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## MATERIAL AND METHOD

Honey is one of the most falsified food commodities in the world and for this reason, the development of new and reliable analytical approaches, able to differentiate among distinct honey origins and varieties, represents a continuous preoccupation among researchers and control laboratories. This because, honey is considered as a superfood due to its nutritional and medicinal properties directly related to the botanical origin of the raw material used by bees in the honey production.

Up to now, the acknowledged methods for honey authenticity control are: melissopalynology analysis (pollen profiles), which gives information about floral and geographical origin, together with physicochemical and organoleptic analysis while data information on honey aroma can be obtained by gas chromatography (volatile compounds) and liquid chromatography (flavonoids, amino acids and oligosaccharides). The state-of-the-art methods for detection of honey adulteration through the addition of C4 sugar are elemental analysis-isotope ratio mass spectrometry (EA-IRMS) and a combination of EA-IRMS and liquid chromatography coupled to isotope ratio mass spectrometry (EA/LC-IRMS).

A total of 101 authentic honey samples, originated from Romania and France, were collected during April – September, 2018; the harvesting period being directly related to the flowers blooming periods from the collection sites. The freshly harvested honey samples were stored in glass pots (180mL) containing around 250g of honey (depending on honeys densities). Romanian and French samples were collected directly from the producers, the geographical origin of these samples covering the most important melliferous areas of both countries. The distribution, according to the floral origin and country, was: i) Romania: acacia -18; linden - 9; honeydew - 6; colza - 6; sunflower - 6; coriander -1; yellow bedstraw -1; thyme -1; raspberry -1; amorpha -1 and ii) France: acacia -18; linden - 3; honeydew - 6; colza - 6; sunflower -5; lavender - 6; chestnut - 5; coriander -2.



## RESULTS AND DISCUSSION

Isotopic fingerprint of water ( $\delta^2\text{H}$  and  $\delta^{18}\text{O}$ ) from different food matrices represents one of the most effective markers for geographical origin recognition. The results for the entire set of honeys are grouped in Fig. 1. The global meteoric water line (GMWL) is also plotted on this figure; it represents the linear relationship between  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$ . It can be observed in Fig. 1 that for some honey samples the water isotope ratios are higher than the highest point provided in the GMWL official curve (2.5 ‰) indicating the impact of the drying step, performed by the bees, which create a water fractionation. Another observation that can be easily noticed is that most of the water honey values are under the GMWL line, a fact that could be the result of this evaporation process. Nonetheless, as all the measurements are located on a line nearly parallel to the GMWL one, it can be reasonably estimated that the fractionation which occur in the hives due to the naturally drying of the honey is independent of location. Also, the small deviations, specific for investigated honey samples, from GMWL are given by the climatic and meteorological particularities of each area from which honey samples were collected and which are directly related to the local meteoric water line (LMWL). The obtained isotopic differences only result from the initial isotopic composition of the water absorbed by the plants. As expected, differences in terms of these two isotope values between French and Romanian honeys, due to the different geo-climatic conditions, which prevail in the two countries were observed (Fig. 1). A higher variability of honey isotopic composition was obtained for French samples as compared with those from Romania. This can be due either to a larger variability of geographical regions from which French samples were collected or to a longer harvesting period which was covered by the French honeys, which conducted to a larger meteorological variation. It appears from Fig. 1 that, even a clear discrimination is observable among the average values of Romanian and French honeys, there are some overlap between the samples originated from the two countries.

