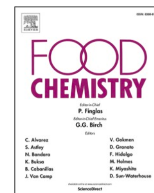
Food Chemistry 355 (2021) 129629

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Polymer film as starch azure container for the easy diastase activity determination in honey

Lara González-Ceballos [a], Miguel A. Fernández-Muiño [b], Sandra M. Osés [b], M. Teresa Sancho [b], Saturnino Ibeas [a], Jose Antonio Reglero Ruiz [a], Saúl Vallejos [a,*]

[a] Department of Chemistry, Facultad de Ciencias, Universidad de Burgos, Plaza de Misael Bañuelos s/n, 09001 Burgos, Spain
[b] Department of Biotechnology and Food Science, Universidad de Burgos, Plaza de Misael Bañuelos s/n, 09001 Burgos, Spain

ARTICLE INFO

Keywords:
Honey
Diastase activity
Starch azure
Polymer
Film

ABSTRACT

A new original application for a polyacrylic film based on the monomers 2-(dimethylamino)ethyl methacrylate (**NNDA**), 2-hydroxyethyl acrylate (**2HEA**) and methylmethacrylate (**MMA**) as a starch azure container has been set up for a simple determination of honey diastase activity. The proposed method is based on the correlation of reducing sugars generated during the enzymatic process with the Schade reference assay. The polyacrylic film is charged with starch azure acting as a container for this substance; thus, the starch does not interfere in the measurement of reducing sugars, so that the diastase activity is easily calculated. The method has been contrasted with Schade method, showing good correlation and differences under 0.4% between methods in some honey samples. The polyacrylic film has great potential for the routine honey diastase activity assessment in small laboratories, dramatically reducing analysis time and cost.

1. Introduction

Honey is a sweet food made by bees, whose composition includes a variety of compounds (Bogdanov, 2017) among them sugars, organic acids, minerals, vitamins, amino acids, polyphenols, substances responsible for aroma and flavours and enzymes (diastase, invertase, glucose oxidase, catalase, and others). This study focuses on honey diastases that are currently enzymes of paramount importance in honey control (Thrasyvoulou et al., 2018).

Diastase hydrolyses starch into dextrin and finally into reducing sugars (**RS**), in this case, glucose. The activity of this enzyme in honey is one of the indicators of honey quality since it is related to ageing, as well as to inadequate processing and storage procedures. Honey usually does not fulfil the conditions for commercialisation after being overheated, reheated or/and stored for a long time (Pasias, Kiriakou, & Proestos, 2017; Tosi, Martinet, Ortega, Lucero, & Ré, 2008), because diastase activity has dropped below the legal limit (OJEC, 2001). Thus, a fast method to accurately analyse the diastase activity is currently of utmost importance.

Nowadays, the most used methods for the determination of the diastase activity in honey are two, namely Schade and Phadebas assays (Bogdanov, 2009; Stoldt & Uwe Trapp, 2019) However, the first is tedious and time-consuming, and the latter is expensive for a significant number of analytical determinations and has not yet been broadly accepted as a reference method.

This work has been developed using Schade procedure as the reference assay, which is based on a previous starch standardisation, and requires partial shipments of starch samples by the supplier, verification of the appropriateness of the starch, and correction by the supplier (if applicable) until a valid batch (starch) is found. The validity of the batch depends on whether the absorbance of the blue complex that starch forms in the presence of lugol is within a narrow absorption range at a given wavelength (660 nm, absorbance between 0.5 and 0.55; see **ESI S1**), and once achieved, the user usually must purchase the entire batch, which is costly. After checking that the starch fulfils every requirement, the experimental assay of Schade method can be carried out. The diastase activity of a honey sample is determined by adding the standardised starch. The decrease of the concentration of starch in the sample is monitored at different times, following by UV–Vis spectrophotometry a blue complex formed in the presence of lugol (more information in **ESI S2**). Using Schade method, the time for sample analysis is variable from 45 min to several hours, depending on the diastase activity of the honey, which makes the procedure tedious, and complicated when planning the analysis of a great number of samples. In fact, some authors (Sak-Bosnar

* Corresponding author.
E-mail address: svallejos@ubu.es (S. Vallejos).

https://doi.org/10.1016/j.foodchem.2021.129629
Received 22 December 2020; Received in revised form 19 February 2021; Accepted 14 March 2021
Available online 18 March 2021
0308-8146/© 2021 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

& Sakač, 2012; Sakač & Sak-Bosnar, 2012) have already developed direct and alternative methods for the determination of diastasic activity based on a platinum redox sensor. Despite being a great advance, the study was based on only 10 kinds of honey, and the method requires the use of relatively expensive equipment, electrodes, and other instrumentation necessary for typical electrochemical analysis, so, these methods are not accessible for beekeeping cooperatives, and are not oriented to small laboratories.

