UV-Vis spectroscopy and chemometric: a simple method to differentiate honey, partially ripe honey and nectars

December 2023

Maldonado, L.M.[1]; Marcinkevicius, K.[1]; Álvarez, A.R.[1]; Salomón, V.M.[1]; Gaggiotti, M.[2]; Messina, N.[3]; Palacio, M.A.[4]; Medina, S.[5]; Maldonado, M.M.[1]; Orellano, E.[2]; Bedascarrasbure, E.L.[6]

ABSTRACT

Honey is a supersaturated solution of sugars that also contains small amounts of enzymes, vitamins, phenolic compounds (*e.g.,* flavonoids), terpenes, organic acids and hydrogen peroxide. The presence of such substances explains honey's biological activity and absorbance of ultraviolet radiation. Honey is one of the three most adulterated foods worldwide. High-complexity and sensitivity techniques are used to detect such adulterations, like isotopic analysis and Nuclear Magnetic Resonance. However, a simpler, high-speed, low cost and more accessible analytical method would be more convenient to perform food analyses, particularly for bee products. In this regard, UV-Vis spectroscopy coupled with chemometric analysis has been widely and successfully used to analyze bee products. Therefore, this study aims to validate a methodology based on the use of UV-Vis spectroscopy coupled with chemometric analysis to differentiate the nectar recently collected by bees from partially mature honey and honey. Samples of different geographical and botanical origins were collected from two locations in Argentina; one trial was carried out in Tucumán (in the main lemon flow) and the other in Entre Ríos (eucalyptus flowering). Spectra between 190 nm and 420 nm were recorded and different projection methods were applied, including principal component analysis, FreeViz, and linear discriminant analysis. From the results, it is concluded that UV-Vis spectroscopy coupled with chemometric analysis allows differentiating recently collected nectar from partially mature honey and honey and that phenolic compounds are highly present in mature honey, which could favourably affect its biological activity.

Keywords: honey, nectar, partially mature honey, UV-Vis spectroscopy, chemometrics.

RESUMEN

La miel es una solución sobresaturada de azúcares que además contiene pequeñas cantidades de enzimas, vitaminas, compuestos fenólicos (por ejemplo, flavonoides), terpenos, ácidos orgánicos y peróxido de hidrógeno. La presencia de estas sustancias explica la actividad biológica de la miel y la absorción de radiación ultravioleta. La miel es uno de los tres alimentos más adulterados a nivel mundial. Para detectar dichas adulteraciones se utilizan

[1]Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria (EEA) Famaillá, Ruta provincial 301 km 32 (4132), Famaillá, Tucumán, Argentina. Correo electrónico: maldonado.luismaria@inta.gob.ar

[2]Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria (INTA) Rafaela, Ruta 34 km 227 (2300) Rafaela, Santa Fe, Argentina.

[3]Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria (EEA) Concordia, Estación Yuquerí, Ruta Provincial 22 y vías del Ferrocarril (3200) Concordia, Entre Ríos, Argentina.

[4]Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria (EEA) Balcarce. Ruta 226 km 73,5 (7620) Balcarce, Buenos Aires, Argentina.

[5]Cooperativa Norte Grande Ltda. Parque Industrial Kanamico, Ruta Provincial 301 km 17,5 (4128) Lules, Tucumán, Argentina.

[6]Instituto Nacional de Tecnología Agropecuaria (INTA), Centro de Investigación de Agroindustria (CIA), Nicolás Repetto y de los Reseros s/n (1686), Hurlingham, Buenos Aires, Argentina.

Doi https://doi.org/10.58149/wtmq-vz93

técnicas de alta complejidad y sensibilidad, como el análisis isotópico y la resonancia magnética nuclear. Sin embargo, un método analítico más simple, rápido, de bajo costo y más accesible sería más conveniente para realizar análisis de alimentos, particularmente para productos apícolas. En este sentido, la espectroscopia UV-Vis junto con el análisis quimiométrico se han utilizado amplia y exitosamente para analizar productos apícolas. Por lo tanto, este estudio tiene como objetivo validar una metodología basada en el uso de espectroscopía UV-Vis acoplada al análisis quimiométricos para diferenciar el néctar recién recolectado por las abejas de la miel parcialmente madura y de la miel. Se recolectaron muestras de diferentes orígenes geográficos y botánicos en dos localidades de Argentina; un ensayo se realizó en Tucumán (en el flujo principal de néctar de limón) y el otro en Entre Ríos (floración de eucaliptos). Se registraron espectros entre 190 nm y 420 nm y se aplicaron diferentes métodos de proyección, incluyendo el análisis de componentes principales, FreeViz y el análisis discriminante lineal. De los resultados se concluye que la espectroscopía UV-Vis acoplada al análisis quimiométrico permite diferenciar el néctar recientemente recolectado de la miel parcialmente madura y de la miel y que los compuestos fenólicos están presentes principalmente en la miel, lo que podría incidir favorablemente en su actividad biológica.

Palabras clave: *miel, néctar, miel parcialmente madura, espectroscopía UV-Vis, quimiometría.*

INTRODUCTION

Honey is a natural product, defined by the Codex Alimentarius as "the natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honeycomb to ripen and mature" (FAO/WHO, 2019). The major components of such supersaturated solution of sugars are the monosaccharides fructose and glucose, whereas minor components are represented by the disaccharides sucrose, maltose and other oligosaccharides (White, 1957). Honey also contains small quantities of other substances such as enzymes, vitamins, phenolic compounds (*e.g.*, flavonoids), terpenes, organic acids, and hydrogen peroxide (Crane, 2009; Mabry *et al*., 1970; Machado De-Melo *et al*., 2018). Such a variety of components of different nature explains the capacity of honey to absorb ultraviolet radiation and its biological activity (Cianciosi *et al*., 2018; Isla *et al*., 2011; Majtan, 2014).

