al*. (*27*) reported lower values for the extraction of *Butia catarinensis* seed oil, with a yield of 17.2 % (*m*/*m*). As hypothesized, BSO predominantly consists of saturated fatty acids, with a combined concentration of 98.84 mg/mL. Among these, short- and medium-chain fatty acids, including caprylic acid at 22.45 mg/mL and lauric acid at 22.87 mg/mL, are the majority (Table 1). Medium-chain triacylglycerols are less likely than long-chain triacylglycerols to accumulate in the human body due to their rapid absorption into the metabolism, which helps prevent mass gain. These fatty acids are beneficial in situations requiring a rapid energy source or when there are digestion challenges (*28*).

Among the unsaturated fatty acids, a high concentration of oleic acid (C18:1) at 33.21 mg/mL and linoleic acid (C18:2) at 30.61 mg/mL was observed. Soybean oil, widely used in food, is primarily composed of linoleic acid (53.2 %) (*23*). In this context, regarding linoleic acid content, BSO exhibits

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a considerable concentration (30.61 mg/mL). These fatty acids, commonly found in palm fruit oils, have generated significant interest due to their fatty acid profile, but their application in oleogel has not yet been studied (*28*). Similar results, however, with higher percentages of lauric acid were found by Vieira *et al.* (*29*), with a value of 39.17 % in *Butia capitate* oil. Unsaturated fatty acids, such as oleic acid (20.73 %), were also found in *Butia capitata* stone oil (*29*). Pereira *et al*. (*27*) reported that oil extracted from *Butia catarinenses* seeds exhibited the highest concentration of lauric acid (39.56 %), followed by oleic acid (11.34 %), capric acid (10.42 %), and caprylic acid (10.08 %). The composition and quantification of fatty acids depend on various factors, including edaphoclimatic conditions, raw material variety, cultivation methods, origin, storage conditions, and the technologies and solvents used (*1*).

The presence of saturated fatty acids provides stability to oxidation, as there are no double bonds in the chain (*29*). Lauric acid, present in significant amounts in BSO, is a short-chain saturated fatty acid known for its antimicrobial properties (*29*). Fats with a fatty acid profile characterized by high percentages of unsaturated and non-trans acids remain liquid at room temperature (*30*). Therefore, oleogel based on BSO, which offers a balanced proportion of healthy fatty acids such as oleic acid and linoleic acid (Table 1), is relevant for replacing hydrogenated vegetable fats, thereby reducing trans fatty acids in cookie formulations. Moreover, BSO, with a high percentage of linoleic acid (omega 6) and oleic acid (omega 9), is considered significant for food and health, given its roles in cell membranes and brain function, which impact the central nervous system (*1*).

The choice of vegetable oil impacts the thermal, textural, and rheological properties of the oleogel, thereby influencing its characteristics and concentration. According to Patel (*31*), oils with varying concentrations of saturated and unsaturated fatty acids yield oleogels with different properties. Oils with higher saturated fatty acid content produce oleogels with a firmer texture. Conversely, when the oil used for oleogel preparation has a high level of unsaturated fatty acids, the concentration of beeswax must be increased.

Metabolomics analysis of beeswax

Like many other lipids, beeswax consists of a mixture of different class components, considered a complex mixture with over 300 components. Each class comprises compounds with varying chain lengths, including hydrocarbons, free fatty acids, fatty acid and alcohol esters, diesters, and exogenous substances (*32)*. Among the chemical compounds in beeswax, fatty acid esters are the main components, formed by combining long-chain fatty acids with long-chain alcohols. Beeswax also contains various hydrocarbons, including medium and long-chain alkanes, such as triacontane (31.85 %), present in the nonpolar fraction (Table S1), and eicosane, predominantly found in the polar fraction (30.31 %) (Table S2). In addition to esters, beeswax contains free fatty acids, including

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palmitic acid (24.18 %), linoleic acid (8.26 %), and stearic acid (2.59 %) (Table S2). Long-chain alcohols, such as heptacosanol (1.08 %) and tetracosanol (2.54 %), formed by the reduction of fatty acid esters, are also present in beeswax. These compounds are part of the nonpolar fraction, with tetratriacontanoic fatty acids comprising 21.92 % and triacontane 31.85 %, making them the major organic compounds.

The use of waxes, particularly beeswax, for structuring edible oils is due to their gel-forming properties at low concentrations and high oil-binding capacity (*33*). Špaldoňová *et al.* (*34*) presented the chromatographic profile displayed for beeswax, which predominantly consists of fatty acid methyl esters with 15-37 carbon atoms, with palmitic acid methyl ester identified as the major compound.

Volatile organic compounds in BSO and beeswax

VOCs are molecules characterized by high vapor pressure, moderate hydrophilicity, and low molecular mass. These compounds play a key role in the sensorial characteristics of foods, influencing aroma and contributing to product acceptance. However, no studies have been conducted on the volatile profile of the oil extracted from *Butia* seed.

The BSO presented six volatile organic compounds, four ketones (2-pentanone, 2,3-pentadione, butylacetone, and sulcatone), one acid (hexanoic acid), and one ester (ethyl hexanoate) (Table 2). The ketones identified in this study are natural compounds commonly found in various plant-derived products. Ethyl hexanoate, with a low aroma threshold, was the main volatile compound in BSO (23.34 mg/mL). According to Hoffmann *et al.* (*1*), this compound was also found in the pulp of *Butia odorata* and is largely responsible for the aroma associated with this species of *Butia*. Hexanoic acid, a medium-chain volatile fatty acid found in *Butia* pulp, is responsible for off-flavor (unpleasant aroma) (*1*).