In this research, we propose a new original application for a simple polyacrylic film based on 2-(dimethylamino)ethyl methacrylate (**NNDA**), 2-hydroxyethyl acrylate (**2HEA**) and methylmethacrylate (**MMA**) as a starch azure container for the easy determination of honey diastase activity. Our proposal is based on the correlation of our proposed method with the reference method. Additionally, our proposed method does not follow the decrease of the starch concentration, but the increase of reducing sugars concentration generated during the enzymatic process, i.e., the starch standardisation process is not necessary, which is a significant advantage.

The use of the polyacrylic film is mandatory for the quantification of **RS**, which is carried out by Fehling method (Fehling, 1849) (more information in **ESI S3**). Without the polyacrylic film, starch would interfere in the measurement of reducing sugars, and the analysis would be impossible. However, the studied polyacrylic film can be charged with starch azure and can act as a container for this substance. In this way, the starch azure does not interfere in the measurement of reducing sugars, and the diastase activity can be calculated. The reason for using starch azure instead of conventional starch is the naked eye verification of the charging process with starch of the polyacrylic film. Fig. 1 shows a graphical summary of the proposed method.

2. Materials and methods

2.1. Honey samples

This study was carried out with 20 representative honeys harvested in 2019 in Castilla-León, a Spanish area located in the northern Iberian Plateau that held the highest number (3827) of apicultural undertakings in Spain in 2018, representing 16% of the total apicultural undertakings of this country (Ministerio de Agricultura, 2019). Honeys' botanical origins were determined by both melissopalinology (Louveaux, Maurizio, & Vorwohl, 1978; Terradillos, Muniategui, Sancho, Huidobro, & Simal-Lozano, 1994; Von Der Ohe, Persano Oddo, Piana, Morlot, & Martin, 2004), and sensory analyses (Marcazzan, Mucignat-Caretta, Marina Marchese, & Piana, 2018; Persano Oddo & Piro, 2004; Piana et al., 2004), there is 1 chestnut (Fagaceae type *Castanea sativa* Miller) honey (sample 1), 3 honeydew honeys (samples 2–4), 1 lavender (Lamiaceae type *Lavandula latifolia* Medik.) honey (sample 5), 5 ling heather (Ericaceae type *Calluna vulgaris* (L.) Hull) honeys (samples 6–10) and 10 multifloral honeys (samples 11–20). The sediment of the samples showed that the most important secondary pollen types were Leguminosae type *Trifolium* spp., Leguminosae type *Genista* spp., Rosaceae type *Rubus* spp., Compositae type *Helianthus annuus* L. and Ericaceae type *Erica* spp. All honey samples were fresh, and were tested as soon as they were received.

2.2. Materials

All materials and solvents were commercially available and used as received unless otherwise indicated. The following materials and solvents were used: methylmethacrylate (**MMA**) (Aldrich, 99%), N,N-(dimethylamino)ethyl methacrylate (**NNDA**) (Aldrich, 98%), 2-hydroxyethyl acrylate (**2HEA**), (Aldrich, 96%), ethylene glycol dimethacrylate (**E**) (Aldrich, 98%), hydrochloric acid (VWR, 36%), methanol (Aldrich, 99.8%), starch azure (Aldrich), dimethyl sulfoxide (DMSO) (VWR, 99%), copper (II) sulfate pentahydrate (MERCK, $\geq$ 98%), potassium sodium tartrate tetrahydrate (Aldrich, 99%), sodium hydroxide (Aldrich, 97%), methylene blue (PANREAC), sodium chloride (Aldrich, 99%), sodium acetate (Aldrich, 99%), glacial acetic acid ($\geq$99%), wheat starch (GUINAMA), potassium iodide (Aldrich, $\geq$99.99%), iodine (Aldrich, $\geq$99.99%). Azo-bis-isobutyronitrile (AIBN, Aldrich, 99%) was recrystallised twice from methanol.