Honey is one of the three most adulterated foods worldwide, only after olive oil and dairy products. It is adulterated through different practices, including the addition of sugary syrups (derived from corn, sugar cane, sugar beet, rice, and others), passage through ion exchange resins to eliminate residues and reduce its colour, misassignment of botanical/geographical origin, and by mechanical dehydration of immature honey (García, 2018; Se *et al*., 2019; Stefas *et al*., 2021). The method used to detect adulterations depends on the specific situation (Apimondia, 2020). In general, highly sensitive and complex techniques such as isotopic analysis and Nuclear Magnetic Resonance are applied (Amadei Enghelmayer *et al*., 2022). In this regard, UV-Vis spectroscopy is an analytical method widely used in chemical laboratories due to its simplicity, speed, low cost, and accessibility. Although its low selectivity, it has been successfully used in the analysis of foods, and when associated with chemometrics, it is particularly applied to analyze bee products (Ansari *et al*., 2018; Maldonado *et al*., 2020; Martelo-Vidal and Vázquez, 2016).

The honey maturation process within the colony, which goes from the collection of nectar by foraging bees to the capping of honey, is much more complex than it appears. Since differences in chemical composition and biological activity have been verified between immature honeys and mature honeys (Guo *et al*., 2020; Maldonado *et al*., 2011), the present study aims to validate a methodology based on UV-Vis spectroscopy coupled with chemometric analysis to differentiate the nectar recently collected by bees from partially mature and honey for each colony.

MATERIALS AND METHODS

Samples collection

Samples of nectars, partially mature honeys and honeys with different botanical and geographical origins were collected from two different locations within Argentina: Tucumán, where the nectar flow occurs at the beginning of the beekeeping season, and in Entre Ríos at the end of the beekeeping season.

The samples from Tucumán were specifically collected in a town called Sauce Huacho (Latitude: -27.021940, Longitude: -65.436065) in the department of Famaillá. The hives were located very close to a commercial lemon (*Citrus limon*) plantation, in a typical natural forest of the foothill area of Tucumán, where the predominant plant species include Sauce Criollo (*Salix humboldtiana*), Afata (*Cordia trichotoma*), Guaran (*Tecoma stans*), Tipa (*Tipuana tipu*), and Pacará (*Enterolobium contortisiliquum*).

The samples from Entre Ríos were collected at Estación Experimental Concordia of the Instituto Nacional de Tecnología Agropecuaria (INTA; Latitude: -31.374422, Longitude: -58.116903), in Yuquerí Station, Provincial Road 22, and railroad tracks in Concordia. The hives were located in a field surrounded by Eucalyptus of different ages and varieties (mainly hybrids of *Eucalyptus grandis*), and this flower supply is predominant during the period of the year in which the trial was carried out.

Five hives were randomly selected for both trials. The hives were acquired from commercial apiaries applied by Beekeeping Program (INTA-PROAPI) technological path for the production

of Argentinian-quality honey. Each one consisted of eight breeding squares, a population of more than ten squares covered with bees (Category I) from the genetics of INTA-PROAPI. In each hive of the trial, three frames with new combs were selected in the brood chamber for nectar sampling and three new frames of honeycombs for sampling partially mature honey and honey.

To take samples of nectar recently collected by the bees and partially mature honey, the frames of each hive were shaken separately on plastic trays covered with polyethylene film (which are suitable to store food) at the moment of maximum nectar flow. Then, the sample was extracted from the trays using a 20 ml syringe and placed in 50 ml Falcon tubes with a screw cap. The procedure was repeated until the honeycombs were capped in more than 80% by the bees. To take samples of honey, the capped combs were scraped, the honey and wax dragged from the caps, and collected in jars with lids of 250 g to 500 g capacity. All the samples were labelled and immediately refrigerated at -20°C until they were analysed. The following coding was used: firstly a number identifying the hive sampled (1, 2, 3, 4, 5), followed by acronyms according to the type of sample: honey (hny or HNY), partially ripe honey (prh or PRH), nectar (nct or NCT) and finally a number to indicate the sampling order (1, 2, 3). Lower case letters were used for the trial performed in Tucumán, whereas capital letters were for the one performed in Entre Ríos. More details are indicated in table 1.

UV-Vis spectra obtention and pre-treatment

The honey was manually separated from the capping wax. Then, nectar, partially ripe honey and honey samples were diluted with 80% ethanol (prepared by diluting ethanol 96% vol. with distilled water), filtered through Whatman N° 1 filter paper, and an aliquot placed in quartz cuvettes with 1 cm optical path. Spectra were determined in quintuplicate using a spectrophotometer (Hewlett Packard model 8452A, USA), absorbances between 190 nm and 420 nm were recorded at 2 nm intervals (Marcinkevicius *et al*., 2021; Roshan *et al*., 2013), and exported to Microsoft Excel 2016; absorbances for each wavelength were organized in columns and the samples in rows. Subsequently, spectra were pre-processed, which included baseline correction and normalization (SNV) (Noviyanto *et al*., 2016) using Orange software version 3.31.1 (https://orangedatamining.com/) (Demšar *et al*., 2013).

Chemometric analysis

Three methods based on projections were applied. Firstly, an exploratory analysis was performed using Principal Component Analysis (PCA) to determine if the samples could be separated by their spectral information. Then, using FreeViz projection, the relationship between wavelengths and type of samples was analyzed. Finally, through Linear Discriminant Analysis (LDA) of means, the capacity of spectra to differentiate samples of nectar, partially mature honey and honey was established. The wavelength range for the analysis was narrowed between 196 nm and 420 nm to rule out possible interference from the air, quartz cuvette defects, and other possible causes (Perkampus *et al*., 2013). Thus, 113 variables were initially considered. For PCA and LDA analyses, the statistical software Infostat version 2019 was used (Di Rienzo *et al*., 2011), whereas, for FreeViz projection, Orange software version 3.31.1 was used.

RESULTS

UV-Vis spectra of the samples

The spectra are the result of the sum of absorptions of various types of molecules present in small quantities in honey, such as phenolic compounds, organic acids, enzymes, and vitamins. Each compound has a characteristic absorption spectrum and its intensity varies with the wavelength.