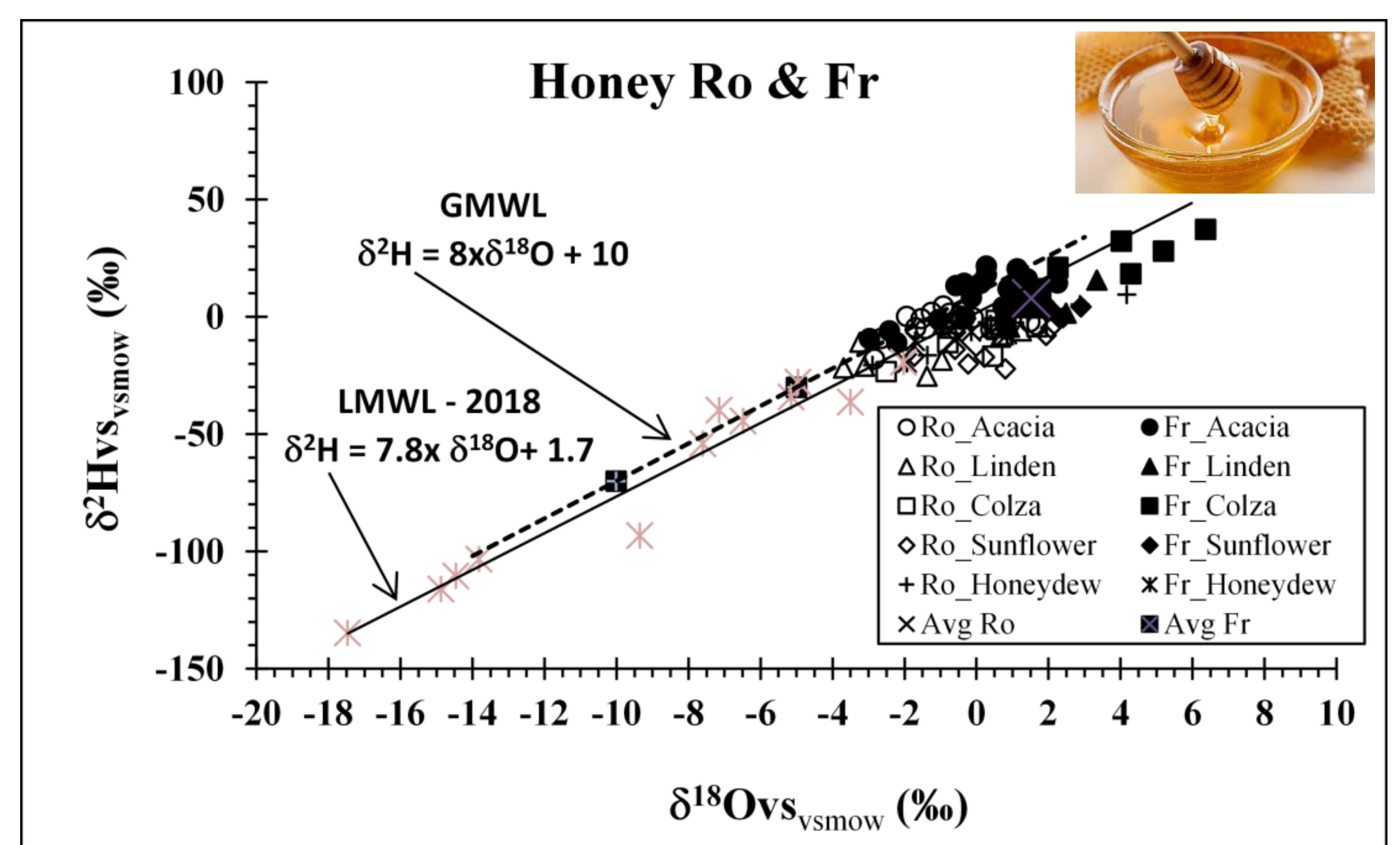


Fig.1. Distribution of  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values for Romanian and French honey samples (all samples).

