SHORT COMMUNICATION

NMR-based metabolomic approach to differentiate organic and conventional Italian honey

R. Consonni* , F. Bernareggi, L.R. Cagliani

National Research Council, Institute for Macromolecular Studies (ISMAC), Lab. NMR, v. Corti 12, 20133 Milan, Italy

* CORRESPONDING AUTHOR FOOTNOTE

Roberto Consonni

Tel.: +39-02-23699578 Fax: +39-02-23699620

Email: roberto.consonni@ismac.cnr.it

Abstract

Honey represent a well appreciated food product endowed with several beneficial properties. In these last decades organic agriculture highly impacted social and political thought, leading to think that organic foods are healthier than the conventional ones. As far as we know, there are no studies applied to the differentiation of organic and conventional honey productions; the present study demonstrate the capability of ^{1}H NMR spectroscopy to address this important issue.

Polyflower, chestnut an acacia honeys have been differentiated on the basis of the water soluble minor components, by taking the advantage of multivariate statistical analysis performed on Orthogonal Signal Correction filtered ^{1}H NMR data. HMF content was also quantified to evaluate the freshness of samples.

KEYWORDS: Honey, organic food, NMR spectroscopy, metabolites.

1. Introduction

Honey is a well appreciated natural product produced by honey bees (*Apis mellifera L.*) from various secretions of plants. The largest producer is China, with 474.000 tons of honey in 2014, followed by Europe with 161.000 tons (http://www.fao.org/faostat/en). Within Europe, Romania is the largest producer with 35.00 tons in 2015 and Italy resulted the fifth producer with 23.000 tons (https://ec.europa.eu/agriculture/organic/eu-policy/eu-legislation/brief-overview_en). Different botanical varieties of honey are present on the market worldwide, according to the pollen composition, namely monofloral or polyfloral honey. To date about 300 varieties of honey have been identified (http://www.honey.com/honey-at-home/learn-about-honey/honey-varietals) whose composition and properties have been extensively characterized in these last years (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1999:222:0001:0028:EN:PDF). Additionally, "conventional" or "organic" honeys are available within Europe, according to beekeepers practices. These practices involve both the beekeeping and the environmental conditions tested by the bee to collect the nectar, that need to be reciprocally consistent. The differences between the two practices are mainly based on the presence of chemical compounds within the environment; as a matter of fact, in the organic beekeeping practice, only natural products are allowed. For example, natural phytochemicals, like thymol or eucalyptol could be adopted against the *Varroa Destructor* parasite, clean wax paper (in terms of deprived of any chemical contamination) must be used for the deposition of honey within the beehive, and only pollen or honey could integrate the feeding of bees and the location of the beehives, as indicated in the relatively recent EC 1804/99 directive (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1999:222:0001:0028:EN:pdf) and D.M. 91436 4/08/2000 (http://www.orsacampania.it/normativa/trasversale/Decreto%20Ministeriale%20n.%2091436%20del%204%20agosto%202000.pdf), which are indicating the general rules for "organic farming". In 2007 the European Council of Agricultural Ministers agreed on a new Council Regulation (Council Regulation EC No. 834/2007, http://eur-lex.europa.eu/eli/reg/2007/834/oj) setting out the principles, aims and overarching rules of organic production and defining how organic products were to be labelled. This last EC regulation promotes the logistic characteristics where the organic beekeeping farms must be placed: the areas need to ensure nectar and pollen from organic crops or from spontaneous flora and forests, and beekeeping farms will be kept far away from sources that may cause the contamination of honey or may affect bees' health. Additionally, Commission Regulation n° 889/2008 (http://www.ifoam-eu.org/sites/default/files/page/files/ifoameu_reg_regulation_dossier_201204_en.pdf) establishes that beekeeping farms should have, on an area of 3 km around the apiary, only organic crops or spontaneous vegetation, the wax used for honeycombs must be organic and it is prohibited the use of synthetic chemical insecticides during honey extraction. Foods derived from organic practices may be therefore labelled "organic" (in Italy "biological") only if at least 95% of their agricultural ingredients meet the necessary standards. Labelling is regulated by the EC n. 2000/13 (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2000:109:0029:0042:IT:pdf) directive, in respect to the consumer protection, and within the European boundaries, the new label has been introduced by the EC n.271/2010 (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:084:0019:0022:EN:pdf) regulation, to better highlight organic production system and in compliance with control rules and certification organisms. In the case of "conventional" foods, any ingredients which meet organic standards can be listed as organic. To ensure credibility, the code number of the certifying organization for organic food must be provided. During these last years, some researchers already focused their studies in the differentiation of organic vs conventional foods like fruits (Llano, Muñoz-Jiménez, Jiménez-Cartagena, Londoño-Londoño, & Medina 2018; Cuevas, Pereira-Caro, Moreno-Rojas, Munoz-Redondo, Ruiz-Moreno 2017; Kobi, Martins, Silva, Souza, Carneiro, Heleno, Queiroz, & Costa, 2018; Hohmann, Christoph, Wachter, & Holzgrabe 2014; Hohmann, Monakova, Erich, Christoph, Wachter, & Holzgrabe 2015; Marti, Leiva-Brondo, Lahoz, Campillo, Cebolla-Cornejo, & Rosello 2018), vegetables (Hoefkens et al. 2009; Pacifico, Casciani, Ritota, Mandolino, Onofri, Moschella, & Valentini, 2013; Merlini, Pena, da Cunha, de Oliveira, Rostagno, & Antunes 2018), dairy (Erich et al. 2015) and very recently roasted coffee (Consonni, Polla, & Cagliani 2018). Another important issue in honey characterization is the determination of hydroxymethylfurfural (HMF), a furanic derivative mainly produced by the sugar degradation, specifically due to the dehydration process of hexoses in acid medium, and as intermediate of the Maillard reaction (Ribeiro De Oliveira Resende et al. 2012), linking the HMF concentration to ageing and heating processes respectively (Sodré, Marchini, Moreti, Otsuk & Carvalho, 2011). HMF is assumed as a freshness indicator for honey, even though its presence could naturally occur in honeys from warm climate areas, such as tropical and subtropical countries. On the other hand HMF has potential harmful properties; as a matter of fact in vitro studies showed its mutagenic, genotoxic, cytotoxic, and carcinogenic effects (Janzowski, Glaab, Samini, Schlatter, & Eisembrand, 2000; Teixido, Santos, Puignou, & Galceran, 2006); the effects on humans are instead not completely clarified (Capuano & Fogliano 2011; Islam, Khalil, Islam, & Gan, 2014). For the above considerations, the European Union (Directive, 2001/110/EC) recommended a HMF content lower than 40 mg Kg^{-1}. Exceptions are represented by honeys with low enzymatic levels for which the limit is set to 15 mg Kg-1, and by honeys from tropical regions for which the limit is set to 80 mg Kg-1. The aim of this study is to fill in this literature lack concerning the discrimination between conventional and organic honeys. In this view, the use of NMR metabolomics and chemometrics is here presented to investigate whether the water soluble metabolite profile would provide evidences in the differentiation of samples of the same botanical origin. In particular the most popular honeys available on the Italian market, namely polyfloral, acacia and chestnut, have been considered and the HMF content was quantified in order to monitor their freshness and quality aspects.

2. Material and methods

2.1. Samples

A total of 56 honey samples of different geographical and botanical (19 of acacia, 18 of chestnut and 19 of polyfloral) origin were collected on the Italian market from trusted producers. Among them 28 were organic and 28 were conventional. All the organic honey sample have been verified by the Italian authorized control organization, certified by Mi.P.A.A.F. (Ministery of farming, food and forestry policies) and labelled accordingly. The geographical origin of samples are summarized in Table 1, including available details on the minimum durability date (MDD) reported on the labels. Each sample was prepared in duplicate to minimize possible sample inhomogeneity; about 100 mg of honey were dissolved in 600 μL of deuterated water (Sigma-Aldrich, 99.96 atom % D, Milan, Italy). Water solution of honeys were not corrected for the occasional small pH deviations. Buckets were adjusted to include these deviations only for organic acids whenever necessary. Quantification of HMF content in all honey samples was performed on the basis of a calibration curve calculated using water solutions of 5-methyl-furfurale (5MF) in the concentration range of 100-5 ppm (Fig. S1) . 5MF was obtained from Sigma-Aldrich.

