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Sample preservation for determination of organic compounds: microwave versus freeze-drying

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Abstract

In search of a reliable drying method, which might be used even under field conditions, microwave drying was compared to freeze-drying of plant material. Leaves of *Ananas comosus* and *Avicennia germinans* as well as buds and phloem of *Acer pseudoplatanus* were used and checked for one or more of the following substances: sugars, sugar alcohols, organic and amino acids, total nitrogen, and glycinebetaine.

With most samples good agreement was achieved between the two drying methods. Only in the case of the *Ananas comosus* leaves, which exhibited low pH and high water content, did appreciable differences occur in organic and amino acids. Besides that, sucrose was the compound most susceptible to alterations, which was especially evident when leaves of *Sambucus nigra* were dried in the two different compartments (condenser compartment, drying bell jar) of the freeze-dryer in use.

For *Ananas comosus* leaf samples it was shown that microwaving can also be used prior to extraction of tissue sap.

Key words: Microwave, freeze-drying, drying method, tissue sap, organic solutes.

Introduction

Freeze-drying is regarded as the most gentle method to preserve plant material for analyses of various organic substances. However, this procedure is either time-consuming or expensive depending on the apparatus used. Moreover, neither the machinery nor the necessary supplies (liquid nitrogen, dry ice) are easily available in remote places. Therefore, an alternative method was sought that would allow drying of reasonable amounts of plant material in a short time in the field.

Reports in the literature (Walsh *et al.*, 1989; Paparozzi and McCallister, 1988; Tiedemann *et al.*, 1984) and suggestions from Professor CA Atkins (University of Western Australia) indicated that drying plant material in a simple commercially available microwave oven should be tested. Power supply by a portable generator suffices to run such equipment and microwaves are known to cause rapid denaturation of enzymes by the heat produced during oscillation of dipolar molecules.Therefore, microwave and freeze-drying was compared for several kinds of substances (sugars, polyols, organic acids, amino acids) in quite different plant materials ranging from succulent CAM plants to the different organs of trees.

Material and methods

Plants of *Ananas comosus* (L.) Merill var. Española Roja and *Avicennia germinans* (L.) Stearn were grown in the glasshouse, samples of *Acer pseudoplatanus* L. and *Sambucus nigra* L. were collected in the field. Phloem material of *Acer pseudoplatanus* was produced by removing the dead parts of the bark and punching out little cylinders with a cork borer. In all cases the plant material was cut into little pieces by the use of razor blades to produce a bulk of homogenous material. After determining the fresh weight, samples were put immediately into either the microwave or the freeze-dryer.

Drying procedure

Plant material was dried in a domestic microwave (Bosch 571, Stuttgart) at a power of approximately 600 W. Depending on

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the type of material (water content, structure) drying took 15–25 min. To prevent damage of the material (turning into charcoal) it is essential always to have a beaker with water inside the instrument. This is not necessary if the microwave is used only for a short 'killing' of the material as described below for tissue sap preparation.

Samples for freeze-drying were immediately exposed to −52 °C in the condenser compartment of a CHRIST Alpha 1–4 freeze-dryer. After freezing, one lot of samples of *Sambucus nigra* was moved to the drying bell jar. All other samples were lyophilized in the condenser compartment.

Tissue sap preparation

Parallel samples to those exposed to drying were put into tightly shutting Eppendorf vials and either put into a deep-freezer following the method of Smith and Lüttge (1985) or exposed in the microwave for 3× 10 s. Between treatments vials were turned around and checked for their temperature. When the vials had cooled down a hole was made in the bottom, the vials fitted into centrifuge tubes and centrifuged for 15 min at $3000 \times g$.

