Nectar measurement with refractometer and microcapillaries

Refractometer measures the content of solved substances (for EFN mainly mono- and disaccharides) through BRIX, scale is in weight percent

Capillary provides data on volume in µl:

1 L water = 1 kg
1 ml water = 1 g
1 µl water = 1 mg

- ⇨ a BRIX of 10 means 10 % of the weight are sugars,
- ⇨ given a volume of 1 µl this means: 1 mg * 10 / 100 = 0.10 mg

Example calculation for three measurements, which are pooled:
((BRIX [%] * Vol [µl]) + (BRIX [%] * Vol [µl]) +(BRIX [%] * Vol [µl])) / 100 = Total amount of sugars [mg]

Excel spreadsheet (example)

	A	B	C	D	E	F	G	H	I
1	Plant	Leaf	BRIX1	Vol 1	BRIX2	Vol 2	BRIX3	Vol 3	Sugar
2									*
3									
4									
5									

* enter here: = (c2*d2+e2*f2+g2*h2)/100

In case the values are really small, multiply with 1000 and provide data in µg

!!: As sucrose (disaccharide) has the same weight as 1 glucose + 1 fructose, the weight percent data are suitable to provide information on mol monosaccharides, which result from potentially complete hydrolysis of existing di- or trisaccharides

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As the combined measuremts of volume and concentration provides data on the total amount of dissolved sugars, you can add water to the nectaries to facilitate collection – if necessary. This is how it works: Place ca. 5 µl water on a nectary using a micropipette to resolve and harvest the EFN. Skilled "EFN-collectors" press the water droplet not completely out of the pipette but move it back and forth. This dissolves the sugar in the EFN efficiently.
In case the plant produces only little amounts of EFN per leaf or nectary this way can combine the EFN from several nectaries in the same water droplet.
Once you have collected the EFN, place the droplet on the screen of the refractometer (calibrate using pure water!!). Close the refractometer and read/note the BRIX value. Then remove the EFN with the microcapillary from the refractometer (screen AND lid) and determine the volume. Write down both values in a way to make sure that they (a) belong together (thus, represent the same portion EFN) and (b) that you later can identify which value is the BRIX and which is the volume. If the concentration of the EFN was high, repeat the process as discribed above. „High" however is relative – but you should always determine a defined BRIX and then sample the same nectary until this you have values below this cutoff. For acacias use 1 as cutoff, for lima bean 0.5 or lower.

Reference

Ballhorn D.J., Kay J.* & Kautz S. (2014). Quantitative effects of leaf area removal on indirect defense of lima bean (Phaseolus lunatus) in nature. J Chem Ecol. 40:294-296.