2.2. NMR data acquisition and processing

NMR spectra have been recorded at 300 K on Bruker Advance II 500 spectrometer (Bruker Biospin, GmbH Rheinstetten, Karlsruhe, Germania), operating at 11.7 T and equipped with a 5 mm reverse Z gradient probe. Monodimensional spectra have been acquired with TOPSPIN 1.2 (Bruker TOSPSPIN 1.2®) employing a solvent presaturation scheme, with 128 scans over 32K of data and a spectral width of 7500 Hz. A resolution enhancement function was applied before Fourier transformation. All spectra have been phased, baseline corrected and referred to α-glucose anomeric proton at 5.17 ppm. A limited region around the residual water signal was removed and the spectral region was split into small integrated intervals with fixed length (0.04 ppm) in the a) full spectral region, within 0.0-9.50 ppm interval, b) low intensities signal, within 0.00-3.01 and 5.47-9.50 ppm intervals, and referred to the total area value in both cases. Heteronuclear bidimensional experiments, HSQC and HMBC, have been recorded with 256 scans each, and with

6000 Hz and 30000Hz for ^{1}H and ^{13}C dimension respectively. Direct and long range coupling constants were 145 Hz and 8 Hz respectively.

2.3. Statistical methods

Multivariate statistical analysis was performed by using SIMCA-P 13.03 software (Soft Independent Modeling of Class Analogy; Umetrics, Umea Sweden). Principal Component Analysis (PCA) and two classification approaches have been used such as Partial Least Square-Discriminant Analysis (PLS-DA) and Orthogonal Projection to Latent Structures-Discriminant Analysis (OPLS-DA), performed with Unit Variance as data pretreatment. Model validation was also checked by means of random permutation test on the Y block to overcome randomness safely or over-fitting within the model. The number of latent components was determined by cross-validation technique. T2 and Distance to the Model (DModX) tests were applied to check for the presence of outliers and to evaluate the model applicability domain for all samples. The use of Orthogonal Signal Correction (OSC) filter was also investigated to remove the uncorrelated variables to response Y, from the X matrix, providing a PLS-based solution.

3. Results and Discussion

The ^{1}H NMR spectra of water extracts from honey samples is characterized by the presence of major soluble metabolites; among them carbohydrates have a dominant role, while other components like organic acids and amino acids in lower content. The detected carbohydrates are constituted by mono- up to tetra-saccharides, according to previous determinations (Consonni, Cagliani, & Cogliati, 2012; Consonni, & Cagliani, 2015; Schievano, Tonoli, & Rastrelli, 2017). In this respect the expansion of Fig 1A. represent the typical NMR profiles of the aromatic region for the three botanical origins of the investigated honeys. Specific molecules appeared selectively represented in each botanical variety. Noticeably, kynurenic acid and formiate, in full accordance with previous data (Beretta, Caneva, & Maffei, 2007), are well represented in both conventional and organic chestnut honeys, phenyalanine, and tyrosine characterized polyflower honey and in lesser extent also present in chestnut, while acacia appeared containing only very low level of kynurenic, phenylalanine and formiate, and a relatively higher content of uracile. The anomeric region represented in Fig 1B, is the most characteristic NMR region for honey dissolved in water, where all the anomeric protons of different saccharidic isoforms are represented. The two dominant monosaccharides, glucose and fructose are detectable in their respective isoforms: glucose in the α-pyranosidic configuration at 5.15 ppm and β-pyranosidic at 4.56 ppm, while the two most abundant isoforms of fructose at 4.03 ppm (β-furanosidic) and at 3.93 ppm (β-pyranosidic). Interestingly, the open chain *keto* isomer of fructose was detected as well, being in a relatively uncrowded region occurring at 4.46 and 4.57 ppm, also confirmed by TOCSY and HSQC correlations and in full accordance with the literature data (Barclay, Ginic-Markovic, Johnston, Cooper, & Petrovsky 2012). A part from these two monosaccharides, di-, tri and tetra-saccharides have been recognized and already assigned in an our previous work (Consonni, Cagliani, & Cogliati, 2012). Finally, Figure 1C represents the aliphatic region of ^{1}H NMR spectra of honey dissolved in water; in this NMR region, the presence of proline is evident for all botanical varieties, representatively lower in acacia, as well as alanine. Additionally, organic acids like succinate, malate, acetate and lactate have been recognized while few compounds remain unassigned and labelled with “u”. A small amount of ethanol was also detected for very few samples. As commonly occurring due to the complexity of ^{1}H NMR spectra and the large dataset produced by the bucket integration, spectra have been analyzed by multivariate data analysis, in order to reduce the complexity but also to establish possible markers responsible for conventional and organic honey samples differentiation.

Unsupervised models (Principal Components Analysis) have been unsuccessfully explored to evaluate whether ^{1}H NMR spectral regions would allow samples differentiation. The results obtained did not support the samples discrimination according to the agronomical practice used, and therefore discriminant analysis appeared more appropriate, especially in order to determine possible metabolites responsible for the differentiation. Similarly, the application of “soft data pretreatment” (like centering, unite variance etc) appeared not suited to discriminate conventional against organic honeys samples properly. The NMR data quite often present structured noise that would shadow the relevant information. The use of OSC filters designed to remove undesirable systematic variation within the dataset, appeared to be highly efficient even when dealing with small perturbation (Blaise, Navratil, Emsley, & Toulhoat, 2011). In the present study, the application of a single OSC filters allowed to remove uncorrelated variables respectively from the entire NMR dataset. PLS-DA was performed for each botanical origin for all samples, after the verification of domain consistency for all samples and the application of a single OSC filter on the entire bucketed ^{1}H NMR spectrum. The three score plots of PLS-DA models are represented in Fig. S2A-C by scoring the first two latent variables. All models have been validated testing the non-casualty, as confirmed by 200 cycles of random permutation of Y variables (permutation test) showed in Fig. S3A-C. All the PLS-DA models resulted well modelled in discriminating organic against conventional honey samples. In particular they resulted stable and with high predictive capability, as denoted by the explained variability and prediction capability values (Acacia model: R^2X=73.7%, R^2Y=98.6%, Q^2=93.2%; polyfloral model: R^2X=78.4%, R^2Y=74.9%, Q^2=49.0%; Chestnut model: R^2X=88.4%, R^2Y=98.4%, Q^2=95.8%;). The analysis of the corresponding loadings revealed only saccharides as the variables responsible for samples differentiation, and in particular the anomeric and the sidechain protons, as demonstrated by the VIP plot (Fig. 2A-C), being these latter the more intense signals affecting the ^{1}H NMR spectrum. In order to evaluate possible resonances as potential markers for the single honey varieties, selected spectral regions of ^{1}H NMR spectrum, particularly those were low intense resonances are present, have been therefore used to check their contribution in samples differentiation. The PLS-DA models obtained with the same dataset treatment performed when only low intensities signals have been used, allowed a clear-cut differentiation of samples and are represented in Fig 3A-C. The statistical data for the models are as following: acacia model: two latent components, R^2X=39.1%, R^2Y=99.7%, Q^2=98.5% (66.39% noise); polyfloral model: two latent components, R^2X=64.3%, R^2Y=82%, Q^2=66.5% (39.17% noise).; chestnut model: three latent components, $R^2X=79.9\%$, $R^2Y=99.7\%$, $Q^2=98.1\%$ (59.01% noise). All the models resulted with a significant prediction capability and the non-casualty have been tested for all models, performing 200 cycles of random permutation of Y variables (Fig. S4A-C). The analysis of the corresponding loading plot performed for each botanical origin (Fig. 4A-C), highlighted individual compounds responsible for the samples differentiation.

