A First Approach to the Optical and Antioxidant Properties of Propolis Collected at Different Sites of Algarve Region

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Abstract: Propolis is a natural honeybee product known to be beneficial to human health. It has a very complex chemical composition, highly dependent on the respective collection site, although phenolic compounds stands for the major fraction responsible for propolis biological activities. Up to now, all assays for determining composition and biological properties have been done after extraction of the raw material with appropriate solvents. Studies concerning the properties of Portuguese propolis are scarce. In the present work, propolis collected at three different sites in the Portuguese province of Algarve, differing in both the soil and vegetation, were compared in respect to their optical and anti-oxidant properties. Two main goals were attempt: 1) to check the antioxidant activity of the methanol extracts of propolis and 2) to test the applicability of the Visible/Near infrared spectroscopy (Vis/NIRS) on the propolis raw material and to determine the best conditions to obtain the respective reflectance spectra, in order to establish in the long-term a rapid and non-invasive technique for propolis screening collected in the Algarve area. The amount of total phenols in propolis ranged from 1,710 to 4,195 mg/ml. The highest phenols levels were predominantly found in Maqui samples. The hierarchy of ability for scavenging DPPH radicals was maqui area > mountain area > transition area. Additionally, Maqui samples were shown to be the best antioxidants, since they were able to promote more easily the reduction of Fe(III) to Fe(II). The Vis/NIR diffuse reflectance spectra obtained differed according to the collecting sites, although all of them exhibited a peak around 875nm. Mountain North samples had the highest R values at all wavelengths, followed by the Mountain South. Minimal values were shown by the Transition Zone samples, particularly from 650 upwards. Maqui samples R spectra presented intermediate values.

Keywords: Diffuse reflectance, Vis/NIR, quality, antioxidant, propolis.

1. Introduction

Propolis or bee glue is a resinous dark-coloured material collected by honeybees from different parts of plants (branches, flowers, pollen and buds) and mixed with bee wax and salivary secretions in the hive. Honeybees use propolis as a hive sealer as well as to prevent the decomposition of organisms previously killed by them after invasion of the hive [1-3].

Propolis chemical composition is complex. This natural product is predominantly constituted by 50% resin (composed of flavonoids and related phenolic acids), 30% wax, 10% essential oils, 5% pollen and 5% various organic compounds. The composition and contents of these components depend on various factors: season, vegetation of the area, geographical origin and the state of propolis (fresh or aged) [4,5].

The biological properties of propolis (antibacterial, antiviral, anti-inflammatory, antifungal, antiepithetotoxic, antioxidant and antitumoral) [1,2,6-9], mainly due to the presence of polyphenols (including flavonoids, phenolic acids and their esters), also differ with the geographic area [7,10].

Propolis has been used in folk medicine as an antiseptic and cicatrising in wound treatment, as a mouth disinfectant and also as an antipyretic agent. The importance of propolis in cosmetics and as a constituent of health food has increased and, at present; it is one of the most popular natural products, being commercially available throughout the world [4]. In this way it is necessary to ensure the consistency of pharmacological and clinical research, to understand the biological mechanisms of action of the components of propolis as well as to
achieve a reliable standardisation on propolis types and to enhance product quality control. In Portugal, namely in Algarve, very few is known about propolis, in spite of the economic importance of beekeeping in this country.

The aims of the present study were to compare propolis collected at different sites of Algarve (Portugal): 1) The antioxidant activity of the methanol extracts of propolis; 2) The applicability of the Visible/Near infrared Spectroscopy (Vis/NIRS) on the propolis raw material and to determine the best conditions to obtain the respective reflectance spectra. This last goal will allow in the long-term to establish in a rapid and non-invasive technique for propolis screening collected in the Algarve area. This would be attempted by the correlation of the Vis/NIR diffuse reflectance spectra obtained from propolis raw material from different origins with the respective chemical composition, using partial least squares (PLS) [11].

2. Material and Methods

2.1. Material

Samples were collected on the countryside of the Mediterranean province of Portugal, Algarve. They were collected from three different zones comprehending different soil and vegetation properties: mountain-like [1-10], transition-like [11-16] and maqui-like [17-24].

2.2. Colour determination

Colour was measured with a Minolta Chroma Meter CR-300 series, CE Minolta, Japan, using the L* value, which measures lightness corresponding L=0 to black colour and L=100 white colour.