The main volatile compound in beeswax was D-limonene (22.50 %), followed by decanal (9.48 %), alpha-pinene (9.29 %), nonanal (8.30 %), and beta-pinene (8.23 %) (Table 2). In their study on sunflower oleogel with beeswax, Sobolev *et al*. (*35*) identified alcohols such as 1-hexanol, 1-octanol, and 1-nonanol, along with the terpene D-limonene. According to Felicioli *et al*. (*36*), the high concentration of decanal aldehyde present in beeswax may be associated with antimicrobial activity against *Pseudomonas aeruginosa*. On the other hand, even in small amounts, limonene present in beeswax may contribute to antibacterial activity against *Staphylococcus aureus*. Similarly, nonanal may be related to the antibacterial properties of beeswax against Listeria monocytogenes, even in low concentrations.

Oxidative and hydrolytic stabilities of the BSO and oleogels

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The oxidative and hydrolytic stabilities of the BSO and oleogels were evaluated using the peroxide and acidity indices, respectively (Table 3). The peroxide content in the oleogel with 5 % beeswax was higher than in the BSO ($p<0.05$). This result depends on factors such as the number of double bonds in the molecule and the quantity of fatty acids present. Evaluating lipid oxidation is essential for determining the extent of oil oxidation and, consequently, its quality and chemical composition. The BSO extraction process using Soxhlet extraction and the oleogel production process at 90 ºC, which involves heating, are key factors influencing the peroxide and acidity levels in the oil. According to the CODEX Alimentarius Commission (*37*), both BSO and oleogels are within the acceptable consumption limit, which is a maximum of 10–15 mmol/kg oil. The initial phase of oil oxidation occurs when fatty acids react with oxygen to form odorless compounds, such as peroxides (*38*).

The acidity index was extremely low for BSO and higher for the oleogel with 1% beeswax ($p<0.05$). Increasing the beeswax content to 5 % in the oleogels effectively reduced the acidity index (Table 3) compared to other oleogels and to the BSO. Beeswax has emulsifying properties that can help stabilize the oleogel matrix. This stabilization can reduce the exposure of the oil to potential degradation processes, thus lowering the acidity index by minimizing hydrolysis and oxidation reactions that typically generate free fatty acids. The recommended acidity index for refined oils and fats should not exceed 0.6 mg KOH/g (*39*), indicating that both BSO and oleogels are suitable for food applications.

Oil binding capacity and gel visual stability of the oleogels

The OBC values of the oleogel produced with beeswax and BSO are shown in Fig. 1. There was an increase in the OBC with the concentration of beeswax, with the highest value observed for the oleogel with 5 % beeswax (99.9 %) and the lowest OBC for the oleogel produced with 1 % beeswax (45.1%). Hwang *et al*. (*33*) investigated the influence of the beeswax concentration on oleogel production, ranging from 0.5 to 15 %. These authors reported that very high concentrations of wax result in a strong flavor and make the emulsion firm, which hinders its incorporation into products and increases the final price of the product with the oleogel. In another study, Zheng *et al*. (*20*) produced an oleogel with oil rich in diacylglycerol. They used ethylcellulose as a gelling agent, along with the addition of γ-oryzanol as an antioxidant, achieving OBC results above 98.5 %. However, increasing the concentration of ethylcellulose in the oleogel did not result in a significant increase in OBC.

Other important factors include the source of vegetable oil, the oleogelator used, and their concentrations, all of which can affect the thermal, textural, and rheological properties of the oleogel. Patel (*31*) analyzed five different oils with varying levels of saturated and unsaturated fatty acids,

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yielding different results. This author concluded that oils with higher saturated fatty acid content form oleogels with a firmer texture. BSO has a higher content of saturated fatty acids (98.84 mg/mL) compared to unsaturated fatty acids (63.82 mg/mL). A higher percentage of saturated fatty acids leads to the formation of a stronger oleogel (*10*).

In the visual analysis conducted on the first day, the oleogel with 1 % beeswax had complete disintegration of the gel, with oil flowing through the tube wall (resulting in 100 % oil release) (Fig. S1). From the first day to the 90th day, the oleogels stabilized with 3 and 5 % beeswax showed no oil loss, remaining stable throughout storage. These results demonstrate the effectiveness of beeswax as stabilizers, in concentrations above 3 %, in absorbing and retaining BSO during storage at room temperature (20 ± 3 ºC). Thus, oleogels produced with 3 and 5 % beeswax exhibited stable structures for at least 90 days.

Thermal properties and FTIR analysis

The DSC curves of BSO, beeswax, and oleogel, in the temperature range of -20 to 150 °C, are shown in Fig. 2. All thermograms displayed only one endothermic event. For BSO, the endothermic event occurred between 12°C and 16.5°C, with an enthalpy variation of 4.2 J/g. The oleogel exhibited an endothermic event starting at 16 to 20 ºC, and peaks wider than that of beeswax, with an enthalpy variation of 4.56 J/g. For beeswax, the endothermic event occurred around 65.5 °C (melting), with an enthalpy variation of 5.59 J/g.