Specifically, conventional acacia honey (Fig. 4A) resulted characterized by buckets due to organic acids like lactate at 1.26 ppm, succinate at 2.47 ppm, and acetate at 1.87 ppm, in addition to buckets at 2.28 ppm and 1,87 due to proline, while buckets at 1.20 ppm most likely due to isopropanol Organic acacia honeys resulted enriched in bucket at 8.27 ppm due to formiate and bucket at 1.76 ppm. This unknown doublet labelled as "u1", is particularly abundant in chestnut honeys, and shows an homonuclear correlation at 3.98 ppm whose direct attached carbon resonating at 30.6 ppm. Two additional long range heteronuclear correlations with carbons at 64.7 ppm and 97.4 ppm, are observed, but this compound is not yet assigned. Loading plot of polyflower honeys represented in Fig. 4B, highlight buckets due to organic acids like succinate at 2.47ppm, formiate at 8.27 ppm, kynurate at 7.76 ppm and acetate at 1.87ppm, together with buckets at 1,10 ppm due to ethanol and buckets due to proline at 1.87 ppm; conversely, lactate (bucket at 1.26 ppm) isopropanol at 1.20 ppm and unknown compound at 1.07 ppm characterized organic polyflower honey. Finally, loading plot of chestnut model (Fig. 4C), highlighted again organic acid like succinate (with bucket at 2.47 ppm) acetate (at 1.87 ppm)and lactate at 1.26 ppm for conventional honey, while formiate (bucket at 8.27 ppm) and all buckets including all kynurate signals (7.76ppm, 7.47 ppm, 8.12 ppm and 6.86 ppm) the characteristic metabolite of chestnut honey. Summarizing, the presence of organic acids in conventional honeys appears as a common characteristic, for both acacia and chestnut, while in the case of polyflower honeys, a mixed contributions is observed for the less abundant set of metabolites, in full agreement with the botany characteristics of this type of honey (De-Melo, de Almeida-Muradian, Sancho, & Pascual-Mate, 2018). Quantitative evaluation of HMF content I the investigated samples is reported in Table 2. This evaluation confirmed that the level of HMF measured, is largely below the acceptance limit, being within the range of xx.

4. Conclusion

The present study confirmed the capability of NMR measurements in assessing the characterization of complex matrices, like honey, and additionally highlight the possibility to discriminate Italian organic and conventional honeys for three different botanical origins. In addition, to the best of our knowledge, this represent the first investigation involving the differentiation of organic and conventional honey samples. The beneficial use of OSC filters allowed the removal of structured noise, typically present when NMR spectral regions of low abundant constituents are concerned, thus selecting only the useful data for the discrimination of samples. The HMF content determination confirmed that all the samples satisfied the law requirements of quality. This study represent the first attempt in the differentiation of farming condition for honey. Even though confined to the investigation of Italian honeys, it led to the possibility to expand at European level, with adequate sampling and choice of labelled organic honeys.

Acknowledgments

The “Scientific Foundation A. De Marco” is acknowledged for financial support of L. R. Cagliani.

Conflict of interest

The authors declare no conflict of interest.

References

Barclay, T., Ginic-Markovic, M., Johnston, M.R., Cooper, P. & Petrovsky N. (2012) Observation of the keto tatutomer of D fructose in D2O using ^{1}H NMR spectroscopy. *Carbohydrate Research, 347*, 136-141.

Beretta, G., Caneva, E., & Maffei, R. (2007) Kynurenic acid in honey from arboreal plants: MS and NMR evidence. *Planta Medica, 73*, 1592-1595.

Blaise, B. J., Navratil, V., Emsley, L., & Toulhoat, P. (2011). Orthogonal filtered recoupled-STOCSY to extract metabolic networks associated with minor perturbations from NMR spectroscopy. *Journal of Proteome Research, 10,* 4342-4348.

Capuano, E., & Fogliano, V. (2011) Acrylamide and 5-hydroxymethylfurfural (HMF): A review on metabolism, toxicity, occurrence in food and mitigation strategies. *LWT- Food Science and Technology, 44,* 793-810.

Cuevas, F. J., Pereira-Caro, G., Moreno-Rojas, J. M., Munoz-Redondo, J. M., Ruiz-Moreno, M. J. (2017). Assessment of premium organic orange juices authenticity using HPLC-HR-MS and HS-SPME-GC-MS combining data fusion and chemometrics. *Food Control, 82,* 203-211.

FAOSTAT (http://www.fao.org/faostat/en)

https://ec.europa.eu/agriculture/organic/eu-policy/eu-legislation/brief-overview_en

The National Honey Board. (2015). Honey varietals. [Online] Retrieved from http://www.honey.com/honey-at-home/learn-about-honey/honey-varietals.

EC n. 1804/99 directive http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1999:222:0001:0028:EN:PDF

D.M. 91436 4/08/20005 http://www.orsacampania.it/normativa/trasversale/Decreto%20Ministeriale%20n.%2091436%20del%204%20agosto%202000.pdf

Consonni, R., Polla, D., Cagliani, L.R., (2018) Organic and conventional coffee differentiation by NMR spectroscopy, *Food Control*, *94*, 284-288.

Consonni, R. & Cagliani, L.R. (2015). Recent developments in honey characterization. *RSC Advances*, *5*, 59696-59714.

Consonni, R., Cagliani, L.R. & Cogliati, C, (2012). NMR Characterization of saccharides in Italian honeys of different floral sources. *Journal of Agricultural and Food Chemistry*,*60*, 4526-4534.

Council Regulation (EC) n. 834/2007 http://eur-lex.europa.eu/eli/reg/2007/834/oj

Council Regulation (EC) 889/2008 http://www.ifoam-eu.org/sites/default/files/page/files/ifoameu_reg_regulation_dossier_201204_en.pdf

De-Melo, A.A.M., de Almeida-Muradian, L.B., Sancho, M.T., Pascual-Mate, A. (2018) Composition and properties of *Apis mellifera* Honey: a review. *Journal of Apicultural Research*, *57*, 5-37.

De Oliveira Resende Ribeiro, Da Silva Carneiro, C., Teixerira Màrsico, E., Lima Cunha, F., Conte Junior, C.A. & Borges Mano, S. (2012). Influence of the time/temperature binomial on the hydroxymethylfurfural content of flora honeys subjected to heat treatment. *Ciencia ae Agrotecnologia*, *36*, 204-209.

EC n. 2000/13 directive (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2000:109:0029:0042:IT:PDF. Accessed

EC n. 271/2010 regulation (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:084:0019:0022:EN:PDF. Accessed

Erich, S., Schill, S., Annweiler, E., Waiblinger, H. U., Kuballa, T., Lachenmeier, D. W., & Monakova, Y. B. (2015). Combined chemometric analysis of ^{1}H NMR, ^{13}C NMR and stable isotope data to differentiate organic and conventional milk. *Food Chemistry*, *188*, 1-7.

Hoefkens, C., Vandekinderen, I., De Meulenaer, B., Devlieghere, F., Baert, K., Sioen, I., … Van Camp, J. (2009). A literature-based comparison of nutrient and contaminant contents between organic and conventional vegetables and potatoes. *British Food Journal*, *111*, 1078-1097.

Hohmann, M., Monakova, Y. B., Erich, S., Christoph, N., Wachter, H., & Holzgrabe, U. (2015). Differentiation of organically and conventionally grown tomatoes by chemometric analysis of combined data from proton nuclear magnetic resonance and mid-infrared spectroscopy and stable isotope analysis. *Journal of Agricultural Food Chemistry*, *63*, 9666-9675.

Hohmann, M., Christoph, N., Wachter, H., & Holzgrabe, U. (2014). ^{1}H NMR profiling as an approach to differentiate conventionally and organically grown tomatoes. *Journal of Agricultural Food Chemistry*, *62*, 8530-8540.

Islam, M.N., Khalil, M.I., Islam, M.A., & Gan, S.H., (2014) Toxic compounds in honey. *Journal of Applied Toxicology*, *34*, 733-742.

Kobi, H. B., Martins, M. C., Silva, P. I., Souza, J. L., Carneiro, J. C. S., Heleno, F. F., … Costa, N. M. B. (2018). Organic and conventional strawberries: nutritional quality, antioxidant characteristics and pesticide residues. *Fruits, 73*, 39-47.

Janzowski, C., Glaab, V., Samini, E., Schlatter, J., & Eisembrand, G. (2000) 5hydroxymethylfurfural: assessment of mutagenicity, DNA-damaging potential and reactivity towardscellular glutathione. *Food and Chemical Toxicology*, *38*, 801-809.

Llano, S. M., Muñoz-Jiménez, A. M., Jiménez-Cartagena, C., Londoño-Londoño, J., & Medina S. (2018). Untargeted metabolomics reveals specific withanolides and fatty acyl glycoside as tentative metabolites to differentiate organic and conventional Physalis peruvian fruits. *Food Chemistry, 244,* 120-127.

Marti, R., Leiva-Brondo, M., Lahoz, I., Campillo, C., Cebolla-Cornejo, J., & Rosello, S. (2018). Polyphenol and L-ascorbic acid content in tomato as influenced by high lycopene genotypes and organic farming at different environments. *Food Chemistry, 239,* 148-156.