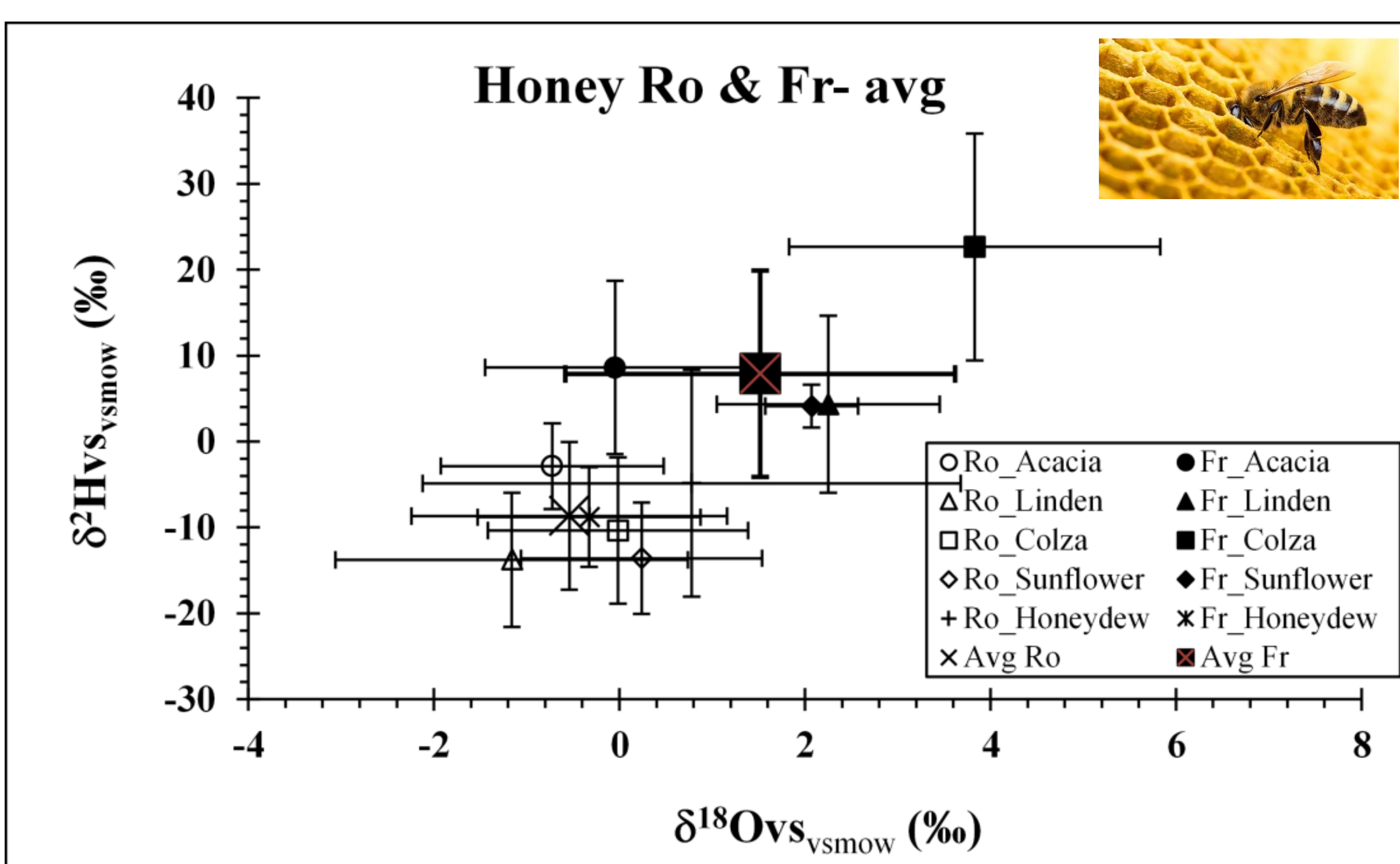


Fig.2. Differentiation between distinct varieties of Romanian and French honeys based on their  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  mean values for each honey type.

In order to go deeper in the classification, the individual positioning of mono-varietal honey is presented in Fig. 2. Only mono-varietal honeys common to the set of Romanian and French honeys and with a sufficient number of samples along with honeydew honeys are presented in Fig. 2. It is observable that considering the floral origin, neat distinction is observable among the two countries illustrating the efficiency of  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  for geographical discrimination.



## CONCLUSION

A new approach for honey authentication with respect to geographical and botanical origin was successfully proposed and tested in this work. For the development of these models, 7 mono-varietal honey samples coming from two countries, Romania and France were used. The applicability of some emerging markers like:  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  of the water extracted from honey, in the development of new efficient recognition models were proved.

Interesting isotopic signature was found for two mono-floral honeys which present a global interest: colza and sunflower honeys. These two plants are cultivated all over the two countries. As shown on Figure 3, the average values for Romanian colza and sunflower honeys are very close and have a similar dispersion, -10.4 and -13.6‰ for  $\delta^2\text{H}$ , 0.0 and 0.2‰ for  $\delta^{18}\text{O}$  respectively for colza and sunflower honeys. This observation can be explained by the sampling area as most of the two types of honeys come from the same geographical areas of Romania. Thus, a similarity in the values can be expected confirming also the lack of power of these two isotopes in discriminating among floral origin. On the opposite, French colza and sunflower honeys present distinct isotopic values with a very different dispersion, 22.7 and 4.1‰ for  $\delta^2\text{H}$ , 3.8 and 2.1‰ for  $\delta^{18}\text{O}$  respectively for colza and sunflower honeys.