The shape of the spectral curves obtained for the samples from the Tucumán and Entre Ríos trials were consistent with those reported for honeys from different botanical and geographic origins (Orfanakis *et al*., 2021; Roshan *et al*., 2013) and are shown in figure 1 a and b. It is observed that both types of honeys absorbed more UV radiation than the partially ripe honeys and the nectars. In addition, the spectra of the Entre Ríos samples, originating from *Eucalyptus*, were of greater intensity than those from Tucumán, originating from *Citrus limon*.

Principal component analysis

The principal component analysis (PCA) made it possible to reduce the number of initial variables under study and generate new ones called principal components (PC). The PC are orthogonal and result from the linear combination of the initial ones, which explains the total variability of the system.

For the trials carried out in Tucumán and Entre Ríos, seven and six components were obtained that explain the total variance, respectively. The distribution of the samples in the space defined by PC1 and PC2 is presented in figure 2 a and b. However, good separation between honey, partially mature honey and different types of nectar was not achieved, particularly between the latter two types of samples. In general, it can be

Sampling	Trial Tucumán (Sauce Huacho)	Trial Entre Ríos (Concordia)	Type of sample
1	17/09/2021	17/02/2022	Nectar (nct1, NCT1) Parcially ripe honey (prh1, PRH1)
2	27/09/2021	15/03/2022	Nectar (nct2, NCT2) Parcially ripe honey (prh2, PRH2)
3	12/10/2021	11/04/2022	Honey (hny3, HNY3)

Table 1. Sampling dates and types of collected samples.

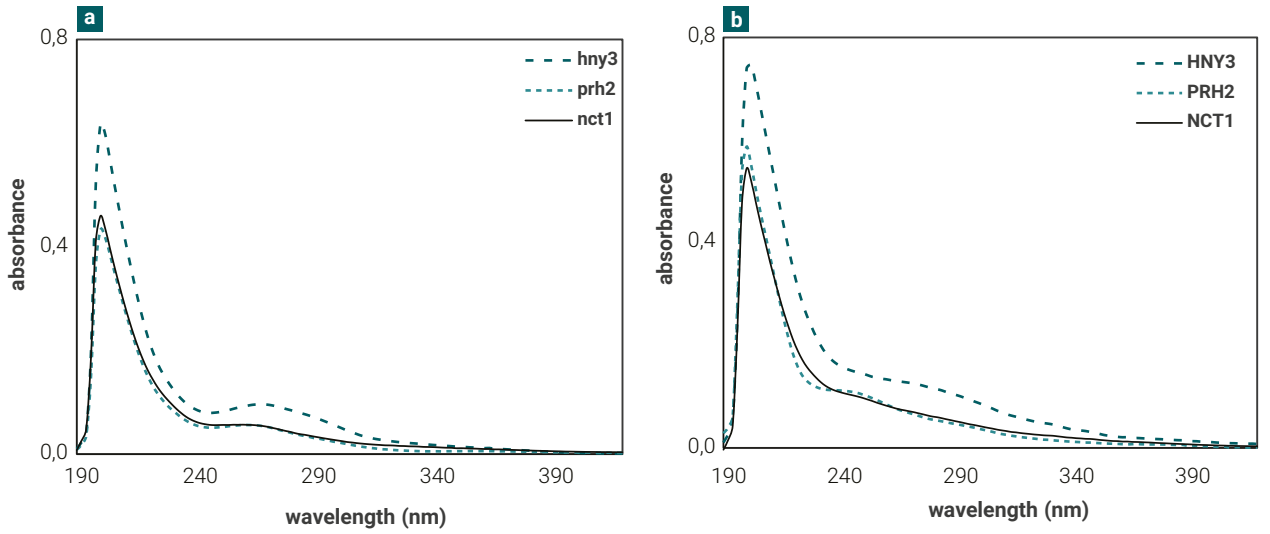

Figure 1. Average UV-Vis spectra of samples from the trials carried out in Tucumán (a) and Entre Ríos (b), Argentina.

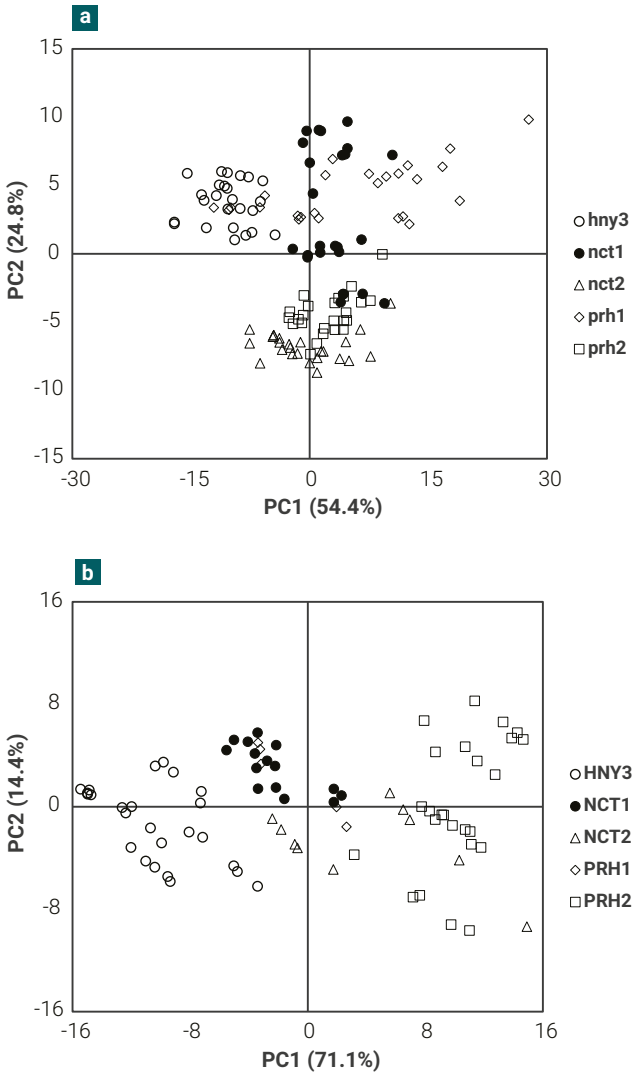

Figure 2. PCA indicating distribution of samples for the trials carried out in Tucumán (a) and Entre Ríos (b) in the three samplings.

seen that the samples tended to separate better according to sampling order (1,2,3).