Merlini, V. V., Pena, F. D., da Cunha, D. T., de Oliveira, J. M., Rostagno, M. A., & Antunes, A. E. C. (2018). Microorganic quality of organic and conventional leafy vegetables. *Journal of Food Quality, vol. 2018*, 1-7.

Pacifico, D., Casciani, L., Ritota, M., Mandolino, G., Onofri, C., Moschella, A., & Valentini, M. (2013). Metabolomics for organic farming traceability of early potatoes. *Journal of Agricultural and Food Chemistry, 61,* 11201-11211.

Schievano, E., Tonoli, M. & Rastrelli, F. (2017) NMR quantification of carbohydrates in complex mixtures. A challenge on honey. *Analytical Chemistry*, *89*, 13405-13414.

Sodré, G.S., Marchini, L.C., Moreti, A.C.C.C., Otsuk, I.P.,& Carvalho, C.A.L., (2011). Pghysico chemical characteristics of honey produced by Apis mellifera in the Picos region, state of Piauì, Brazil. *Revista Brasileira de Zootecnia, 40*, 1837-1843.

Teixido, E., Santos, F.J., Puignou, L., & Galceran, M.T. (2006) Analysis of 5-hydroxymethylfurfural in foods by gas chromatography-mass spectrometry. *Journal of Chromatography A, 1135*, 85-90.

Figure captions

Fig. 1A-C. ^{1}H NMR spectra of conventional (C) and organic (B) water extracts of honey of different botanical origin are represented in couple: chestnut (bottom), polyflower (middle) and acacia (top). Panels A-C reports aromatic, anomeric and aliphatic ^{1}H NMR regions.

Symbols as following: FOR, formiate; KYN, kynurenic acid; Phe, phenyl alanine; Tyr, tyrosine; URA, uracile; SUC, succinate; MAL, malate; ACE, acetate; LAC, lactate; Pro, proline; Ala, alanine; KOJ, kojibiose; NIG, nigerose; TUR, turanose; MLT, maltose, MLTU, maltulose, MLT3, maltotriose,; MLT4, maltotetraose; SUC, sucrose; GEN, gentiobiose; IMLT, isomaltose; IMLT3, isomaltotriose; IMLT4, isomaltotetraose; PAL, palatinose, MEL, melezitose; RAF, raffinose; MLB, melibiose; u, unknown.

Fig. 2A-C. VIP of PLS-DA models for acacia (A), polyflower (B) and chestnut (C) honeys obtained after application of mean centering as data pretreatment and one OSC filter performed on the entire ^{1}H NMR spectrum.

Fig. 3A-C. Score plots of PLS-DA models performed on selected NMR regions containing only low intensity resonances. Acacia (A), polyflower (B) and chestnut (C) honeys obtained after application of CTR as data pretreatment and one OSC filter.

Fig 4A-C. Loading plots of PLS-DA models performed on selected NMR regions containing only low intensity resonances. Acacia (A), polyflower (B) and chestnut (C) honeys obtained after application of CTR as data pretreatment and one OSC filter performed on the NMR regions containing only low intensity signals. The first two latent components have been plotted. A(1) and A(2) are centroids for CONV and ORG samples respectively.

Table 1. List of conventional and organic honeys samples investigated, with the indication of regional origin of samples, beekeeping practice and the minimum durability date (MDD) reported on the labels. n.d. indicate “not declared” regional origin.

Table. 2. HMF quantification for selected honey samples.

Figure 1A

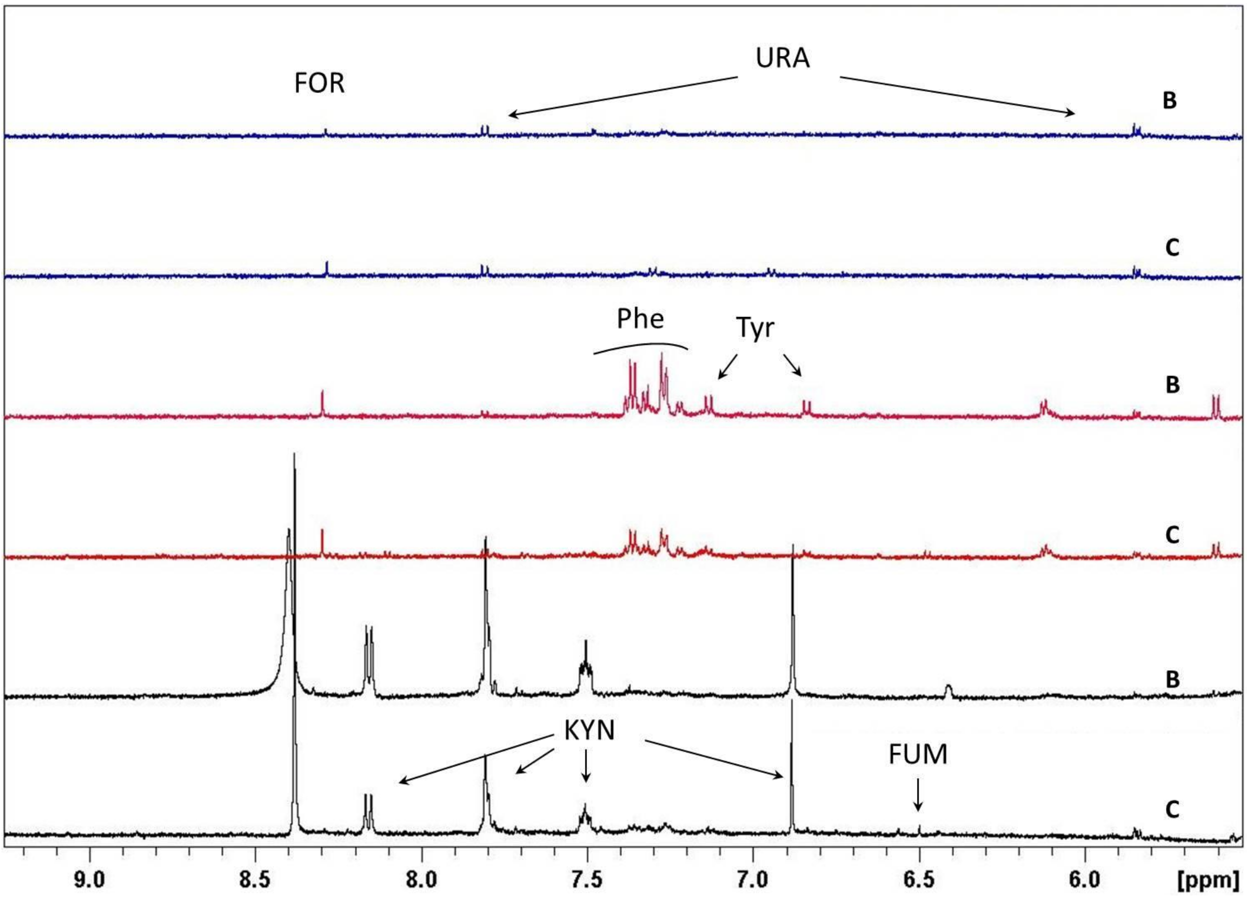
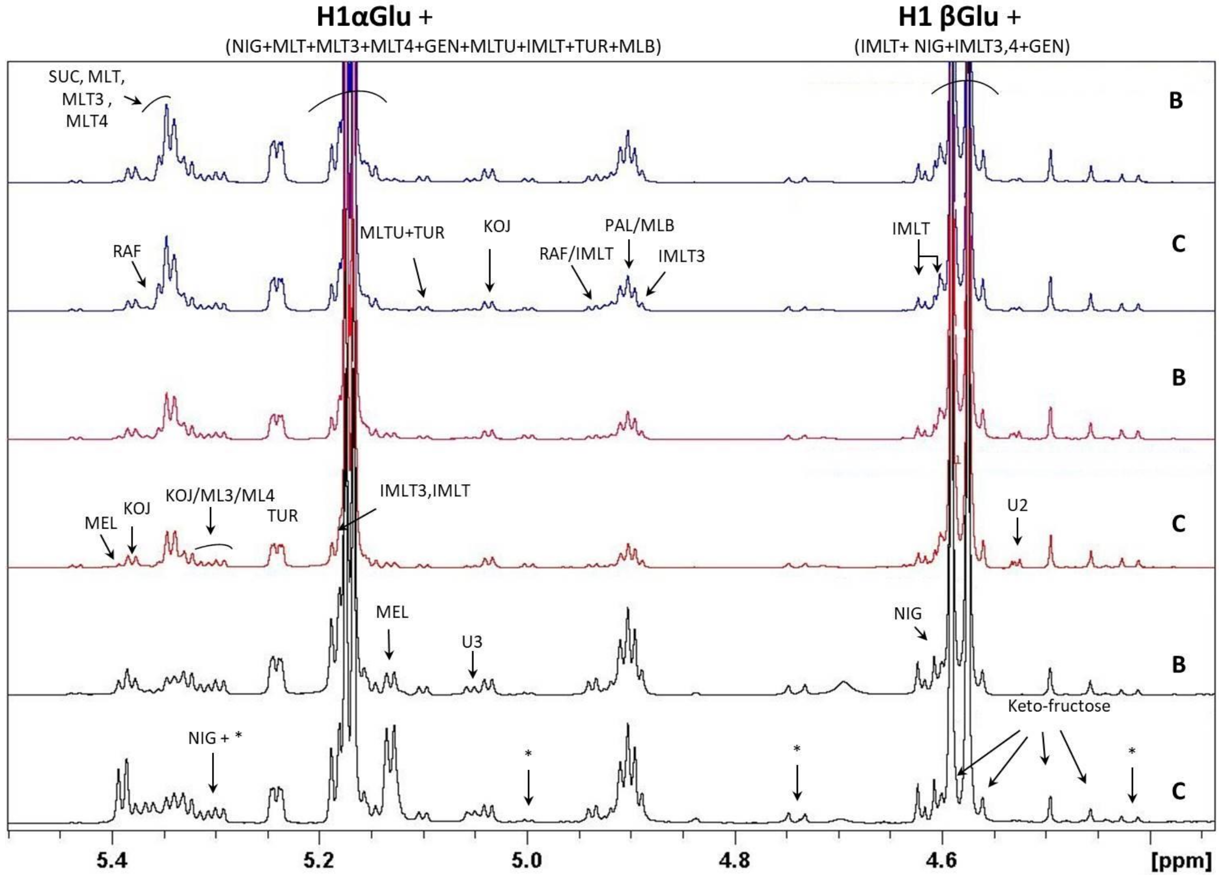