2.3. Starch containing polyacrylic film

The *polyacrylic* film was prepared by bulk radical polymerisation of three commercial monomers: **NNDA**, **2HEA** and **MMA**, in a molar feed ratio of 45/45/10 (**NNDA/2HEA/MMA**) using 1% weight of AIBN as radical thermal initiator, and 5% mol of **E** as the crosslinking agent. This mixture of monomers was chosen due to the excellent workability and controlled swelling of films, as we have optimised in previous works (González-Ceballos et al., 2021; Guembe-García et al., 2020a, 2020b). The polymerisation was carried out at 60 °C, overnight, in a mould comprised between two silanised glasses (100 μm thick), in an oxygen-free atmosphere. The polyacrylic film was removed from the mould, washed three times with methanol, and dipped in HCl (4%). This treatment with HCl protonates the tertiary amine group of **NNDA**, and improves the starch charging process. Finally, the polyacrylic film was washed several times with water until the pH of washing water was 7.

The starch azure charging process was carried out by dipping a 12 mm diameter disc of the polyacrylic film in 5 mL of a starch azure solution (2% in DMSO) for 24 h. The charging process was finished until no colour evolution was visually observed in the polyacrylic film. Finally, the blue-coloured polyacrylic film was dractically dipped in water, and washed with water (3x10 mL). This radical media change from DMSO to water is decisive for the encapsulation of starch azure. The swelling percentage of the film in DMSO is 230%, which allows the entry of starch. However, in water, the swelling of the film is 110%, which generates a very rapid contraction of the volume of the film, which leaves the starch retained inside. Fig. 2 shows the chemical structure of the prepared polymer before and after immersion in starch azure solution, and a real image of the prepared material.

The amount of starch azure in the polyacrylic film was estimated in a

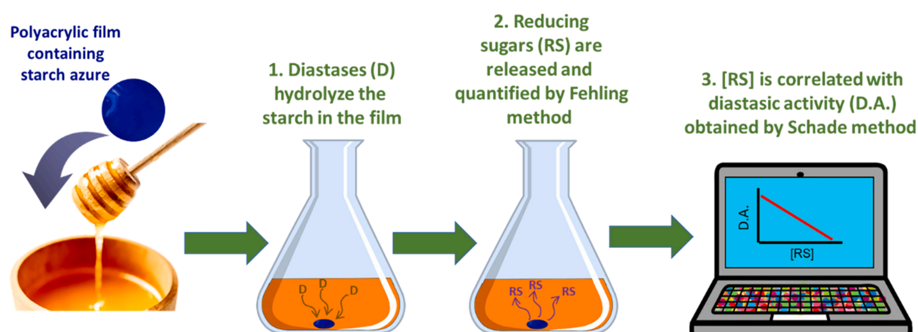


Fig. 1. Graphical abstracts of the proposed method for the determination of the diastase activity of honey, by using a polyacrylic film containing starch azure.

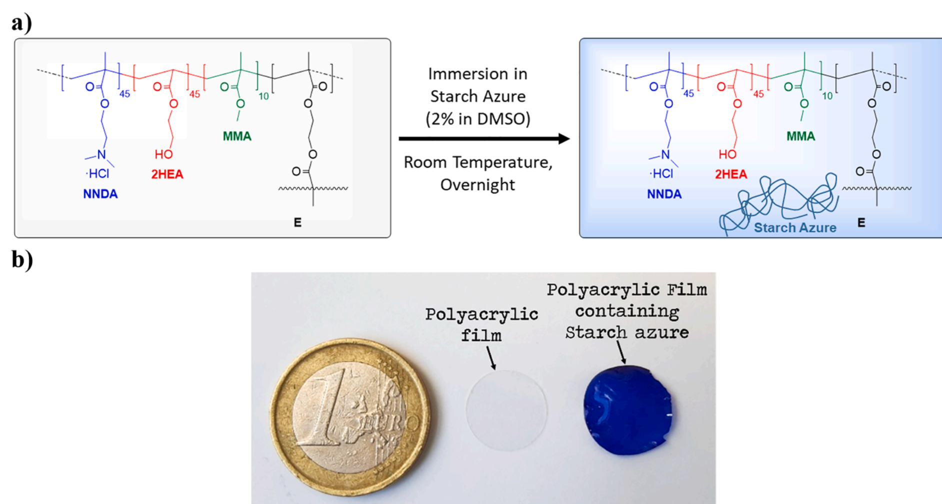


Fig. 2. **a**) Chemical structure of the polyacrylic film before and after immersion in starch azure solution (2% in DMSO). **b**) Image of the polyacrylic film before and after immersion in starch azure solution (2% in DMSO).