Relationship between wavelengths and the type of samples

The FreeViz projection was used to analyze the relationship between wavelengths with honey, partially ripe honey and nectars because UV-Vis spectroscopy is a low selectivity technique. FreeViz projection is based on the attraction of the elements of the same group, repulsion of different groups and the resulting forces are exerted, in this case, on the vectors that represent the wavelengths. The optimized projection is the one in which the best separation between groups and the minimum potential energy of the system are achieved. (Demšar *et al*., 2007). For both regions, the circular and optimized projections are shown in figure 3.

Linear Discriminant Analysis

The total variability with 4 discriminant functions achieved after linear discriminant analysis (LDA) was performed and is explained in table 2. The space defined by canonical functions 1 and 2 explained 81.69% and 95.78% of the total variance for the trial carried out in Tucumán and Entre Ríos, respectively.

The absolute value of the coefficients of the discriminant functions calculated using standardized data allows establishing the weight of each wavelength on the discriminant power. For the trial carried out in Tucumán, the low wavelengths (less than 260 nm) had a great incidence on function 1 (f1) and had a great discriminating capacity, while the range between 320 nm and 420 nm would not have a significant effect and could be neglected to reduce the complexity of the calculation (figure 4 a). Regarding the trial carried out in Entre Ríos, an important contribution to the discriminant power of wavelengths (less than 290 nm) was observed through function 2 (f2); then, a decrease was observed until 370 nm approximately and started increasing to the end of the range (figure 4 b).

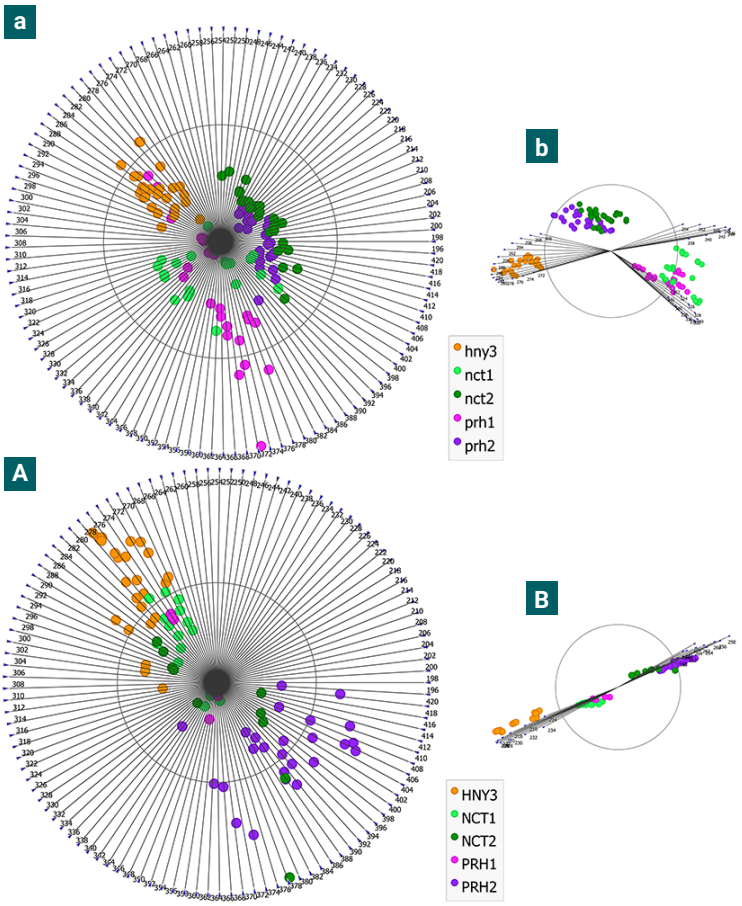

Figure 3. FreeViz projections. Circular (a,A) and optimized (b,B) projections of the relationship between wavelengths and sample types. Tucumán in lowercase and Entre Ríos in uppercase.

Functions	eigenvalues	variance (%)	cumulative variance (%)
Tucumán			
1	277,08	44,82	44,82
2	227,94	36,87	81,69
3	93,51	15,12	96,81
4	19,64	3,18	99,99
Entre Ríos			
1	2294,04	61,28	61,28
2	1291,45	34,50	95,78
3	104,63	2,79	98,57
4	53,48	1,43	100,00

Table 2. Summary of canonical discriminant functions for trials in Tucumán and Entre Ríos.

On the other hand, the univariate analysis of variance indicated that the contribution of the wavelengths in the spectra discriminating capacity was highly significant ($p \leq 0.01$); it was 96.5% and 93.8% of the total range for the trials carried out in Tucumán and Entre Ríos, respectively. From these results, it is necessary to consider the full range for the linear discriminant analysis.

DISCUSSION

UV-Vis spectra of the samples

The samples collected in Tucumán and Entre Ríos locations exhibited spectra with two absorption bands (figure 1 a and b); the first was narrower and with greater intensity at low