Figure 1B



H1αGlu +
(NIG+MLT+MLT3+MLT4+GEN+MLTU+IMLT+TUR+MLB)
H1 βGlu +
(IMLT+ NIG+IMLT3,4+GEN)
SUC, MLT,
MLT3 ,
MLT4
B
RAF
MLTU+TUR
KOJ
RAF/IMLT
PAL/MLB
IMLT3
IMLT
C
B
MEL
KOJ
KOJ/ML3/ML4
TUR
IMLT3,IMLT
U2
C
MEL
U3
NIG
B
Keto-fructose
NIG + *
*
*
*
C
5.4
5.2
5.0
4.8
4.6
[ppm]

Figure 1C

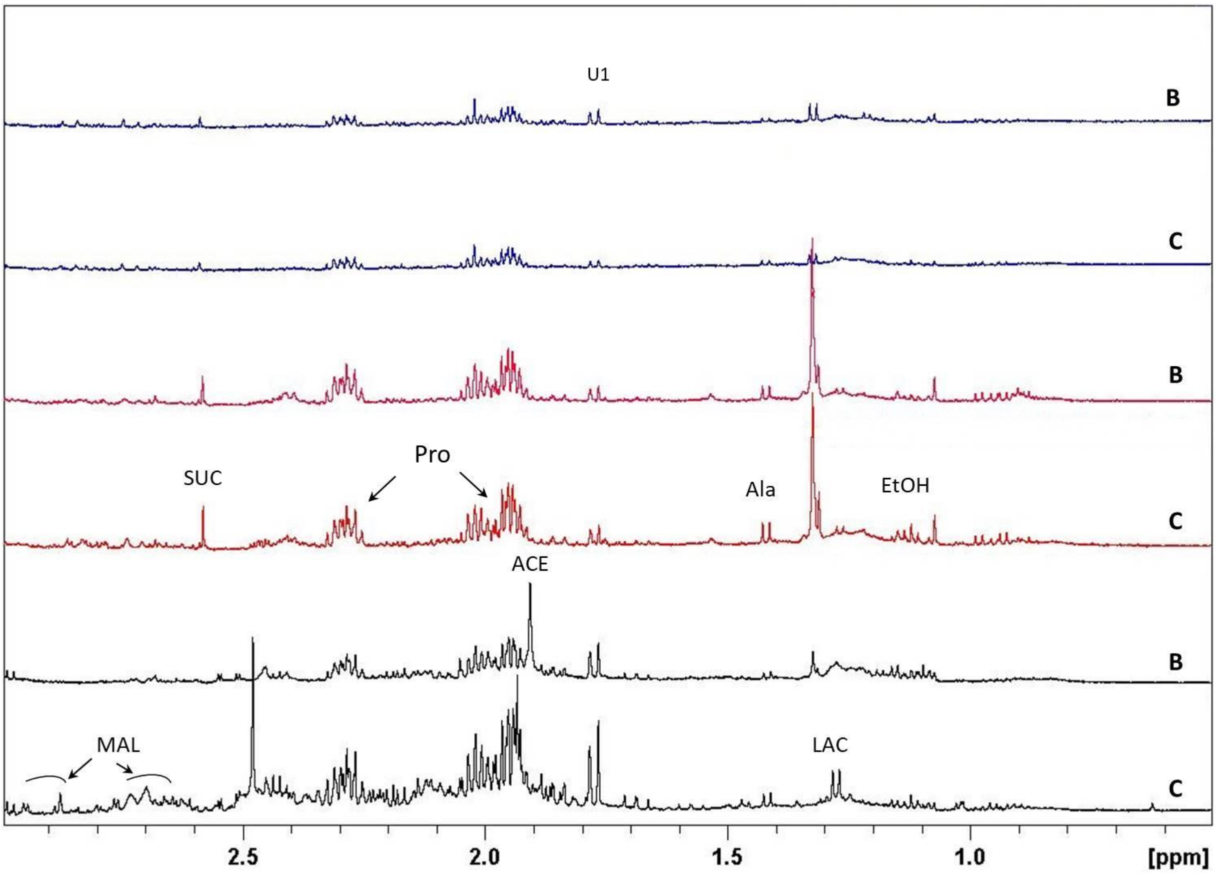


Figure 2A

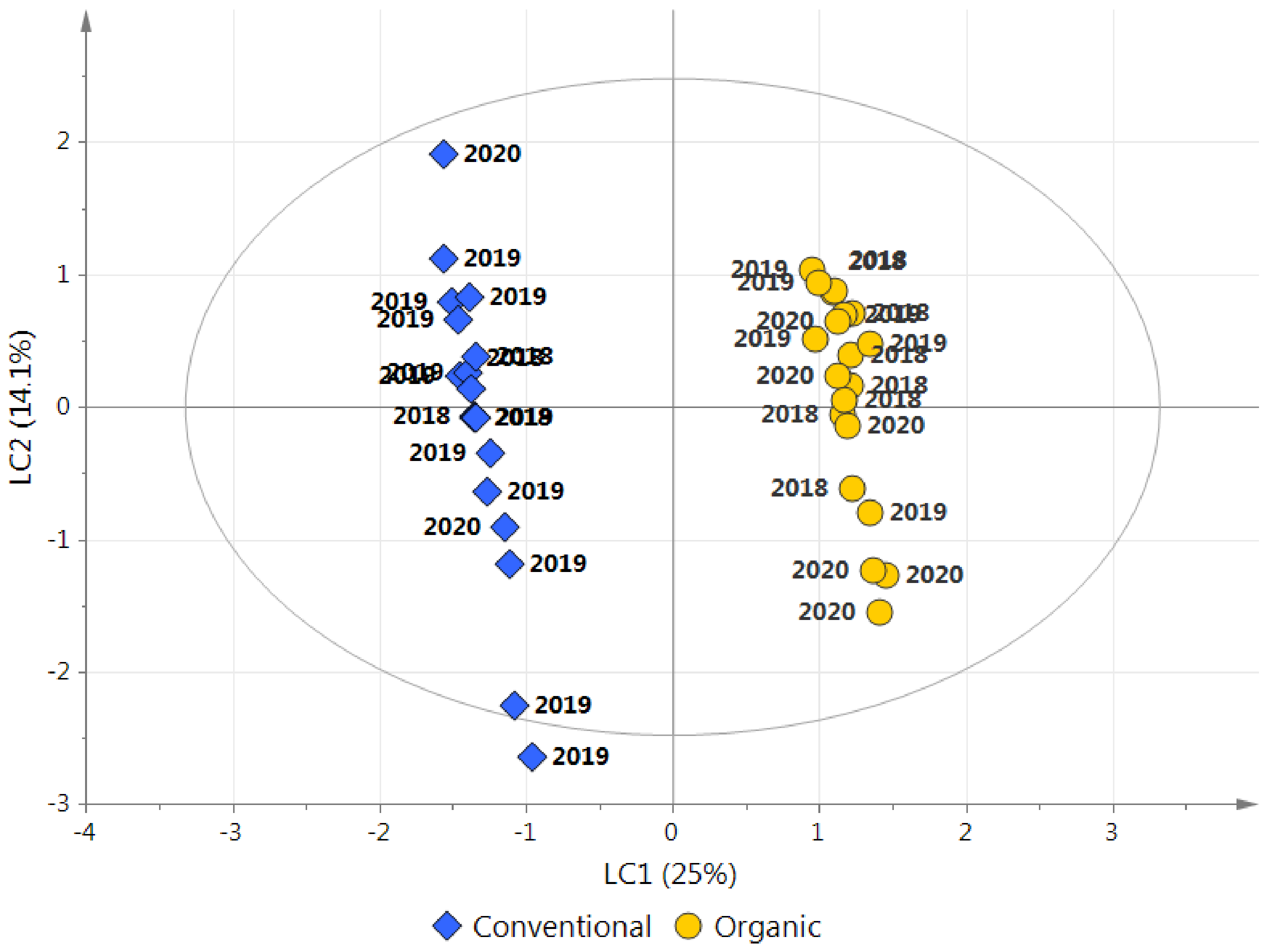
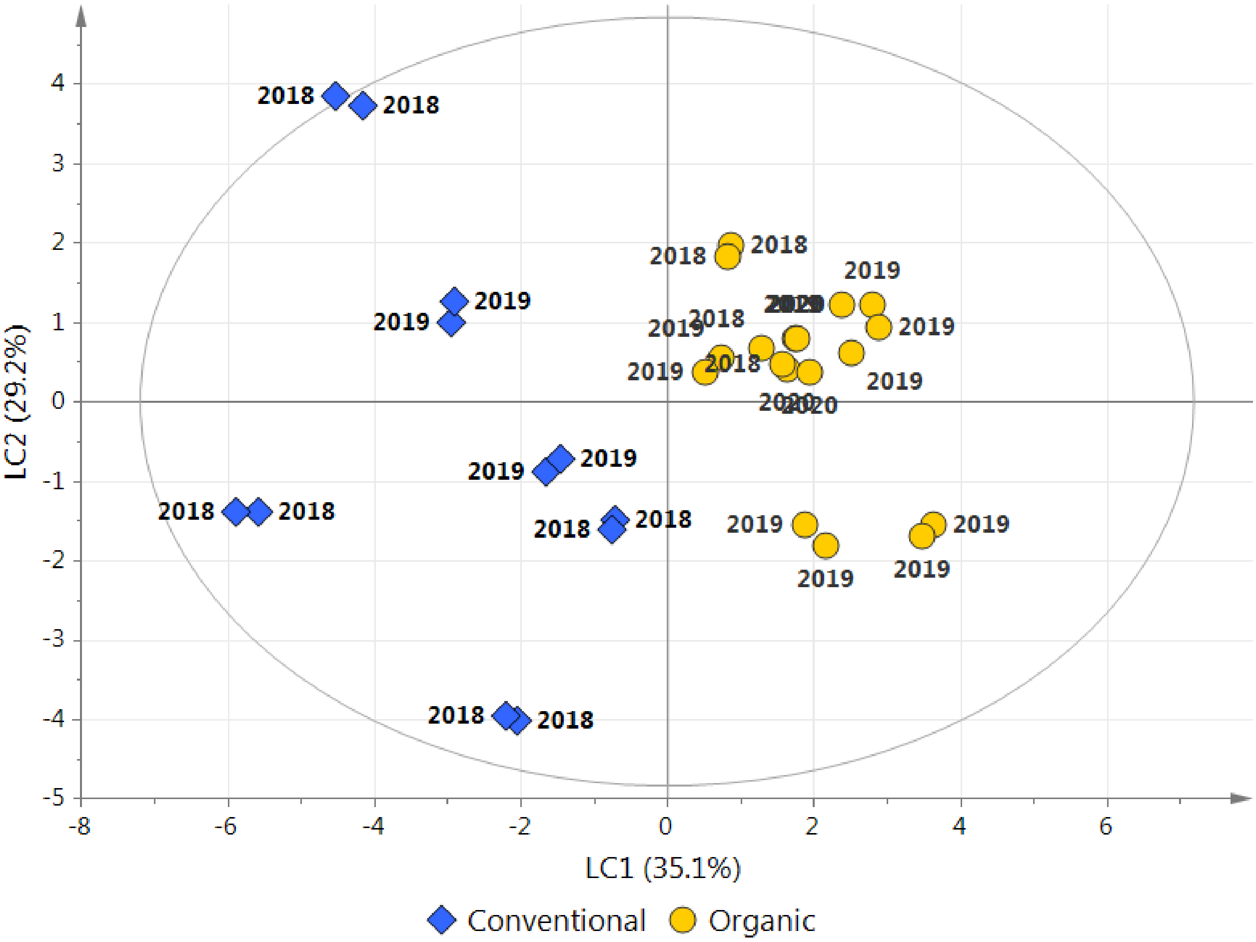


Figure 2B



LC2 (29.2%)
LC1 (35.1%)
4
3
2
1
0
-1
-2
-3
-4
-5
-8
-6
-4
-2
0
2
4
6
2018
2019
2020
Conventional
Organic