14.8 wt%, and was calculated by thermogravimetry (more details in **ESI S4**).

2.4. Instrumentation

UV/Vis spectra were recorded using a Varian Cary 400 Bio UV/Vis spectrophotometer.

Samples were heated using a thermostatic bath Grant W28.

2.5. Methods

2.5.1. After Schade method (reference method, approved by the International Honey Commission, 2009, (Bogdanov, 2009)

Diastase activity was measured in Schade units per gram of honey. One Schade unit is the amount of enzyme that will convert 0.01 g of starch to the prescribed end in one hour at 40 °C under the test conditions.

Briefly, in this method, honey was dissolved in distilled water. Then, 5 mL pH 5.3 acetate buffer and 3 mL 2.9% (w/v) NaCl aqueous solution were added, transferring the solution to a 50 mL volumetric flask and making up to the mark. After 10 mL of the diluted sample was set at 40 °C, 5 mL of starch solution was added, stirring the solution and then starting the timer. At periodic intervals, starting at 5 min, 1 mL aliquots were taken and mixed with lugol (a solution of molecular iodine (I_2) and potassium iodide (KI) in distilled water). The formed blue colour was measured at 660 nm. The absorbance depends on the concentration of starch, which decreases with time due to diastase activity. The analysis continued until an absorbance of 0.235 or lower was obtained. Table 1 shows the diastase activities of measured honeys determined by this method, and the complete procedure is depicted in **ESI S1**.

2.5.2. Polyacrylic films containing starch azure method (PAFSA method)

The interaction of honey with **PAFSA** generates an increment in the concentration of **RS** through the hydrolysis of starch; thus, each honey was tested before and after the interaction with the **PAFSA** for determining the increment of the concentration of RS. For it, a stock solution of each honey was prepared dissolving 2 g of honey in 100 mL of distilled water. After that, two different measurements of the concentration of **RS** were carried out: (1) *Without **PAFSA***. 10 mL of this stock solution was diluted to 500 mL, added to the burette, and tested according to Fehling method, in which the concentration of reducing sugars is measured by titration using methylene blue as a colourimetric indicator (Bicudo de Almeida-Muradian et al., 2020; Bogdanov, 2009) The analysis was performed in triplicate for each honey. (2) *With **PAFSA***: One disc of **PAFSA** was added to the stock solution (50 mL) and stirred for exactly 30 min. Note that three replicates with three different **PAFSA** discs were carried out for each honey. **PAFSA** discs of these replicates were prepared individually, in order to check the good reproducibility of the manufacturing method. After that, a 10 mL fraction was separated and diluted to 500 mL with water, added to the burette, and tested according to Fehling method. The difference in the concentration of **RS** (with **PAFSA** – without **PAFSA**) was correlated with the diastase activity calculated as described in Section 2.5.1.

Table 1
Diastase activity (expressed in Schade units) and increment of reducing sugars concentration (%) of measured honeys. Table shows the average of 3 replicates with each method.