The oleogel had a lower melting point and broader peak than beeswax (Fig. 2). This suggests that BSO contains a variety of compounds, some of which have lower melting points, such as butyric, caprylic, and capric acids (Table 1). According to Li *et al*. (*40*), oleogelators with low molecular mass, such as monoacylglycerol, sodium stearoyl lactylate, rice bran wax, and beeswax, have melting points around 60 °C. Waxes are considered among the most efficient structuring agents for producing oleogels, as they crystallize at low concentrations, forming a crystalline network with a high oil retention capacity (*33*,*41*). Hwang *et al*. (*33*) observed a similar change in the melting peak of an organogel containing natural waxes (sunflower wax, rice bran wax, beeswax, and candelilla wax). They attributed this phenomenon to the dilution of the wax upon addition to the oil, which alters the melting temperature of the oleogel. This results in a decrease in temperature and enhances the band signal.

The thermal stability of oleogels is influenced by the stability of the materials used for their stabilization, with enhanced thermal stability typically observed when polymers are combined. Previous studies have documented that oleogels stabilized with certain lipophilic gelling agents, such as fatty acids and waxes, usually have melting temperatures between 50 and 75 °C. Additionally, the melting temperature varies depending on the specific oleogelator used (*41*). However, in this study,

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the endothermic events of both BSO and the oleogel occurred at similar temperatures (16.5 and 20 ºC, respectively). This suggests that the crystallization observed with the addition of beeswax (5 %, *m*/*m*) was not significant enough to substantially enhance the thermal stability of the oleogel.

Fig. 3 shows the FTIR spectra of BSO and beeswax oleogels at concentrations of 1, 3, and 5 %. When analyzing the pure compounds (beeswax and BSO), characteristic bands specific to each compound were observed. Bands between 2990 and 2800 cm^{-1}, at 2885–2780 cm^{-1}, and at 1750 cm^{-1} for BSO, oleogels, and beeswax related to free lipids as the methyl group ($-CH_3$), methylene group ($-CH_2$), and ester group of fatty acids, respectively (*8*). The bands around 1150 cm^{-1} correspond to the stretching of bonds of aliphatic esters and bending vibrations of CH_2 (*11*); for the spectra of the oleogels with different concentrations of beeswax, not all characteristic bands of beeswax were identified. This may be due to the low concentrations of beeswax used.

Color parameters of the BSO and oleogels

The color parameters of BSO and BSO-based oleogels containing different concentrations of beeswax are shown in Table 3. Lightness was higher for BSO (72.46) and decreased with the addition of beeswax, resulting in values of 53.91, 42.66, and 44.06 for oleogels with 5 %, 3 %, and 1 % beeswax, respectively. The *a** red/green coordinate showed negative values for both BSO and all oleogels, indicating greenish tones. Conversely, the *b** yellow/blue coordinate increased as the beeswax concentration increased. No statistical difference was observed between BSO and the oleogel with 1 % beeswax regarding the b* coordinate ($p>0.05$), nor between the oleogels with 3 % and 5 % beeswax, indicating that the yellow coloration of the oleogels is primarily due to the BSO and not the beeswax. BSO contains carotenoids, antioxidant compounds from secondary metabolism, which impart a yellow-orange color (*1*). The chroma value, which measures color purity, was higher for the oleogel with 1 % beeswax and for BSO, with no significant difference between them ($p>0.05$). However, increasing the beeswax concentration (to 3 % and 5 %) in the oleogels resulted in a reduction in the chroma value.

Texture profile analysis of the oleogels

The texture profile analysis of the oleogels is shown in Table 3. Hardness, defined as the maximum force during the first compression, was highest in the oleogel with the greatest concentration of beeswax compared to the others ($p<0.05$). The oleogel with the lowest beeswax concentration (1 %) exhibited greater springiness than the other formulations ($p<0.05$). Overall, all oleogels produced in this study demonstrated low springiness values, indicating a limited ability to return to their original form after deformation, a characteristic inherent to oleogels. Fats are classified by their plasticity, where lower elasticity is associated with greater plasticity (*33*). The springiness and

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cohesiveness values were quite similar between the oleogels containing 3 and 5 % beeswax, both lower than the oleogel with 1 % beeswax, which had the highest values. In terms of hardness and chewiness, the oleogel with 5 % beeswax showed the highest values, which aligns with the high OBC results observed (Fig. 1).

Beeswax has a diverse chemical composition, containing esters, hydrocarbons (eicosane), free fatty acids (palmitic, linoleic, oleic, stearic), and free fatty alcohols (tetracosanol). These compounds, particularly the esters with long chains, crystallize and form networks that trap the liquid vegetable oils. This crystallization changes the physical properties of the oil, affecting the elasticity and hardness of the oleogel (*33*). The texture profile of oleogels is crucial for their optimal industrial application.

Application of the BSO and oleogels in the cookies

The evaluations of texture, color, mass loss, expansion factor, and specific volume of the cookies made with hydrogenated vegetable fat (control), BSO, and oleogel containing BSO and 5 % beeswax are presented in Table 4 and Fig. S2. Oleogel with 5 % beeswax was selected for cookie production due to its gel stability and high oil-binding capacity, ensuring the maintenance of the cookie's desired texture.