Figure 2C

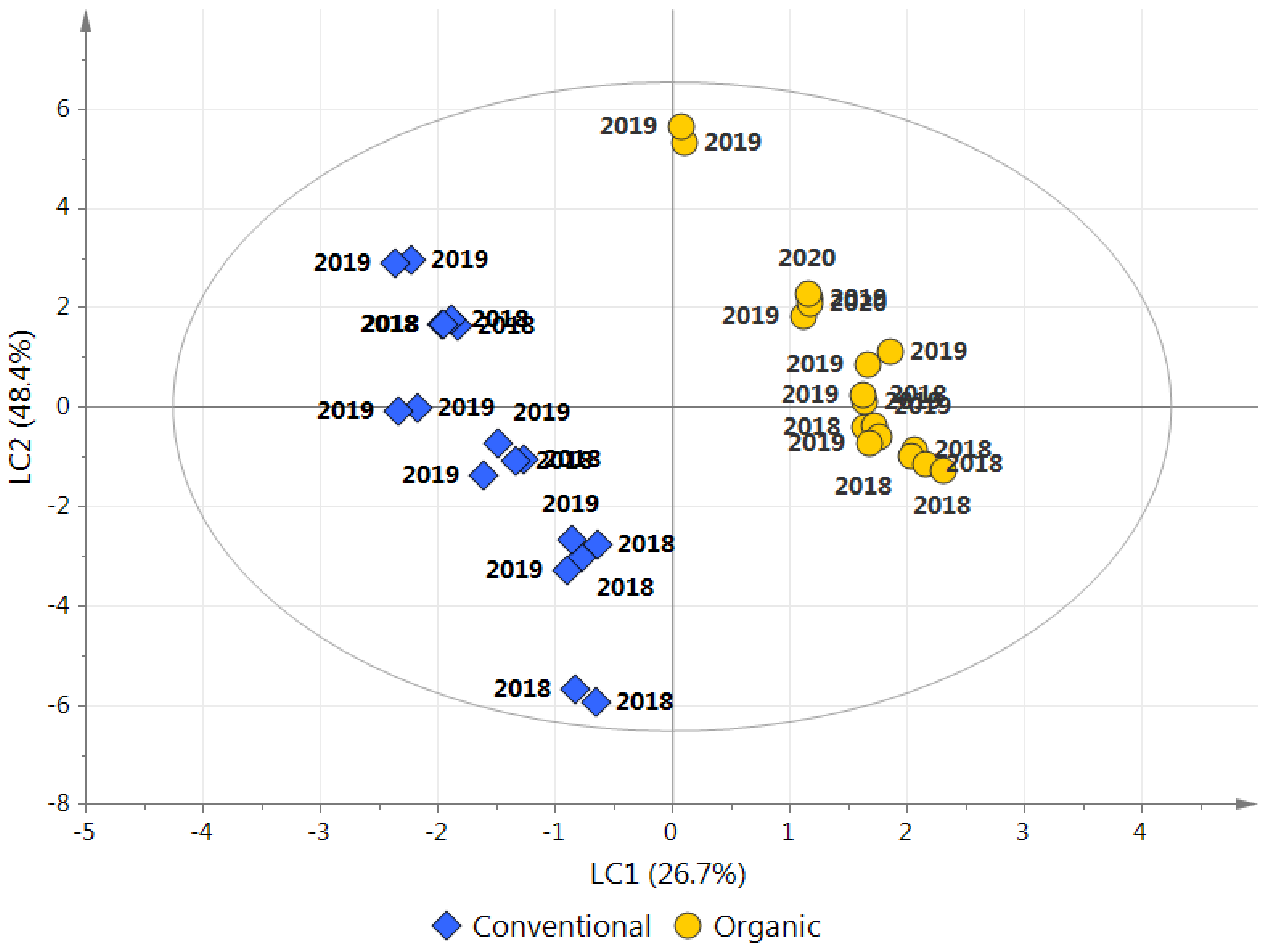
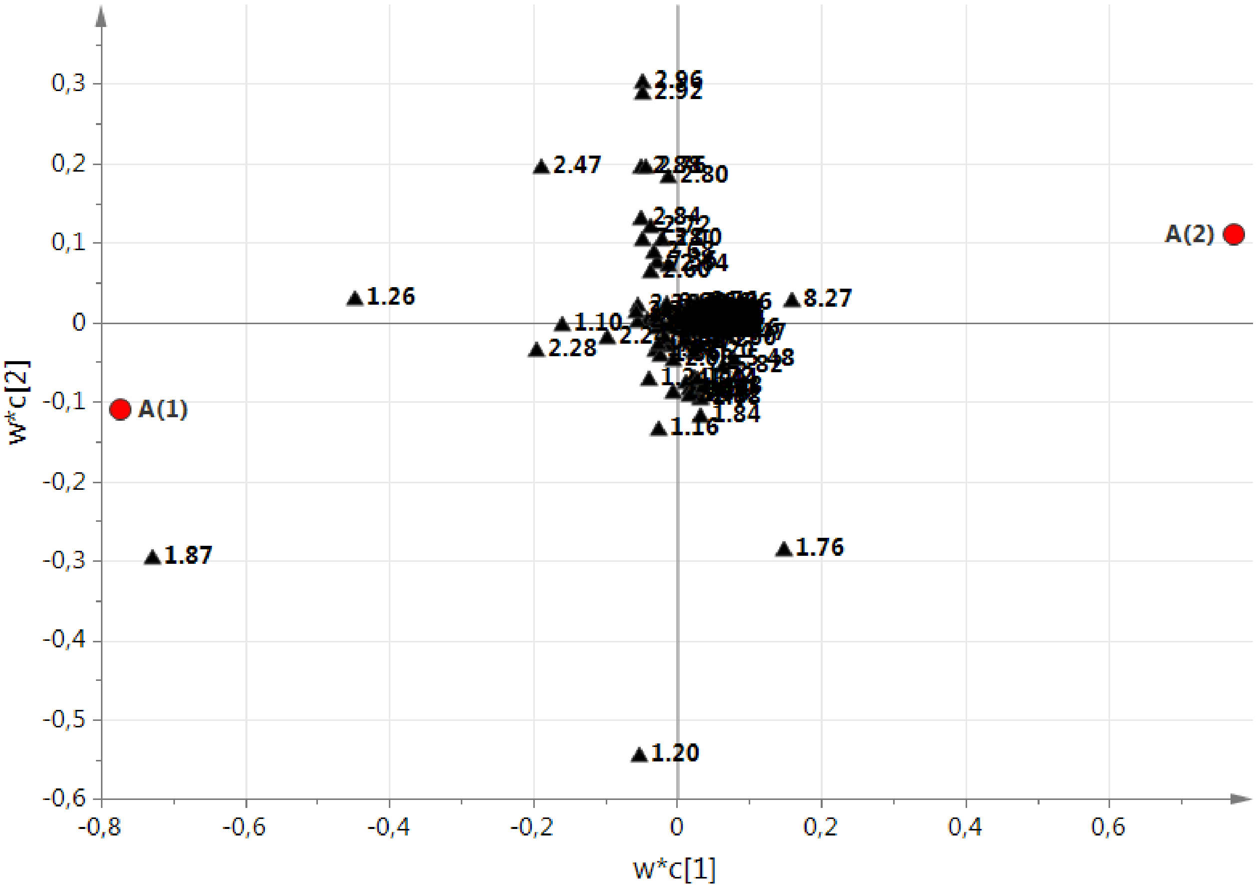
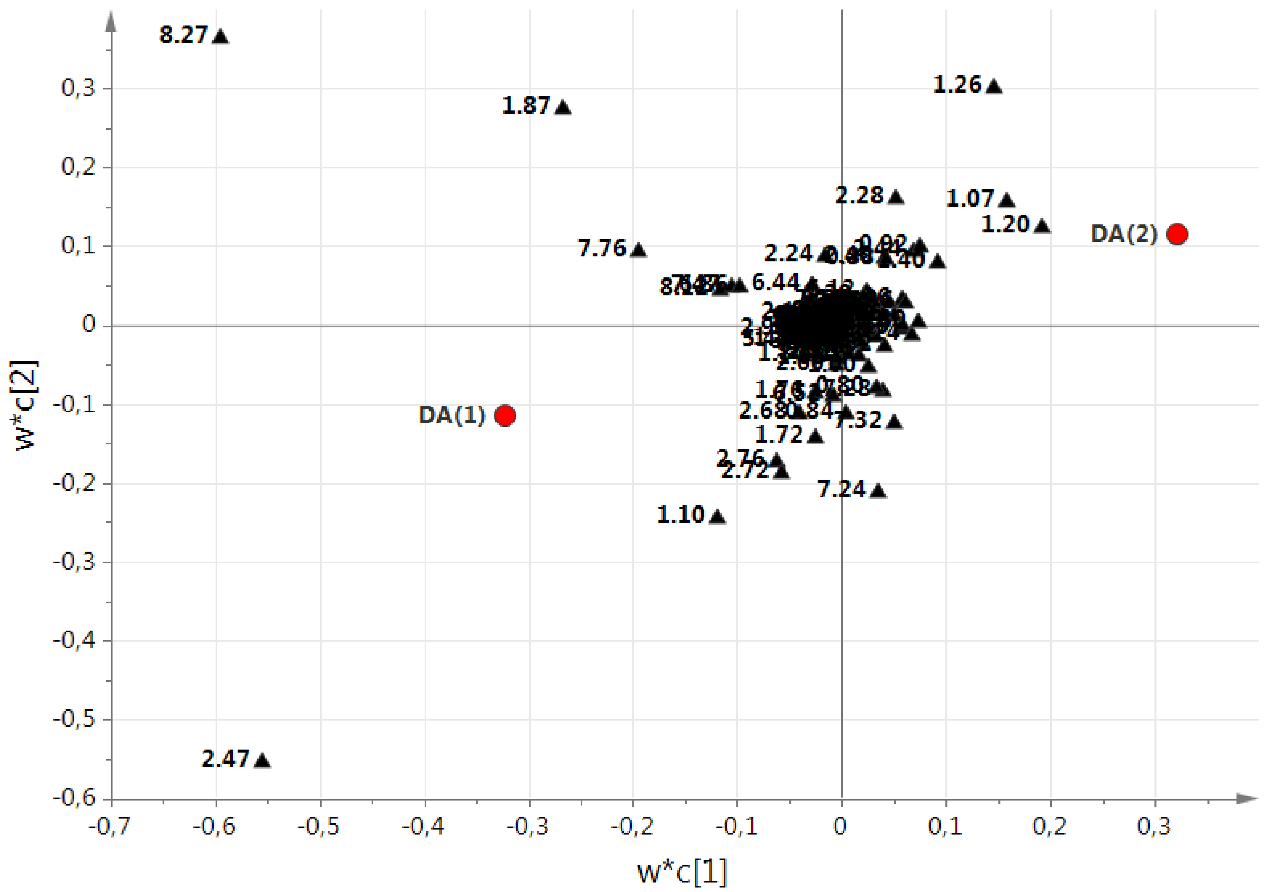