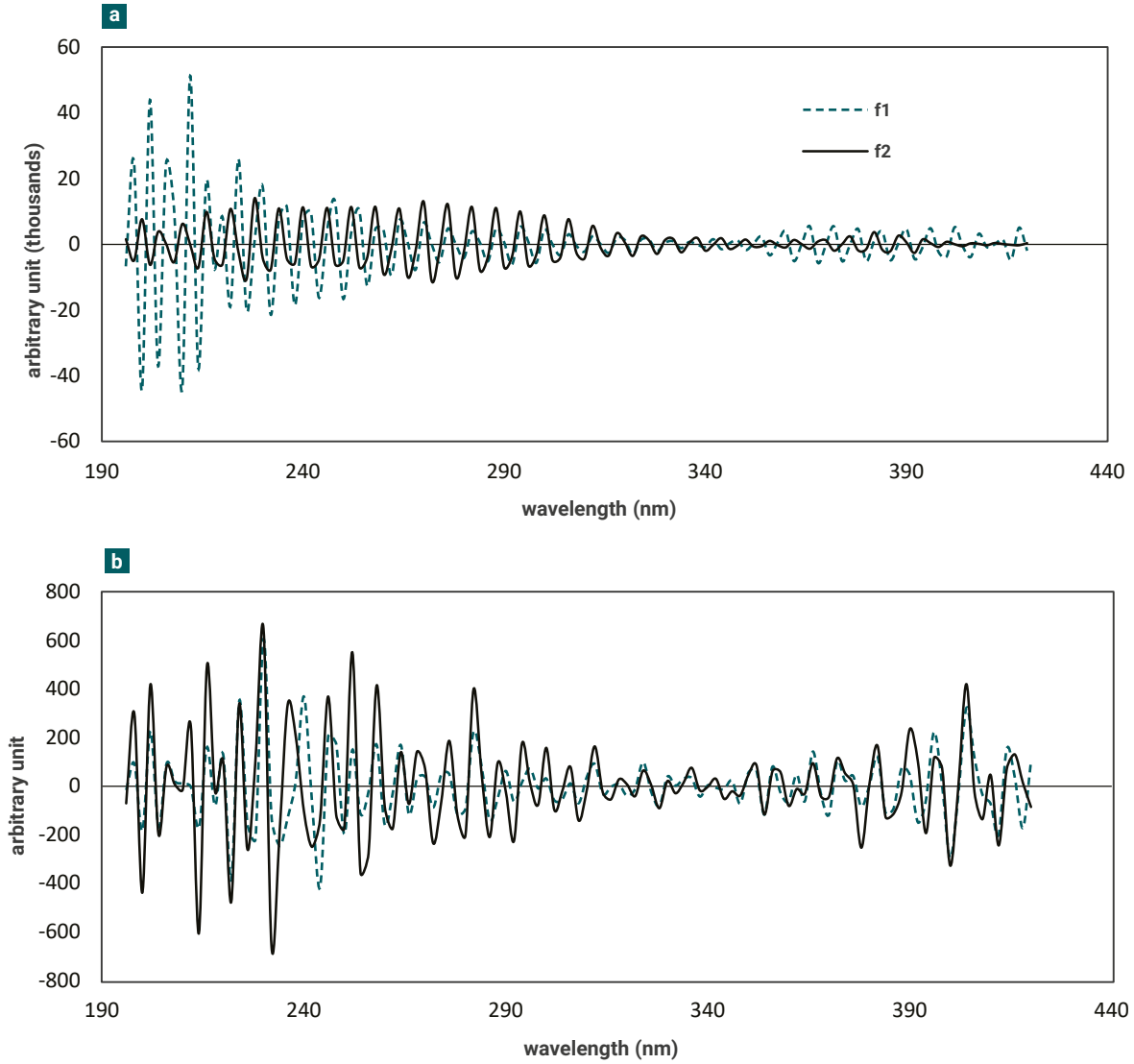

Figure 4. Coefficients of the canonical functions. (a) Trial in Tucumán, (b) Trial in Entre Ríos.

wavelengths, and related to the presence of phenolic compounds and sugars such as glucose and fructose (Orfanakis *et al*., 2021) whereas the second followed the first and was broader, with less intensity and shoulder-shaped, which gradually disappeared to the end of the range analyzed. For wavelengths between 270 and 300 nm, this second band is associated with the presence of aromatic acids (Suhandy and Yulia, 2021), while at 320 nm, absorption may be due mainly to the presence of flavonols and non-flavonoid compounds (Orfanakis *et al*., 2021).

The dispersion was observed in the spectra throughout the analyzed range and for the different types of samples, as indicated for some wavelengths in figure 1. It is typical of the method applied and can also be due to differences among the hives. Dispersion is represented in vertical lines, denoting the maximum and minimum values obtained, plotted alternately; only honey, partially mature honey from the second sampling, and nectars from the first sampling were considered to facilitate visualization. The dispersion was reduced during the pre-processing through SNV normalization that performs the centring of the spectra and the scaling by their standard deviation.

Relationship between wavelengths and the type of samples

Honey was likely to be more related to the wavelengths between 274 nm and 300 nm for the trial carried out in Tucumán, and between 268 nm and 312 nm for the one carried out in Entre Ríos, as observed in the circular projections in figure 3 (a) and (A), respectively. This outcome indicated a higher contribution of phenolic compounds (Mabry *et al*., 1970). On the other hand, results for partially ripe honey and nectars suggested that they have greater bonding with a wider range of wavelengths, which corresponded to other types of molecules. More research needs to be carried out to establish the nature of such types of compounds. Finally, the circular projections also showed that some nectars and partially ripe honey could be associated with the same rank as the honey; yet, the optimized projections showed that they were well-differentiated groups and that there were no overlaps (figure 3 (b.B)).