The cookie made with oleogel exhibited the lowest hardness, followed by the cookie with BSO, and the one with hydrogenated vegetable fat (control). Hardness refers to the ability to resist deformation under applied force. The fracturability (or brittleness) of a cookie is the distance it deforms before breaking under stress (*35*,*42*). Fracturability has been noted by other authors, with results being lower for cookies containing oil gels modified with olive oil, soybean oil, linseed oil, and wax, compared to the present study (*42*).

In this study, cookies made with BSO exhibited lower hardness than those made with hydrogenated vegetable fat. The reduced hardness of the cookie produced with oleogel and pure BSO may be attributed to the chemical composition of BSO and the beeswax present in the oleogel formulation. The primary components of beeswax are fatty acid esters, which act as emulsifiers, along with monoglycerides and fatty acids (stearic acid and oleic acid) (*33*). The presence of these emulsifiers can improve the incorporation of air into the dough and thus reduce its hardness.

No significant difference was observed in the fracturability of the cookies ($p>0.05$). Fracturability is related to crispness and is a key factor for cookies. Fat affects the softness of the cookies by lubricating the dough, while sugar impacts the characteristics of fracturing or breaking. The thermal stability of the materials and their melting temperature used in cookie production also influence the texture properties (*23*).

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The fracturability (or brittleness) of the biscuit is defined as the deformation (in millimeters or centimeters) that the sample undergoes until it breaks under particular conditions (*42*). Despite the relatively small thickness of the biscuit, a force 10 to 12 times greater is required for it to fracture. Although the force is not directly measured in this parameter, it significantly affects the positioning of the probe, which travels a greater distance before causing a rupture. It is important to note that the texture analysis was conducted 1 h after baking. During this time, the biscuits had already cooled and had a dry structure with no residual moisture. This condition contributed to the increased hardness and the reduced fracturability (*42*).

Hydrogenation, or the use of saturated fats, is necessary to structure liquid oil into semi-solid plastic pastes for use in spreads, margarine, and shortenings (*42*). However, hydrogenated fats are composed primarily of saturated fatty acids, such as palmitic and stearic acid (*23*). Bakery products have been the focus of studies aiming to replace hydrogenated fats with structured oils, due to the global rise in overweight and obesity associated with the high consumption of unhealthy, energy-dense diets, including processed foods (*8*). Zhao *et al*. (*43*) used oleogels in an attempt to replace commercial fat in biscuits; he concluded that biscuits with corn oil oleogel exhibited characteristics comparable to the control (visual appearance, hardness, and firmness), resulting in healthier biscuits with a high content of unsaturated fats.

There was no significant difference in the color parameters between cookies made with oleogel, BSO, and hydrogenated vegetable fat ($p>0.05$). Visual analysis showed that all cookies had a spherical shape with some small grooves on the surface, and the color (Table 4, Fig. S2) was predominantly yellow (Fig. S1, Fig. S2). The cookie made with oleogel exhibited the least mass loss during baking, followed by the cookie with BSO ($p<0.05$), whereas the cookie with hydrogenated vegetable fat had the highest mass loss ($p<0.05$). These results indicate that using oleogel increased mass yield during baking. Goldstein and Seetharaman (*44*) reported a relationship between cookie height and moisture content in cookies made with a monoglyceride emulsion, where greater height was associated with increased moisture. Moisture loss affects cookie volume and expansion capacity, which may explain the lower expansion factor observed in the cookie made with BSO (4.01, $p<0.05$) (Table 4), as no gel was formed to retain moisture during baking.

The cookie made with oleogel had the highest specific volume, while cookies with BSO and the control did not differ from each other ($p>0.05$). There was an inverse relationship between specific volume and hardness: the cookie with oleogel had the highest specific volume (1.29 g/cm^3) and the lowest hardness (7381.62 g). In contrast, the cookie made with hydrogenated vegetable fat had the lowest specific volume (0.81 g/cm^3) and the highest hardness (11258.12 g) compared to the cookies with BSO and oleogel. These results may be related to the higher mass loss observed in this cookie, 14.06 % ($p<0.05$) (Table 4). Specific measurements of volume, thickness, and width in cookies are

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influenced by several factors, including ingredient quality, texture (softness or hardness), types and quantities of ingredients, and processing conditions.

Regarding the expansion factor, there was a significant difference between all cookies, with the highest expansion factor observed for the control cookie. Factors such as cookie diameter, thickness, and expansion factor are commonly used to assess product quality. According to Jacob and Leelavathi (*42*), the spreading of the cookie dough is related to the viscosity of the dough since a dough with low viscosity takes longer to stop spreading in the mold and harden, leading to larger sizes. However, cookies with very high or very low expansion factors cause problems in the processing of industrialized foods, resulting in products with low or high mass.

Oleogelation is a promising method, as it does not alter the unsaturated fatty acid profile of liquid oils. The replacement of 100 % hydrogenated fat by BSO oleogel with beeswax aims to reduce the content of saturated and hydrogenated fats, in addition to improving the lipid profile of cookies, since there is a high content of polyunsaturated fatty acids such as linoleic and oleic acids (Table 1). Furthermore, the oleogel production process does not use chemical reagents to transform liquid oils into thermo-reversible and three-dimensional gel networks with solid-like properties.