2.3. Vis/NIRS

Vis/NIRS measurements were performed in the wavelength band between 445 and 930nm with an optical spectrometer (USB4000, Ocean Optics, USA), using an Integrating Sphere (IS). Light from a tungsten-halogen source (HL-2000-FHSA, Ocean Optics, USA) is sent to the propolis raw material, compressed in a transparent Petri dish through an optical fibre and the re-emerging light is collected by a second fibre and sent to the spectrometer. For the acquisition, processing and calibration, specific software was used (Spectra Suite, Ocean Optics, USA). The diffuse reflectance (R) spectra from each from each propolis sample was calculated automatically taking in account the raw sample spectrum (RS), the dark spectrum (RD) (effect of the detector’s inherent dark current) and the reference spectrum (RR) (taken from a Spectralon white surface, WS-1, Ocean Optics, USA), according to the mathematical expression R=100x(RS- RD)/(RR- RD). All Vis/NIRS measurements are carried out in a dark room at 20 ±2 oC and took about 10s. Data manipulation and auxiliary calculations were performed in MATLAB 5® [13].

2.4. Extraction of propolis

Propolis (1g) was copped into small pieces and extracted with 10 ml of water at 80ºC for 3h. Following centrifugation, the residue was further extracted with methanol (10ml) under reflux for 3h. The methanol suspension was separated by centrifugation. All extractions were performed in triplicate (A, B, C).

2.5. Total phenols content

Total phenols content in samples was determined by the Folin-Ciocalteau colometric method [14]. Samples solutions (0.25 ml) was mixed with 1 ml of the Folin Ciocalteau (1:10) and 1.25 ml of Na2CO3 (75g/ml), and the absorbance was measure at 760 nm after 30 minutes incubation at room temperature (±25oC). Total phenols content was expressed as mg/ml (gallic acid equivalents) from calibration curve. All determinations were performed in triplicate.

2.6. Free radical scavenging activity (DPPH)

The reaction mixture contained 1ml of 60 M methanolic solution of DPPH and test samples (25 µl). After 5 min incubation at room temperature (±25oC), the absorbance was recorded at 517 nm. Absorption of a blank sample containing the same amount of methanol and DPPH solution acted as negative control. All determinations were performed in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to the following formula: Scavenging effect % = [(A0 – A1) / A0] * 100 where A0 was the absorbance of the blank sample and A1 was the absorbance in the presence of the sample for each reading time. Gallic acid represented the positive control [15].

2.7. Fe (III) to Fe(II) reductive capacity

The Fe(III) reductive capacity of the extracts was assessed spectrophotometrically [16]. Each sample (0.25 ml) was mixed with 0.625 ml phosphate buffer (200mM, pH 6.6) and 0.625 ml of a 1% (w/v) potassium hexacyanoferrate [K3Fe(CN)6] solution. After 20 min at 50ºC, 0.625 ml (10%, w/v) trichloroacetic acid (TCA) were added and the...
mixture was centrifuged for 10 min (3000 rpm). Finally, a 0.625 ml aliquot was mixed with 0.625 ml distilled water and 0.125 ml (0.1%, w/v) FeCl₃ and the absorbance was recorded at 700 nm. Samples were evaluated at a final concentration of total phenols at 0.0175 mg/ml and BHA at 4.17E-05 to 3.33E-03 mg/ml was used as reference samples.

3. Results and discussion

Propolis has a grey to black colour. In order to measure its colour it was chosen the L* value which measures lightness. In the table 1 we can see the mean L* values in the locations where propolis was collected. Additionally, no statistically significant differences (p<0.05) were found in the colour parameters of the various propolis samples (table 1).

Table 1. Colour, L* value which measures lightness corresponding L=0 to black and L=100 to white

<table>
<thead>
<tr>
<th>Mountain North</th>
<th>Mountain South</th>
<th>Transition zone</th>
<th>Maqui North</th>
<th>Maqui South</th>
</tr>
</thead>
<tbody>
<tr>
<td>25.77±1.30</td>
<td>26.43±1.53</td>
<td>26.67±0.98</td>
<td>25.97±1.33</td>
<td>27.99±2.52</td>
</tr>
</tbody>
</table>

As expected from the very dark colour exhibited by all raw propolis samples, the feasibility of this analysis turned out quite difficult and the respective Vis/NIR diffuse reflectance (R) spectra presented very low values at all wavelengths (Fig. 1). Unfortunately, the wavelengths below the visible range, that could be directly related to the phenols content shown to be higher abundant in the Maqui samples (table 2), were excluded, because of the high noise to signal ratio.