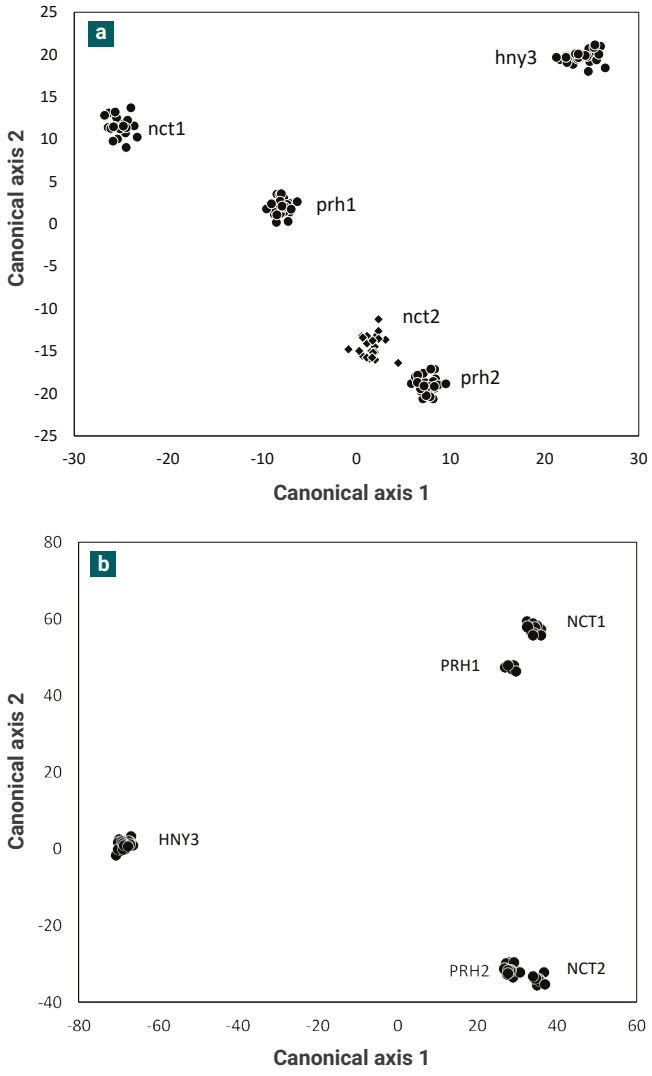

Figure 5. LDA applied to samples from the trial carried out in Tucumán (a) and Entre Ríos (b).

Linear Discriminant Analysis

LDA is a supervised method, so before the analysis, we defined the groups to be differentiated/classified and the belonging of each sample to a specific group. For both trials, LDA allowed grouping of the samples of honey, partially ripe honey and nectar among themselves, clearly differentiating the groups by their nature and sampling (figure 5 a and b).

The validity of the result was verified using the cross-classification test (table 3). A 0% error indicated that all the samples assigned to each group were correctly classified. Moreover, these results indicated that changes in the chemical composition occur during the transformation processes from nectar to partially ripe honey, and from this one into honey (Eyer *et al*., 2016; Guo *et al*., 2020; Semkiw *et al*., 2008), which in term caused changes in the resulting UV-Vis spectra that could be evidenced by chemometric analysis although it is not easy to perceive them directly.

Considering the scales of the canonical axes 1 and 2 for both trials, it is also observed that the distances of the nectar and partially mature honey groups were greater compared to the honey group in the trial carried out in Entre Ríos. This result suggested that the chemical changes that occurred during the process of transformation of nectar into honey were greater in those kinds of honey that originated from *Eucalyptus* than in those from *Citrus lemon*. Likewise, in both trials, the nectar of samples 1 and 2 were also clearly differentiated, indicating that changes may have occurred in the chemical composition of nectar collected over time.

CONCLUSION

This study demonstrated that the methodology based on UV-Vis spectroscopy coupled with chemometric analysis allows differentiating nectars, partially ripe honeys and honey from different geographical and botanical origins. Furthermore, the wavelength range between 196 nm and 420 nm was identified to be highly significant for the discrimination ability. Even differences between different types of nectars collected on different sampling dates could be detected using the described methodology. Efforts are now focused on analyzing the possibility to apply UV-Vis spectroscopy coupled with chemometric analysis to discriminate among authentic honey, that is, honey naturally matured in the honeycombs from other kinds of honey obtained by artificial dehydration of nectar and partially mature honey.

ACKNOWLEDGEMENTS

Beekeeping Program (INTA-PROAPI)

PE I017 “Development of the organized, sustainable and competitive beekeeping sector”

INTA-NEXCO Agreement

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

REFERENCES

ALBA, M.M. 2013. Análisis quimiométrico de whiskys comerciales empleando absorciometría molecular UV-Vis. Universidad Nacional de La Pampa. https://repo.unlpam.edu.ar/handle/unlpam/2010

ALVAREZ-SUAREZ, J.; GASPARRINI, M.; FORBES-HERNÁNDEZ, T.; MAZZONI, L.; GIAMPIERI, F. 2014. The Composition and Biological Activity of Honey: A Focus on Manuka Honey. Foods, 3(3), 420–432. https://doi.org/10.3390/foods3030420

Amadei Enghelmayer, M.; Maldonado, L.; Salomón, V.M.; Álvarez, A.; Gaggiotti, M. 2022. La detección de las adulteraciones de la miel. In: DINI, C.B.; GARCÍA, N. (Eds.). Manual de Buenas Prácticas en alimentación de abejas. Un aporte a la calidad de la miel argentina. 20-35 pp.

ANSARI, M.J.; AL-GHAMDI, A.; KHAN, K.A.; ADGABA, N.; EL-AHMADY, S.H.; GAD, H.A.; ROSHAN, A.; MEO, S.A.; KOLYALI, S. 2018. Validation of botanical origins and geographical sources of some Saudi honeys using ultraviolet spectroscopy and chemometric analysis. Saudi Journal of Biological Sciences, 25(2), 377-382. https://doi.org/10.1016/j.sjbs.2017.09.005

APIMONDIA. 2020. Declaración de Apimondia sobre el Fraude en la Miel. (Available at: https://www.apiservices.biz/documents/articuloses/declaracion_apimondia_fraude_miel_v_2.pdf verified on December 08, 2023).

BARBEIRA, P.J.S.; PAGANOTTI, R.S.N.; ÁSSIMOS, A.A. 2013. Development of a multivariate calibration model for the determination of dry extract content in Brazilian commercial bee propolis extracts through UV-Vis spectroscopy. Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy, 114, 441–448. https://doi.org/10.1016/j.saa.2013.05.009

Tucumán							
Group	hny3	nct1	nct2	prh1	prh2	Total	Error(%)
hny3	25	0	0	0	0	25	0
nct1	0	25	0	0	0	25	0
nct2	0	0	25	0	0	25	0
prh1	0	0	0	25	0	25	0
prh2	0	0	0	0	25	25	0
Total	25	25	25	25	25	125	0
Entre Ríos							
Group	HNY3	NCT1	NCT2	PRH1	PRH2	Total	Error(%)
HNY3	25	0	0	0	0	25	0
NCT1	0	15	0	0	0	15	0
NCT2	0	0	10	0	0	10	0
PRH1	0	0	0	5	0	5	0
PRH2	0	0	0	0	25	25	0
Total	25	15	10	5	25	80	0

Table 3. Cross classification (apparent error rate).