CONCLUSIONS

The BSO, considered an unexplored source, presented saturated fatty acids, such as lauric acid and caprylic acid, as well as unsaturated acids, such as oleic and linoleic acids. The main volatile component of BSO was D-limonene. The different concentrations of beeswax added to the oleogel promoted changes in the types of interactions between BSO and beeswax, altering the properties of the oleogel. Oleogels containing 3 and 5 % beeswax maintained their stability over a period of 90 days of storage. The increase in the proportion of beeswax increased the hardness of the oleogels, while the production process did not adversely affect the BSO quality. Oleogel formulated with 5 % beeswax and *Butia* seed oil was utilized in cookies as a replacement for hydrogenated vegetable fat. Cookies containing oleogel exhibited reduced hardness and mass loss, demonstrating potential as a substitute for saturated and trans fats in food products.

However, challenges remain regarding scale-up production, stability under varying industrial storage conditions, and regulatory approval for new lipid structures. Future studies should also explore its functional performance in diverse food systems and processing environments, aiming to validate its technological viability and consumer acceptance. A formulation with biological activity such as antimicrobial and antifungal properties would be ideal for applying oils in bakery products.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPLEMENTARY MATERIALS

All supplementary materials are available at www.ftb.com.hr.

AUTHORS' CONTRIBUTION

Cristina Jansen-Alves Elder and Pacheco da Cruz participated in the design and execution of experiments, data processing and interpretation, and in the preparation and writing of the manuscript. Rosinei Silva Santos, Camila de Oliveira Pacheco, Carem Perleberg, and Helen Cristina dos Santos Hackbart helped with the design of the experiments, data processing, and interpretation. Claudio Martin Pereira de Pereira and Alvaro Renato Guerra Dias in data interpretation and manuscript review. Elessandra da Rosa Zavareze was involved in designing the experiments and preparing and revising the manuscript. All authors have read and approved the final version of the manuscript.

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Table 1. Fatty acid profile in *Butia* seed oil (BSO)

Fatty acid	Usual nomenclature	*γ*/(mg/mL)	*w*/%
C4:0	Butyric acid	10.20	6.43
C8:0	Caprylic acid	22.45	14.15
C10:0	Capric acid	12.56	7.92
C11:0	Hendecanoic acid	0.13	0.08
C12:0	Lauric acid	22.87	14.41
C13:0	-	0.18	0.11
C14:0	Myristic acid	8.81	5.55
C16:0	Palmitic acid	6.22	3.92
C18:0	Estearic acid	11.25	7.09
C18:1	Oleic acid	33.21	20.93
C18:2	Linoleic acid	30.61	19.29
C20:0	Arachidic acid	0.09	0.06
C24:0	Lignoceric acid	0.08	0.05
ΣSFA		94.84	59.78
ΣMUFA		33.21	20.93
ΣPUFA		30.61	19.29
ΣTotal		158.66	100.00

Σ=sum of SFA (saturated fatty acids), MUFA=monounsaturated fatty acids, PUFA=polyunsaturated fatty acids

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Table 2. Volatile organic compounds (VOC) identified in *Butia* seed oil (BSO)

VOC	IS	IRL	IRL exp	Reference ion (NIST11)	Experimental reference ions	*γ*/(mg/mL)
2-Pentanone	96	654	-	43.00(100.00)86.00(20.19)41.00(11.89)	43.00(100.00)86.05(28.97)41.00(11.95)	0.42
2,3-Pentadione	91	790	-	43.00(100.00)29.00(61.00)100.00(11.89)	43.00(100.00)57.00(58.41)100.00(26.66)	1.27
Butylacetone	93	853	938	43.00(100.00)58.00(60.19)71.00(13.63)	43.00(100.00)58.00(36.58)71.05(13.04)	6.80
Hexanoic acid	96	884	1020	74.00(100.00)87.00(31.69)43.00(31.29)	74.05(100.00)43.00(43.17)87.00(37.95)	3.44
Sulcatone	94	938	1071	43.00(100.00)41.00(53.70)69.00(35.09)	43.00(100.00)41.00(60.56)69.05(37.68)	1.01
Ethyl hexoate	98	984	1092	88.00(100.00)99.00(55.96)43.00(44.44)	88.00(100.00)99.05(56.81)43.00(54.35)	23.34

IS=index of similarity from literature Mass Spectral Library NIST (National Institute of Standards and Technology) v. 11, IRL=index of retention from literature NIST11, IRL exp=index of retention experimental (base on homologous series n-alkane C8-C40). Reference ions (relative intensity, base peak=100 %) from literature NIST11

Table 3. Color parameters of the *Butia* seed oil (BSO) and oleogels with different concentrations of beeswax, and texture profile of oleogel

Parameter	BSO	Oleogels	
		3 % beeswax	5 % beeswax
*L**	(72.46±2.15)^{a}	(42.66±1.32)^{b}	(44.06±0.99)^{b}
*a**	(-2.94±0.35)^{a}	(-0.90±0.24)^{c}	(-1.41±0.17)^{b}
*b**	(13.23±1.37)^{a}	(6.86±0.11)^{b}	(5.22±0.86)^{b}
*C**	(13.55±1.41)^{a}	(6.92±0.14)^{b}	(5.42±0.85)^{c}
Hardness/g	_	(59.71±3.56)^{b}	(228.01±11.17)^{a}
Springiness	_	(0.99±0.00)^{a}	(1.00±0.00)^{a}
Cohesiveness	_	(0.38±0.02)^{a}	(0.36±0.02)^{a}
Chewiness	_	(22.59±0.90)^{b}	(81.66±0.80)^{a}