Sample number	Botanical origin	Schade Method Diastase activity (Schade units)	PAFSA Method Δ [Reducing Sugars] (%)
1	Chestnut	19.0 ± 0.3	2.90 ± 0.18
2	Honeydew	14.7 ± 0.5	3.48 ± 0.18
3	Honeydew	37.0 ± 0.1	1.10 ± 0.19
4	Honeydew	30.0 ± 0.1	1.90 ± 0.17
5	Lavender	9.8 ± 0.9	3.80 ± 0.15
6	Ling heather	14.1 ± 2.1	3.30 ± 0.20
7	Ling heather	19.4 ± 0.6	3.00 ± 0.19
8	Ling heather	10.1 ± 1.1	3.80 ± 0.18
9	Ling heather	27.3 ± 4.9	1.90 ± 0.16
10	Ling heather	35.3 ± 0.1	1.30 ± 0.17
11	Multifloral	17.1 ± 2.8	3.00 ± 0.19
12	Multifloral	17.2 ± 2.2	3.10 ± 0.17
13	Multifloral	17.0 ± 2.8	3.10 ± 0.17
14	Multifloral	28.3 ± 3.2	2.20 ± 0.19
15	Multifloral	24.4 ± 1.9	2.40 ± 0.16
16	Multifloral	20.3 ± 1.6	2.70 ± 0.19
17	Multifloral	16.6 ± 0.5	3.30 ± 0.17
18	Multifloral	27.0 ± 0.1	2.40 ± 0.16
19	Multifloral	22.1 ± 2.2	2.40 ± 0.17
20	Multifloral	28.6 ± 2.3	2.30 ± 0.17

3. Results and discussion

3.1. Correlation of methods

In this work, we propose an easy, low cost and rapid method for the determination of diastase activity of honeys, as a substitute for the reference method, which is based on a tedious standardisation of starch samples.

The diastase activity of 20 different honeys was determined by the **Shade** method, and the concentration of the reducing sugars was calculated by **PAFSA** method. Results are shown in

Fig. 3 shows the good correlation between both methods, i.e., **PAFSA** method can replace the reference method for the determination of the diastase activity of honeys. These results are also supported by a statistical analysis, in which we have compared the standard deviations and the averages of both methods, and we can affirm that there is no statistically significant difference neither between standard deviations nor between averages of the Schade and **PAFSA** methods.

A typical procedure for a generic titration curve involves the preparation of several freshly prepared solutions with a pattern (target species), and the measurement of these solutions with the appropriate technique. Then, the food sample is prepared and measured in the same way, and the titration allows the determination of the target species. However, the food sample contains hundreds of compounds which could interfere in the measurement, and usually, an interference study is mandatory because the titration using aqueous solutions does not include all these compounds. In our case, this interference study was not necessary, since the titration was not carried out with prepared aqueous samples, but with real samples of honey, which contained all the compounds that are usually found in honey and which could interfere in the analysis.

Although the work has been carried out with honeys with different botanical origins, and also have a wide range of diastase activity (from 9.8 to 37 Schade units), to get a more accurate equation the procedure should be calibrated with a greater number of honey samples from different origins.

3.2. *Proof of concept*

The obtained equation for the linear fitting (Fig. 3) allows the calculation of the diastase activity of honeys by **PAFSA** method. Each increment of reducing sugars concentration was introduced as "X" variable, and "Y" (diastase activity) was calculated for every honey. Fig. 4 shows the comparison of diastase activity determined both by reference and **PAFSA** methods (numerical values with calculated errors can be found in **ESI S5**).

The results obtained show that the **PAFSA** method provides very similar values to those of the Shade method for the diastase activity of the studied honeys. In the case of sample 15, the difference is less than 0.4%, although in other cases such as samples 19 and 20, the difference is greater, around 10%. However, and as shown in Table 2, the advantages of the **PAFSA** method in terms of time and cost make it a real alternative to the reference method. For example, our method requires 30 min for every honey sample, and the time with **Shade** method can varies from 120 min to 180 min in the measured honeys, depending on the diastase activity.

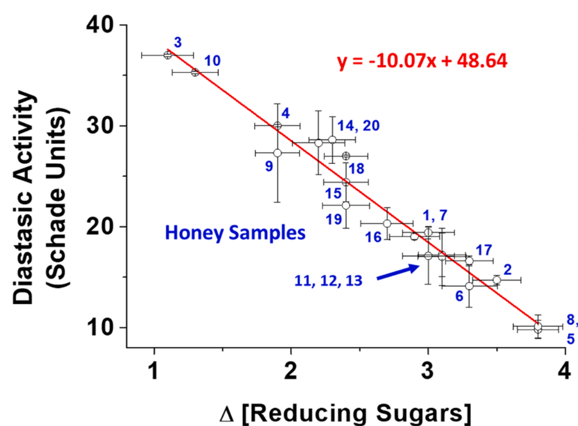


Fig. 3. Graphical representation of the diastase activity obtained with Schade Method against the concentration of reducing sugars obtained with **PAFSA** method.