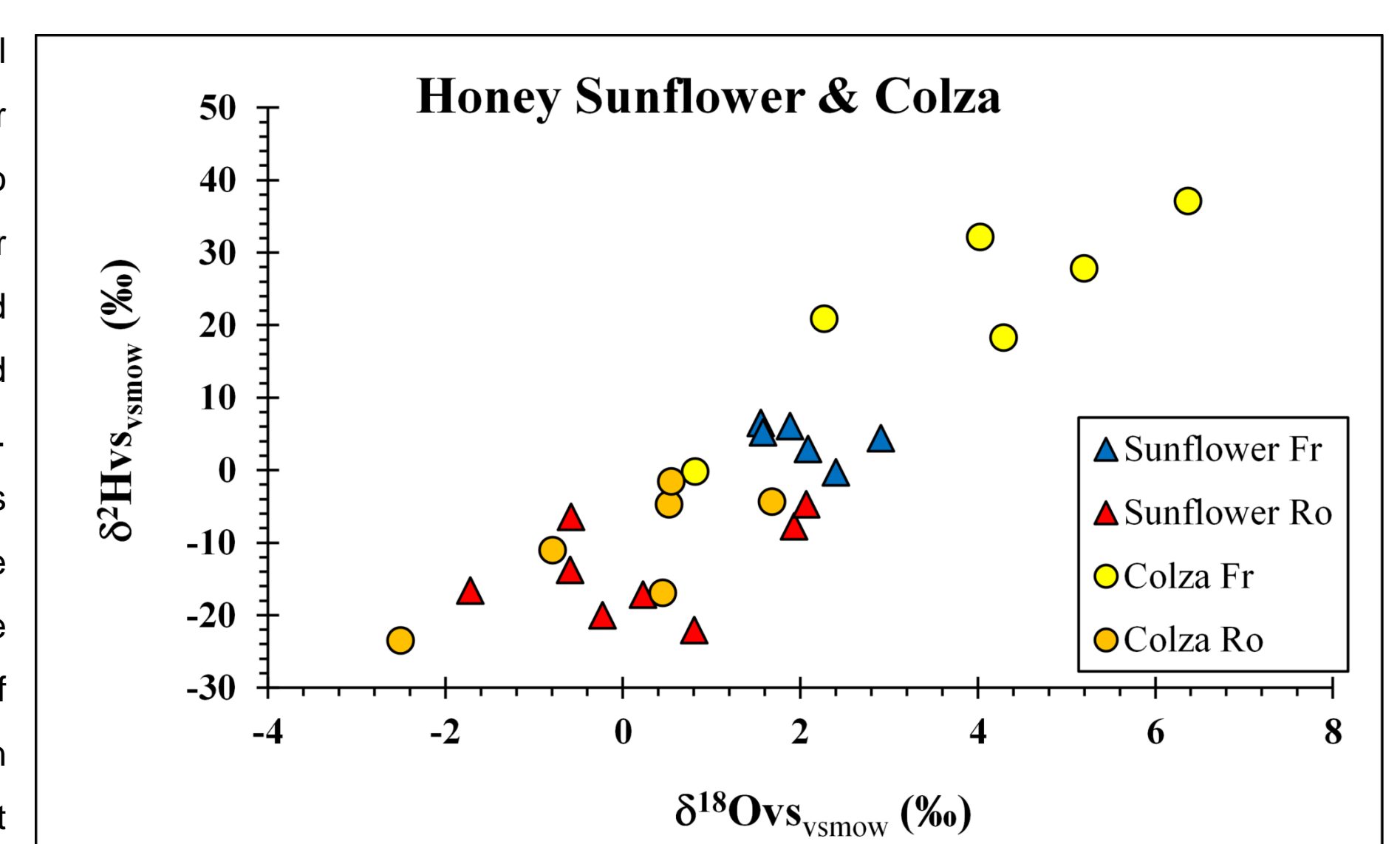


Fig.3

The sunflower honey isotopic ratios are lower than the ones of colza and less dispersed. The dispersion can be explained by the production localization that is concentrated in the south west of France while for colza the production is spread all over the half north of France and as a result, the climatic difference is higher for colza than for sunflower honeys.

The linden variety was characterized by mean values of  $\delta^2\text{H} = -13.8$  ‰ and  $\delta^{18}\text{O} = -1.2$  ‰ for Romanian honeys and  $\delta^2\text{H} = 4.3$  ‰ and  $\delta^{18}\text{O} = 2.3$  ‰ for French samples, respectively. Another interesting feature is given by the result of honeydew honeys, where the difference among the average  $\delta^{18}\text{O}$  of Romanian and French honeydew honeys is 1.5‰ which is relatively low as compared to the investigated mono-floral honeys. Thus,  $2\text{H}/1\text{H}$  and  $18\text{O}/16\text{O}$  isotope ratios showed their potential in discriminating among Romanian and French geographical origin of honey as it is observable on Figs. 1 and 2.

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