Results are expressed as mean value±standard deviation (*N*=3). Different letters in the same row are significantly different for color parameters evaluated by Tukey's test and ($p<0.05$). Different letters in the same row are significantly different for texture profile evaluated by *t*-test ($p<0.05$)

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Table 4. Texture, color and physical parameters of cookies produced with hydrogenated vegetable fat (control), *Butia* seed oil (BSO), and oleogel

Parameters	Cookies		
	Hydrogenated vegetal fat (control)	BSO	Oleogel (BSO and 5 % beeswax)
	Texture profile		
Hardness/g	(11258.12±615.29)[a]	(8972.20±693.87)[b]	(7381.62±504.10)[c]
Fracturability/c	(6.657±0.72)[a]	(6.618±0.69)[a]	(6.614±0.38)[a]
*L**	(79.61±1.59)[a]	(78.55±0.75)[a]	(78.92±1.19)[a]
*a**	(0.50±0.37)[a]	(0.42±0.34)[a]	(0.26±0.45)[a]
*b**	(22.32±1.89)[a]	(22.19±1.14)[a]	(22.56±1.63)[a]
*C**	(22.32±1.89)[a]	(22.38±1.14)[a]	(22.57±1.64)[a]
Mass loss/%	(14.06±0.50)[a]	(12.22±0.77)[b]	(10.82±0.70)[c]
Specific volume (g/cm³)	(0.81±0.02)[b]	(0.92±0.02)[b]	(1.29±0.15)[a]
Expansion factor	(4.79±0.17)[a]	(4.01±0.20)[c]	(4.47±0.18)[b]

Different letters in the same row are significantly different for each parameter evaluated by Tukey's test ($p<0.05$)

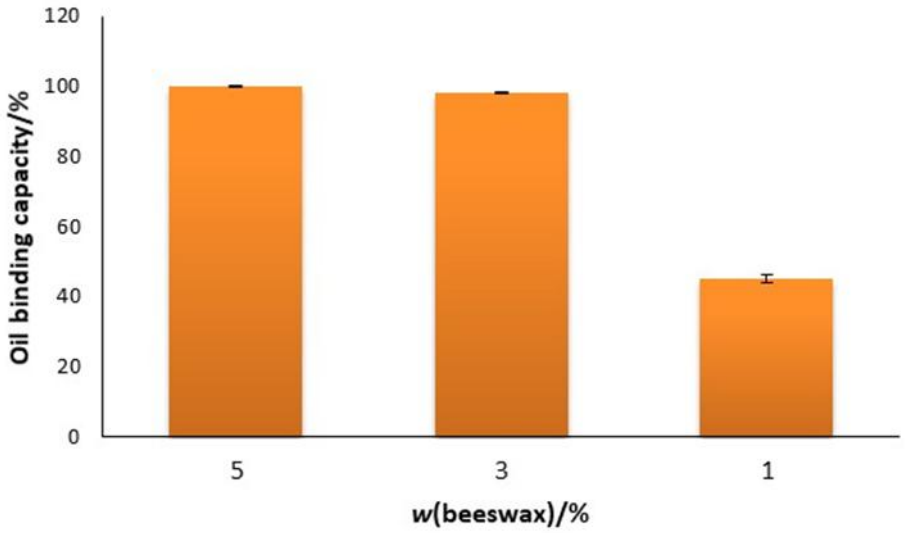

Fig. 1. Oil binding capacity (%) of oleogel *Butia seed* oil (BSO) with beeswax (1, 3 and 5 %)

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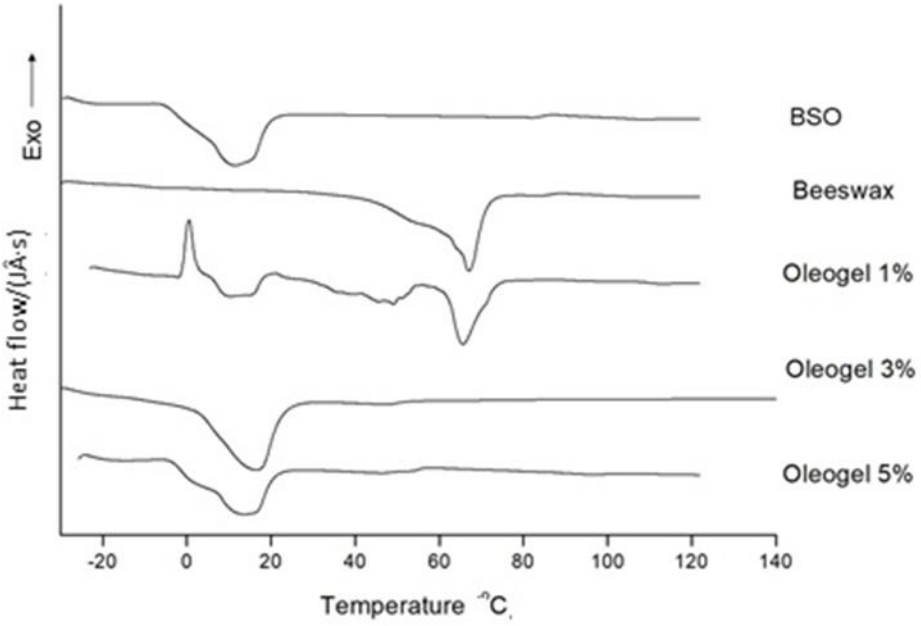