CATALDO, F. 2006. Ozone Degradation of Biological Macromolecules: Proteins, Hemoglobin, RNA, and DNA. Ozone: Science \& Engineering, 28(5), 317-328. https://doi.org/10.1080/01919510600900290

CIANCIOSI, D.; FORBES-HERNÁNDEZ, T.Y.; AFRIN, S.; GASPARRINI, M.; REBOREDO-RODRIGUEZ, P.; MANNA, P.P.; ZHANG, J.; LAMAS, L.B.; FLÓREZ, S.M.; TOYOS, P.A.; QUILES, J.L., GIAMPIERI, F.; BATTINO, M. 2018. Phenolic compounds in honey and their associated health benefits: A review. Molecules, 23(9), 1-20. https://doi.org/10.3390/molecules23092322

CRANE, E. 2009. Bee Products. In Encyclopedia of Insects. https://doi.org/10.1016/B978-0-12-374144-8.00020-5

DEMŠAR, J.; LEBAN, G.; ZUPAN, B. 2007. FreeViz-An intelligent multivariate visualization approach to explorative analysis of biomedical data. Journal of Biomedical Informatics, 40(6), 661-671. https://doi.org/10.1016/j.jbi.2007.03.010

DEMŠAR, J.; CURK, T.; ERJAVEC, A.; GORUP, Č.; HOČEVAR, T.; MILUTINOVIČ, M.; MOŽINA, M.; POLAJNAR, M.; TOPLAK, M.; STARIČ, A.; ŠTAJDOHAR, M.; UMEK, L.; ŽAGAR, L.; ŽBONTAR, J.; ŽITNIK, M.; ZUPAN, B. 2013. Orange: Data Mining Toolbox in Python. Journal of Machine Learning Research, 14, 2349-2353. (Available at: http://jmlr.org/papers/v14/demsar13a.html verified on December 08, 2023).

DI RIENZO, J.A.; CASANOVES, F.; BALZARINI, M.G.; GONZALEZ, L.; TABLADA, M.; ROBLEDO, C.W. 2011. InfoStat (versión 24). Universidad Nacional de Córdoba. (Available at: http://www.infostat.com.ar/ verified on December 08, 2023).

DINIZ, P.H.G.D.; BARBOSA, M.F.; DE MELO MILANEZ, K.D.T.; PISTONESI, M.F.; DE ARAÚJO, M.C.U. 2016. Using UV-Vis spectroscopy for simultaneous geographical and varietal classification of tea infusions simulating a homemade tea cup. Food Chemistry, 192, 374-379. https://doi.org/10.1016/j.foodchem.2015.07.022

EYER, M.; NEUMANN, P.; DIETEMANN, V. 2016. A look into the cell: Honey storage in honey bees, Apis mellifera. PLoS ONE, 11(8), 1-20. https://doi.org/10.1371/journal.pone.0161059

FAO/WHO. 2019. STANDARD FOR HONEY CXS 12-19811 Adopted in 1981. Revised in 1987,2001. Amended in 2019. Codex Alimentarius, 1(5), 1-13.

GALBAN, J.; SANZ, V.; MATEOS, E.; SANZ-VICENTE, I.; DELGADO-CAMON, A.; MARCOS, DE S. 2008. Reagentless Optical Biosensors for Organic Compounds Based on Autoindicating Proteins. Protein & Peptide Letters. Vol. 15, Issue 8. 772-778 pp. https://doi.org/http://dx.doi.org/10.2174/092986608785203782

GARCÍA, N.L. 2018. The Current Situation on the International Honey Market. Bee World, 95(3), 89-94. https://doi.org/10.1080/0005772X.2018.1483814

GAŠIĆ, U.; ŠIKOPARIJA, B.; TOSTI, T.; TRIFKOVIĆ, J.; MILOJKOVIĆ-OPSENICA, D.; NATIĆ, M.; TEŠIĆ, Ž. 2014. Phytochemical fingerprints of lime honey collected in Serbia. Journal of AOAC International, 97(5), 1259-1267. https://doi.org/10.5740/jaoacint.SGEGasic

GUO, N.; ZHAO, L.; ZHAO, Y.; LI, Q.; XUE, X.; WU, L.; GOMEZ ESCALADA, M.; WANG, K.; PENG, W. 2020. Comparison of the Chemical Composition and Biological Activity of Mature and Immature Honey: An HPLC/QTOF/MS-Based Metabolomic Approach. Journal of Agricultural and Food Chemistry, 68(13), 4062-4071. https://doi.org/10.1021/acs.jafc.9b07604

ISLA, M.I.; CRAIG, A.; ORDOÑEZ, R.; ZAMPINI, C.; SAYAGO, J.; BEDASCARRASBURE, E.; ALVAREZ, A.; SALOMÓN, V.; MALDONADO, L. 2011. Physico chemical and bioactive properties of honeys from Northwestern Argentina. LWT - Food Science and Technology, 44(9), 1922-1930. https://doi.org/10.1016/j.lwt.2011.04.003

LICHTENBERG-KRAAG, B. 2014. Evidence for correlation between invertase activity and sucrose content during the ripening process of honey. Journal of Apicultural Research, 53(3), 364-373. https://doi.org/10.3896/IBRA.1.53.3.03

MA, T.; ZHAO, H.; LIU, C.; ZHU, M.; GAO, H.; CHENG, N.; CAO, W. 2019. Discrimination of natural mature acacia honey based on multi-physicochemical parameters combined with chemometric analysis. Molecules, 24(14), 16-19. https://doi.org/10.3390/molecules24142674

MABRY, T.J.; MARKHAM, K.R.; THOMAS, M.B. 1970. The Ultraviolet Spectra of Flavones and Flavonols. The Systematic Identification of Flavonoids. Springer Berlin Heidelberg. 41-164 pp. https://doi.org/10.1007/978-3-642-88458-0_5

MACHADO DE-MELO, A.A.; DE ALMEIDA-MURADIAN, L.B.; SANCHO, M.T.; PASCUAL-MATÉ, A. 2018. Composición y propiedades de la miel de Apis mellifera: una revisión. Journal of Apicultural Research, 57(1), 5-37. https://doi.org/10.1080/00218839.2017.1338444

MAJTAN, J. 2014. Honey: An immunomodulator in wound healing. Wound Repair and Regeneration, 22(2), 187-192. https://doi.org/https://doi.org/10.1111/wrr.12117

MALDONADO, L.M.; SALOMÓN, V.M.; ÁLVAREZ, A.R.; BEDASCARRASBURE, E.; ISLA, M.I.; ORDÓÑEZ, R.M.; ZAMPINI, I.C.; CRAIG, A. 2011. Evaluation of physico-chemical parameters and markers of floral origin in lemon blossom honey of Tucumán, Argentina. 42.º Congreso Internacional de Apicultura APIMONDIA.