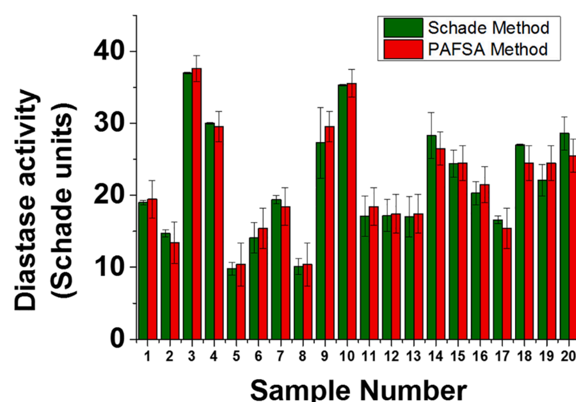


Fig. 4. Comparison of diastase activity determined both by Schade and **PAFSA** methods.

Table 2
Comparative table of **Shade** and **PAFSA** methods.

Method	Time for1 sample	Starch standardisation	Equipment
Schade	2–3 h	Yes	SpectrophotometerThermostatized bathHot-plate
PAFSA	30 min	No	Thermostatized bath

4. Conclusions

Diastase activity is a key parameter on honey analysis since it is related to the quality of honey and possible frauds. Schade assay is the most used method for the determination of the diastase activity in honeys, but it implies a very tedious search to find a proper starch, and usually takes a long time, particularly for honeys with low diastase activities. The use of a polyacrylic film as a starch azure container avoids the starch standardization process, and dramatically simplify the determination of the honey diastase activity. The polyacrylic film allows the measurement of the diastase activity through the quantification of reducing sugars produced during the enzymatic process. This simple methodology is not possible in the presence of starch in solution; thus, the function of the polyacrylic film as starch container is determinant. The polyacrylic film has great potential in the routine quality control of honey samples, effectively reducing analysis time and cost. The reproducibility of the manufacturing process of **PAFSA** is really high, and it can be easily used by technicians in small local laboratories and laboratories of beekeeping cooperatives because only basic glassware equipment is necessary. This work supposes a proof of concept which should be optimised for each user, performing the calibration with a greater number of honey samples from different origins.

CRediT authorship contribution statement

Lara González-Ceballos: Investigation, Writing - original draft. **Miguel A. Fernández-Muiño:** Conceptualization, Methodology. **Sandra M. Osés:** Conceptualization, Methodology, Investigation. **M. Teresa Sancho:** Writing - review & editing, Visualization. **Saturnino Ibeas:** Methodology, Investigation, Validation, Methodology, Investigation, Validation. **Jose Antonio Reglero Ruiz:** Investigation, Methodology. **Saúl Vallejos:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing, Visualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We gratefully acknowledge the financial support provided by FEDER (Fondo Europeo de Desarrollo Regional), and both the Spanish Ministerio de Economía, Industria y Competitividad (MAT2017-84501-R) and the Consejería de Educación—Junta de Castilla y León (BU306P18 and BU041G18) are gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2021.129629.

References

Bicudo de Almeida-Muradian, L., Monika Barth, O., Dietemann, V., Eyer, M., Freitas, A. d. S.d., Martel, A.-C., … Gasparotto Sattler, J. A. (2020). Standard methods for Apis mellifera honey research. *Journal of Apicultural Research, 59*(3), 1–62. https://doi.org/10.1080/00218839.2020.1738135.

Bogdanov, S. (2017). Bee Product Science. In Honey composition. from http://www.bee-hexagon.net; Retrieved November 10, 2020.

Fehling, H. (1849). The quantitative determination of sugar. *Justus Liebigs Annalen Der Chemie, 72*(1), 106–113. https://doi.org/10.1002/jlac.18581060109.

González-Ceballos, L., Cavia, M. D. M., Fernández-Muiño, M. A., Osés, S. M., Sancho, M. T., Ibeas, S., … Vallejos, S. (2021). A simple one-pot determination of both total phenolic content and antioxidant activity of honey by polymer chemosensors. *Food Chemistry, 342*, 128300. https://doi.org/10.1016/j.foodchem.2020.128300.