Fig. 2. Thermograms of *Butia seed* oil (BSO), beeswax, and oleogel containing 1, 3, and 5 % beeswax

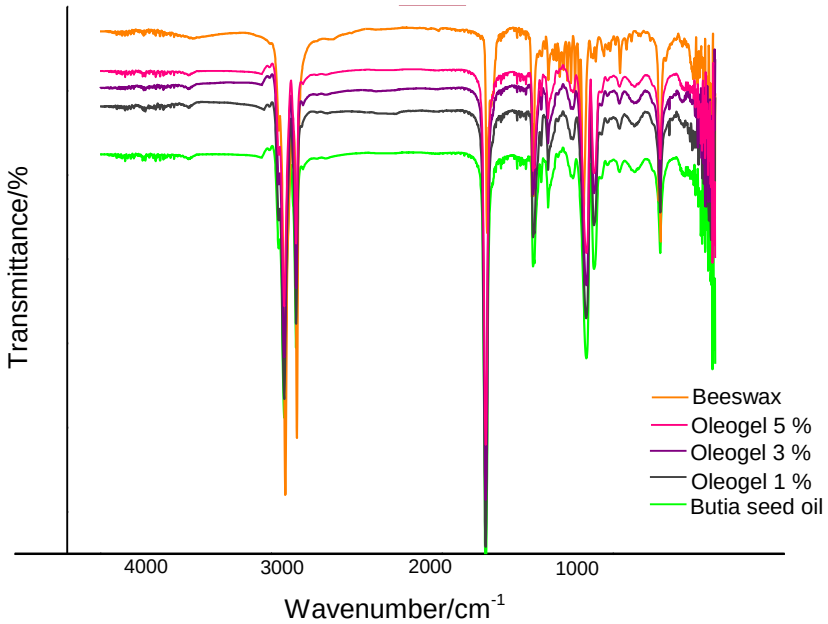

Fig. 3. FTIR spectra for beeswax, oleogels containing 5, 3, and 1 % beeswax and *Butia* seed oil

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Table S1. Polar fraction of the compounds identified in the beeswax

Polar fraction	IS	IRL	IRLexp	Reference ion (NIST11)	Reference ion exp	Quantification/%
13-Octadecenoic acid, (E)-, TMS	93	2194	2227	73.00(100)75.00(93.89)55.00(81.38)	73.05(100)75.00(86.20)55.05(44.33)	1.18
1-Nonadecene	90	1900	1938	97.00(100)83.00(92.94)43.00(91.76)	97.10(100)83.10(86.60)43.05(52.80)	0.42
9-Hexadecenoic acid, (Z)-, TMS	94	1995	2017	117.00(100)73.00(98.60)75.00(97.70)	73.05(100)117.05(96.28)75.00(89.53)	2.08
Arachidic acid, TMS	90	2385	2416	73.00(100)117.00(76.58)75.00(67.22)	117.05(100)73.05(77.09)75.00(59.93)	0.72
Docosane	95	2208	2200	57.00(100)71.00(71.33)43.00(63.42)	57.10(100)71.10(91.67)43.05(58.39)	8.97
Eicosane	95	2009	2010	57.00(100)71.00(77.28)43.00(63.07)	57.10(100)71.10(91.67)43.05(58.39)	30.31
Elaidic acid, (E)-, TMS	94	2194	2215	73.00(100)117.00(95.00)75.00(94.60)	117.05(100)73.05(94.12)75.00(78.03)	14.83
Heneicosane	96	2109	2100	57.00(100)71.00(70.27)85.00(54.95)	57.10(100)71.10(77.11)85.10(55.77)	1.34
Heptacosanol	94	2948	3016	57.00(100)97.00(97.00)83.00(95.20)	97.10(100)83.10(95.93)57.10(79.03)	1.08
Linoleic acid, TMS	94	2202	2208	75.00(100)73.00(97.90)67.00(65.07)	73.05(100)75.00(82.12)67.05(60.60)	8.26
Methyl stearate	91	2077	2102	74.00(100)87.00(74.48)43.00(33.73)	74.00(100)87.05(60.32)43.05(26.18)	1.50
Palmitic acid, TMS	93	1987	2036	117.00(100)73.00(86.09)313.00(68.97)	117.05(100)73.05(81.64)313.20(59.32)	24.18
Stearic acid, TMS	92	2186	2234	117.00(100)73.00(77.08)341.00(67.17)	117.05(100)73.05(77.72)341.25(55.38)	2.59
Tetracosanol	94	2650	2700	57.00(100)83.00(95.40)55.00(94.70)	83.10(100)57.05(78.64)55.05(63.11)	2.54

IS=index of similarity from literature NIST11 (National Institute of Standards and Technology), IRL=index of retention from literature NIST11, IRL exp=index of retention experimental (based on homologous series n-alkane C8-C21). Reference ions (relative intensity, base peak = 100 %) from literature NIST11 and reference ions experimental