However, we have obtained Vis/NIR diffuse reflectance spectra which differed according to the collecting sites, although all of them exhibited a peak around 875 nm (Fig. 1). Mountain North samples had the highest R values at all wavelengths, followed by the Mountain South. Minimal values were shown by the Transition Zone samples, particularly from 650 upwards.

Maqui samples R spectra presented intermediate values. Although these are preliminary studies, we must consider the possibility of a variation in the propolis wax structure, full texture and chemical compounds content other than phenols or flavonoids, accordingly to the collecting sites and the main vegetation present therein. This could cause different absorption, scattering and/or reflection of light by the different propolis samples and thus, different Vis/NIR spectra.

Table 2. DPPH free radical scavenging activity (IC50 values), total phenolics and Fe(III) to Fe(II) reductive capacity of samples.

<table>
<thead>
<tr>
<th>Samples</th>
<th>DPPH IC₅₀ (mg/ml)</th>
<th>Total phenolics (mg/ml)</th>
<th>Fe(II) to Fe(III) reductive capacity (Absorbance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maqui North</td>
<td>0.034 ± 0.002</td>
<td>0.190 ± 0.021</td>
<td>0.233 ± 0.001</td>
</tr>
<tr>
<td>Maqui South</td>
<td>0.082 ± 0.005</td>
<td>0.170 ± 0.019</td>
<td>0.242 ± 0.019</td>
</tr>
<tr>
<td>Transition Zone</td>
<td>1.710 ± 0.083</td>
<td>0.396 ± 0.006</td>
<td>0.300 ± 0.006</td>
</tr>
<tr>
<td>Mountain North</td>
<td>0.010 ± 0.002</td>
<td>0.786 ± 0.081</td>
<td>0.416 ± 0.081</td>
</tr>
<tr>
<td>Maqui South</td>
<td>0.011 ± 0.001</td>
<td>0.884 ± 0.083</td>
<td>0.443 ± 0.083</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>0.003 ± 0.010</td>
<td>0.030 ± 0.001</td>
<td>0.020 ± 0.005</td>
</tr>
</tbody>
</table>

a Each value is the mean ± standard error.
b As Gallic acid equivalents.
c These samples do not reach 50% of activity.

Table 2 shows the total phenols content of samples as well as their capacity for scavenging free radicals (DPPH method) and reductive power. The amount of total phenols in propolis ranged from 1,710 to 4,195 mg/ml. The highest phenols levels were predominantly found in Maqui samples. The sample concentration required to scavenge 50% of the DPPH radicals (IC50 values), are represented in table 2. The hierarchy of ability for scavenging...
DPPH radicals was maqui area > mountain area > transition area. Samples of maqui zone presented the lowest values of IC50 being some of them similar to that of Gallic acid (reference). The maqui area required a lower concentration of extract for scavenging 50% of the DPPH radicals, which suggests that the components within this extracts are stronger radical-scavenging components than those of the remaining areas. Propolis having higher DPPH free radical scavenging activity, indicated by a low IC50 value, demonstrates a high antioxidant activity.

Based on the absorbance values depicted in Fig. 2, all the extracts were capable of catalysing the reduction of Fe (III). Nevertheless, the extracts possessed a value of absorbance that was much lower than that observed for the reference substance (BHA). As observed for DPPH method, also in this assay the samples coming from the Maqui area proven to be the best antioxidants, since they were able to promote more easily the reduction of Fe(III) to Fe(II).

**Figure 2.** Fe (III) to Fe (II) reductive capacity of Portuguese propolis collected at the mountain, transition zone and Maqui sites.

In spite of the relative proximity of the hives (Algarve) it was clear that different propolis samples from diverse locations possessed dissimilar amounts of phenols as well as antioxidant activities. Such results may be partly explained by the different vegetation found in those zones. Also some authors [7] have found that in some few southern Chinese propolis samples had a very small antioxidant activity in contrast to the remaining Chinese samples, distantly located from the former ones.

**4 Conclusions**

In respect to the optical properties of the propolis samples studied, they exhibited Vis/NIR diffuse reflectance spectra which differed according to the collecting sites, although all of them exhibited a peak around 875nm. However, the feasibility of this analysis turned out quite difficult and the respective Vis/NIR diffuse reflectance (R) spectra presented very low values at all wavelength.

**Acknowledgements:**

Cruz Alta, Agricultura, Lda. (Loulé, Portugal), and Dr. Derek Power are acknowledged for providing the propolis used in this study. Ana M. Cavaco has a post-doc fellowship (SFRH/BPD/ 11613/2002) of Fundação para a Ciência e a Tecnologia.

**References**