MALDONADO, L.; MARCINKEVICIUS, K.; BORELLI, R.; GENNARI, G.; SALOMÓN, V.; ISLA, M.I.; VERA, N.; BORELLI, V. 2020. Differentiation of argentine propolis from different species of bees and geographical origins by UV spectroscopy and chemometric analysis. Journal of the Saudi Society of Agricultural Sciences, 19(3). https://doi.org/10.1016/j.jssas.2018.09.003

MARCINKEVICIUS, K.; SALOMÓN, V.M.; GENNARI, G.P.; MALDONADO, L.M. 2021. Detección de adulteraciones de mieles de Tetragonisca fiebrigi por espectroscopía UV y análisis quimiométrico. In: SADA (Ed.), Congreso Argentino de Apicultura. SADA. 86 p.

MARTELO-VIDAL, M.J.; VÁZQUEZ, M. 2016. 3 Advances in Ultraviolet and Visible Light Spectroscopy for Food Authenticity Testing. In: DOWNEY, G. (Ed.). Advances in Food Authenticity Testing. Woodhead Publishing. 35-70 pp. https://doi.org/https://doi.org/10.1016/B978-0-08-100220-9.00003-5

NOVIYANTO, A.; ABDULLAH, W.; YU, W.; SALCIC, Z. 2015. Research trends in optical spectrum for honey analysis. 2015 Asia-Pacific Signal and Information Processing Association Annual Summit and Conference, APSIPA ASC. 416-425. https://doi.org/10.1109/APSIPA.2015.7415305

ORFANAKIS, E.; MARKOULIDAKIS, M.; PHILIPPIDIS, A.; ZOUMI, A.; VELEGRAKIS, M. 2021. Optical spectroscopy methods combined with multivariate statistical analysis for the classification of Cretan thyme, multi-floral and honeydew honey. Journal of the Science of Food and Agriculture, 101(13), 5337-5347. https://doi.org/10.1002/jsfa.11182

PERKAMPUS, H.H.; GRINTER, H.C.; THRELFALL, T.L. 2013. UV-VIS Spectroscopy and Its Applications. Springer Berlin Heidelberg. https://books.google.es/books?id=6ejwCAAAQBAJ

ROSHAN, A.R.A.; GAD, H.A.; EL-AHMADY, S.H.; KHANBASH, M.S.; ABOUSHOER, M.I.; AL-AZIZI, M.M. 2013. Authentication of monofloral yemeni sidr honey using ultraviolet spectroscopy and chemometric analysis. Journal of Agricultural and Food Chemistry, 61(32), 7722-7729. https://doi.org/10.1021/jf402280y

SE, K.W.; WAHAB, R.A.; SYED YAACOB, S.N.; GHOSHAL, S.K. 2019. Detection techniques for adulterants in honey: Challenges and recent trends. Journal of Food Composition and Analysis, 80, 16-32. https://doi.org/10.1016/j.jfca.2019.04.001

SEMKIW, P.; SKOWRONEK, W.; TEPER, D.; SKUBIDA, P. 2008. Changes occurring in honey during ripening under controlled conditions based on pollen analysis and electrical conductivity. Journal of Apicultural Science, 52(2), 45-53.

STEFAS, D.; GYFTOKOSTAS, N.; NANOU, E.; KOURELIAS, P.; COURIS, S. 2021. Laser-Induced Breakdown Spectroscopy: An Efficient Tool for Food Science and Technology (from the Analysis of Martian Rocks to the Analysis of Olive Oil, Honey, Milk, and Other Natural Earth Products). Molecules, 26(16). https://doi.org/10.3390/molecules26164981

SUHANDY, D.; YULIA, M. 2021. The Use of UV Spectroscopy and SIMCA for the Authentication of Indonesian Honeys According to Botanical, Entomological and Geographical Origins. Molecules 26(4), 915. https://doi.org/10.3390/MOLECULES26040915

TAN, J.; LI, R.; JIANG, Z.T. 2015. Chemometric classification of Chinese lager beers according to manufacturer based on data fusion of fluorescence, UV and visible spectroscopies. Food Chemistry, 184, 30-36. https://doi.org/10.1016/j.foodchem.2015.03.085

VANDAMME, L.; HEYNEMAN, A.; HOEKSEMA, H.; VERBELEN, J.; MONSTREY, S. 2013. Honey in modern wound care: A systematic review. Burns, 39(8), 1514-1525. https://doi.org/https://doi.org/10.1016/j.burns.2013.06.014

WHITE, J. W. 1957. The Composition of Honey. Bee World, 38(3), 57-66. https://doi.org/10.1080/0005772x.1957.11094976

XUE, X.; WANG, Q.; LI, Y.; WU, L.; CHEN, L.; ZHAO, J.; LIU, F. 2013. 2-Acetylfuran-3-Glucopyranoside as a Novel Marker for the Detection of Honey Adulterated with Rice Syrup. Journal of Agricultural and Food Chemistry, 61(31), 7488-7493. https://doi.org/10.1021/jf401912u

YARIV, I.; LIPOVSKY, A.; GEDANKEN, A.; LUBART, R.; FIXLER, D. 2015. Enhanced pharmacological activity of vitamin B12 and penicillin as nanoparticles. International Journal of Nanomedicine, 10(1), 3593-3601. https://doi.org/10.2147/IJN.S82482