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Table S2. Nonpolar fraction of the compounds identified in the beeswax

Non-polar fraction	IS	IRL	IRLexp	Reference ion (NIST11)	Reference ion exp	Quantification/%
9-Hexacosene	92	2614	2566	43.00(100)97.00(97.06)57.00(96.53)	97.10(100)43.05(86.25)57.05(69.51)	9.09
E,E,Z-1,3,12-Nonadecatriene-5,14-diol	90	2241	2200	55.00(100)95.00(85.17)81.00(79.74)	81.05(100)55.05(84.98)95.10(75.51)	1.13
Eicosane	96	2009	2000	57.00(100)71.00(77.28)43.00(63.07)	57.05(100)71.10(94.88)43.05(61.42)	1.80
Hexatriacontane	93	3600	3400	57.00(100)71.00(74.40)43.00(54.90)	57.05(100)71.10(99.78)43.05(56.6)	6.66
Methyl elaidate	95	2085	2084	55.00(100)69.00(95.50)74.00(90.20)	55.05(100)69.05(86.55)74.05(72.32)	0.42
Methyl palmitate	93	1878	1920	74.00(100)87(72.07)43.00(32.53)	74.00(100)87.05(55.29)43.05(22.05)	0.88
Methyl stearate	92	2077	2109	74.00(100)87.00(76.48)298.00(35.23)	74.05(100)87.05(61.54)298.25(26.97)	1.84
Neoheptanol	86	875	868	43.00(100.00)85.00(51.01)41.00(29.00)	43.05(100)85.10(40.14)41.05(20.72)	0.22
Nonadecyl trifluoroacetate	94	2110	2100	57.00(100)97.00(97.40)83.00(84.49)	97.10(100)83.10(87.39)57.05(72.44)	2.51
Olean-12-en-3-ol	90	2886	2970	218(100)203(43.84)219.00(18.02)	218.15(100)103.10(54.22)219.15(18.02)	0.36
Oleic acid	90	2175	2171	55.00(100)69.00(81.98)83.00(77.58)	55.05(100)69.05(94.33)83.10(74.97)	1.20
Oleic acid amide	90	2228	2375	59.00(100)72.00(50.30)55.00(22.69)	59.00(100)72.05(77.13)55.05(25.42)	3.69
Palmitic acid	91	1968	1967	60.00(100)73.00(98.10)57.00(84.09)	73.05(100)60.00(66.88)57.05(62.71)	2.93
Tetracosane	94	2407	2400	57.00(100)71.00(68.00)43.00(56.20)	57.05(100)71.10(91.59)43.05(59.00)	12.32

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Tetratriacontane	95	3401	3400	57.00(100)71.00(71.20)43.00(54.40)	57.05(100)71.10(73.68)85.10(63.35)	21.92
Triacontane	95	3003	3000	57.00(100)71.00(72.10)85.00(53.50)	57.05(100)71.10(82.81)85.10(68.39)	31.85
Trimethylsilyl palmitate	93	1987	2041	117.00(100)73.00(86.09)313.00(68.97)	117.05(100)73.05(74.56)313.20(60.08)	1.18

IS=index of similarity from literature NIST11 (National Institute of Standards and Technology), IRL=index of retention from literature NIST11, IRL exp=index of retention experimental (based on homologous series n-alkane C8-C21). Reference ions (relative intensity, base peak=100%) from literature NIST11 and reference ions experimental

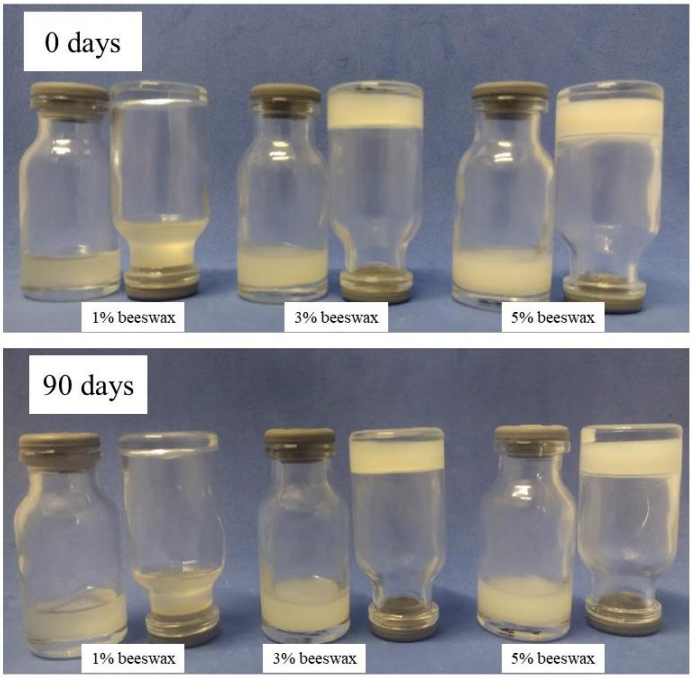

Fig. S1. Visual analysis of 90-day storage stability of oleogel *Butia* seed oil with beeswax (1, 3, and 5 %)

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Fig. S2. Oleogel containing 5 % beeswax and *Butia* seed oil (BSO) (a); cookie with hydrogenated vegetable fat (b); cookie with oleogel (c); cookie with BSO (d)