Guembe-García, M., Peredo-Guzmán, P. D., Santaolalla-García, V., Moradillo-Renuncio, N., Ibeas, S., Mendía, A., … Vallejos, S. (2020a). Why is the Sensory Response of Organic Probes within a Polymer Film Different in Solution and in the Solid-State? Evidence and Application to the Detection of Amino Acids in Human Chronic Wounds. *Polymers, 12*(6), 1249. https://doi.org/10.3390/polym12061249.

Guembe-García, M., Vallejos, S., Carreira-Barral, I., Ibeas, S., García, F. C., Santaolalla-García, V., … García, J. M. (2020b). Zn (II) detection in biological samples with a smart sensory polymer. *Reactive and Functional Polymers, 154*, 104685. https://doi.org/10.1016/j.reactfunctpolym.2020.104685.

Louveaux, J., Maurizio, A., & Vorwohl, G. (1978). Methods of Melissopalynology. *Bee World, 59*(4), 139–157. https://doi.org/10.1080/0005772x.1978.11097714.

Marcazzan, G. L., Mucignat-Caretta, C., Marina Marchese, C., & Piana, M. L. (2018). Una revisión de los métodos para el análisis sensorial de la miel. *Journal of Apicultural Research, 57*(1), 75–87. https://doi.org/10.1080/00218839.2017.1357940.

Ministerio de Agricultura, P. y A. (2019). Ministerio de Agricultura, Pesca y Alimentación. http://publicacionesoficiales.boe.es/NIPO:003191624.

OJEC. (2001). Council Directive 2001/110/EC of 20 December 2001 relating to honey. Official Journal of the European Communities. https://doi.org/https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:010:0047:0052:EN:PDF.

Pasias, I. N., Kiriakou, I. K., & Proestos, C. (2017). HMF and diastase activity in honeys: A fully validated approach and a chemometric analysis for identification of honey freshness and adulteration. *Food Chemistry, 229*, 425–431. https://doi.org/10.1016/j.foodchem.2017.02.084.

Persano Oddo, L., & Piro, R. (2004). Main European unifloral honeys: Descriptive sheets. *Apidologie, 35*(Suppl. 1), S38–S81. https://doi.org/10.1051/apido:2004049.

Piana, M. L., Persano Oddo, L., Bentabol, A., Bruneau, E., Bogdanov, S., & Guyot Declerck, C. (2004). Sensory analysis applied to honey: State of the art. *Apidologie, 35*(Suppl. 1), S26–S37. https://doi.org/10.1051/apido:2004048.

Bogdanov, S. (2009). Harmonised methods of the International Honey Commission. https://www.ihc-platform.net/.

Sak-Bosnar, M., & Sakač, N. (2012). Direct potentiometric determination of diastase activity in honey. *Food Chemistry, 135*(2), 827–831. https://doi.org/10.1016/j.foodchem.2012.05.006.

Sakač, N., & Sak-Bosnar, M. (2012). A rapid method for the determination of honey diastase activity. *Talanta, 93*, 135–138. https://doi.org/10.1016/j.talanta.2012.01.063.

Stoldt, J., Uwe Trapp, T., & Toussai. (2019). Standard for Honey. In Codex Alimentarius. International Food Standards: Vol. CXS 12-198. World Health Organization & Food and Agriculture Organization of the United Nations.

Terradillos, L. A., Muniategui, S., Sancho, M. T., Huidobro, J. R., & Simal-Lozano, J. (1994). An alternative method for analysis of honey sediment. *Bee Science, 3*(2), 86.

Thrasyvoulou, A., Tananaki, C., Goras, G., Karazafiris, E., Dimou, M., Liolios, V., … Gounari, S. (2018). Legislación de criterios y normas de miel. *Journal of Apicultural Research, 57*(1), 88–96. https://doi.org/10.1080/00218839.2017.1411181.

Tosi, E., Martinet, R., Ortega, M., Lucero, H., & Ré, E. (2008). Honey diastase activity modified by heating. *Food Chemistry, 106*(3), 883–887. https://doi.org/10.1016/j.foodchem.2007.04.025.

Von Der Ohe, W., Persano Oddo, L., Piana, M. L., Morlot, M., & Martin, P. (2004). Harmonized methods of melissopalynology. *Apidologie, 35*(Suppl. 1), S18–S25. https://doi.org/10.1051/apido:2004050.