Figure 3A



0,3
0,2
0,1
0
-0,1
-0,2
-0,3
-0,4
-0,5
-0,6
-0,8
-0,6
-0,4
-0,2
0
0,2
0,4
0,6
w*c[1]
w*c[2]
A(1)
A(2)
2.92
2.47
2.80
2.84
1.26
8.27
1.10
2.28
1.84
1.16
1.87
1.76
1.20

Figure 3B



8.27
1.87
1.26
2.28
1.07
1.20
DA(2)
7.76
2.24
6.44
DA(1)
1.72
7.32
7.24
1.10
2.47
w*c[2]
w*c[1]
0,3
0,2
0,1
0
-0,1
-0,2
-0,3
-0,4
-0,5
-0,6
-0,7
-0,6
-0,5
-0,4
-0,3
-0,2
-0,1
0
0,1
0,2
0,3

Fig. 3C

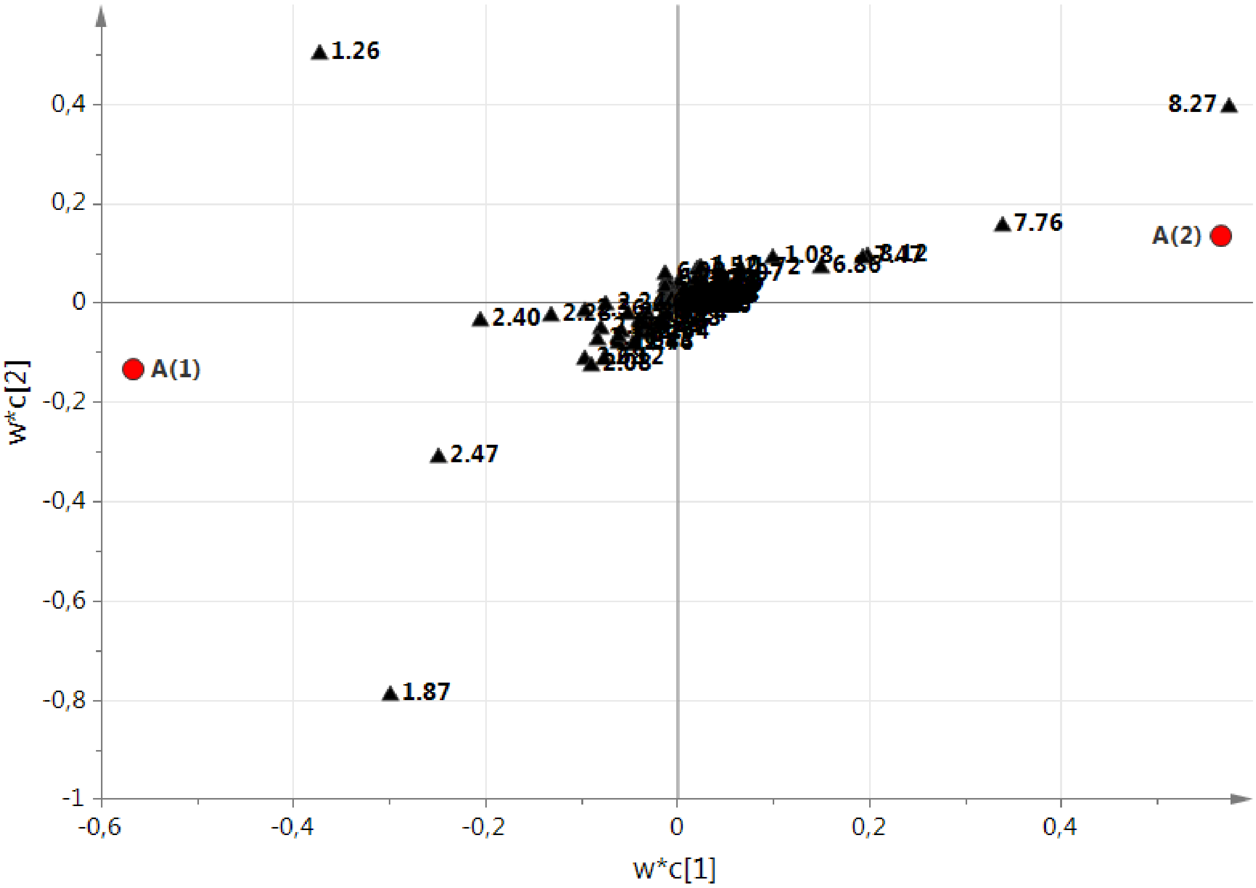

1.26
8.27
7.76
A(2)
1.08
A(1)
2.40
2.47
1.87
0,4
0,2
0
-0,2
-0,4
-0,6
-0,8
-1
-0,6
-0,4
-0,2
0
0,2
0,4
w*c[2]
w*c[1]

Table 1. Geographical origin of samples, beekeeping practice and the minimum durability date (MDD) reported on the labels. n.d. indicate “not declared” regional origin.

N°	BOTANICAL ORIGIN	BEEKEEPING PRACTICE	ITALIAN REGION	MDD
1	Acacia	Conventional	Calabria	2020
2	Acacia	Conventional	n.d.	2019
3	Acacia	Conventional	Piedmont	2018
4	Acacia	Conventional	Piedmont	2019
5	Acacia	Conventional	Piedmont	2019
6	Acacia	Conventional	n.d.	2019
7	Acacia	Conventional	Tuscany	2019
8	Acacia	Conventional	Tuscany	2019
9	Acacia	Conventional	n.d.	2018
10	Acacia	Organic	Lombardy	2018
11	Acacia	Organic	Piedmont	2018
12	Acacia	Organic	Piedmont	2018
13	Acacia	Organic	n.d.	2019
14	Acacia	Organic	Tuscany	2018
15	Acacia	Organic	n.d.	2019
16	Acacia	Organic	n.d.	2019
17	Acacia	Organic	n.d.	2020
18	Acacia	Organic	n.d.	2020
19	Acacia	Organic	n.d.	2020
20	Chestnut	Conventional	n.d.	2019
21	Chestnut	Conventional	Liguria	2018
22	Chestnut	Conventional	Piedmont	2018
23	Chestnut	Conventional	Piedmont	2018
24	Chestnut	Conventional	Sardinia	2019
25	Chestnut	Conventional	n.d.	2018
26	Chestnut	Conventional	n.d.	2019
27	Chestnut	Conventional	n.d.	2018
28	Chestnut	Conventional	n.d.	2019
29	Chestnut	Organic	Liguria	2018
30	Chestnut	Organic	Piedmont	2018
31	Chestnut	Organic	Piedmont	2018
32	Chestnut	Organic	Abruzzo /Piedmont/ Calabria	2019
33	Chestnut	Organic	n.d.	2019
34	Chestnut	Organic	n.d.	2019
35	Chestnut	Organic	n.d.	2019
36	Chestnut	Organic	n.d.	2019
37	Chestnut	Organic	n.d.	2020
38	Polyfloral	Conventional	Abruzzo	2018
39	Polyfloral	Conventional	n.d.	2019
40	Polyfloral	Conventional	Liguria	2018

41	Polyfloral	Conventional	Lombardy	2020
42	Polyfloral	Conventional	Piedmont	2018
43	Polyfloral	Conventional	Piedmont	2018
44	Polyfloral	Conventional	Sicily	2019
45	Polyfloral	Conventional	Tuscany	2019
46	Polyfloral	Conventional	n.d.	2018
47	Polyfloral	Conventional	n.d.	2019
48	Polyfloral	Organic	Piedmont	2018
49	Polyfloral	Organic	Piedmont	2019
50	Polyfloral	Organic	Abruzzo / Marche	2019
51	Polyfloral	Organic	Tuscany/Emilia Romagna	2020
52	Polyfloral	Organic	Tuscany	2018
53	Polyfloral	Organic	n.d.	2019
54	Polyfloral	Organic	n.d.	2019
55	Polyfloral	Organic	n.d.	2019
56	Polyfloral	Organic	n.d.	2020