Analytical procedures

Sugars and polyols: The compounds of the neutral fraction were analysed as their trimethylsilyl ethers by GC. Hot water extracts were fractionated by passage over bonded silica columns (Sepralyte SCX and NH_2). Derivatization and instrument conditions followed Richter *et al.* (1990)

Organic acids and phosphate: Organic acids were eluted from the NH_2- anion exchange column with 1 N HCl. After removing the eluent on a rotary evaporator samples were taken up in a defined volume of aqua dest. and aliquots again taken to dryness. Organic acids were converted to their trimethylsilyl derivatives using *N*-trimethylsilyl-*N*-methyl-trifluoroacetamide in dry pyridine containing biphenyl as an internal standard. Analyses were performed on a Varian 3600 gas chromatograph equipped with a flame ionization detector. The glass column (2 m length, 2 mm inner diameter) was packed with 3% OV 225 on Chromosorb W-HP, 100–200 mesh. A temperature programme was used from 100–230 °C with an increase of 8° min^{-1}.

Test sample: In order to get an estimate for the coefficient of variation (V, standard deviation expressed in percentage of the mean) introduced by the extraction and processing of samples for the two GC methods one and the same powder of *Ananas comosus* leaves was used for 5 repeats of sugar and organic acid analysis. V of the test sample encompassed the variation caused by hot water extraction, ion exchange and GC analysis.

Amino acids: Amino acids were analysed by HPLC using the pre-column derivatization method with phenylisothiocyanate (Bidlingmeyer *et al.*, 1984; Davey and Ersser, 1990).

Glycinebetaine: Determination of quaternary ammonium compounds followed Gorham (1984) using cation-exchange HPLC.

Total nitrogen: Total nitrogen was measured with an automatic Heraeus CN-analyser.

Results

In case of the CAM plant *Ananas comosus* morning samples of high organic content (low pH) were used to compare the two drying methods as well as two different preparations of tissue sap. Interestingly, differences between treatments occurred even in the case of phosphate, which as an inorganic ion was not thought to be prone to changes (Table 1). However, for all compounds determined in the anion fraction microwave drying led to higher values than freeze-drying (Table 1). In the tissue sap preparations only malate differed between the two treatments, but both of these two values were closer to microwaved than to the freeze-dried samples. The same was true for citrate, where (except the microwave sap treatment) the coefficient of variation was higher than for the test sample.

While glucose and fructose agreed quite well in microwaved (drying as well as sap) and freeze-dried samples, the saps produced by freeze-thawing showed significantly higher values (Table 2). On the other side this treatment produced the lowest data for sucrose. Moreover, the three other methods showed the highest variation in this compound, which was also reflected by the difference in the variation between treatments and the test sample (Table 2). As can be seen from the calculation in the last column of Table 2 the differences are not simply explained by hydrolysis of sucrose to hexoses. The number of hexose molecules stemming from glucose, fructose and sucrose was highest in the freeze-thawed sap and lowest in the freeze-dried samples (Table 2).

Due to their low pH and high water content the leaf

Table 1. *Organic acid and phosphate concentrations in morning leaf samples of* Ananas comosus *either dried by microwave or freeze-drying or prepared into tissue saps after microwaving or freeze-thawing*

In the case of the 'test sample' one and the same powder of *A. comosus* leaves was analysed 5 times the same way as the samples. Values represent means ±SD of five replicates. The coefficients of variation (in %) are indicated in parentheses.

Method	Phosphate (mol m^{-3} plant water)	Malate (mol m^{-3} plant water)	Citrate (mol m^{-3} plant water)
Microwave	16.39±1.53 (9.3)	53.19±1.72 (3.2)	30.27±1.93 (6.4)
Freeze drying	11.28±0.79 (7.0)	37.52±1.43 (3.8)	19.85±0.87 (4.4)
Microwave sap	11.48±0.90 (7.8)	49.05±2.45 (5.0)	25.31±0.82 (3.2)
Freeze-thawing sap	13.80±1.67 (12.1)	57.82±2.84 (4.9)	28.12±2.12 (7.5)
Test sample	n.d.	58.27±2.61 (4.5)	160.41±5.34 (3.3)

n.d., Not determined.

Table 2. *Sugar concentrations in morning leaf samples of* Ananas comosus *either dried by microwave or freeze-drying or prepared into tissue saps after microwaving or freeze-thawing*

In the case of the 'test sample' one and the same powder of *A. comosus* leaves was analysed 5 times the same way as the samples. Values represent means ±SD of five replicates. The coefficients of variation (in %) are indicated in parentheses.

Method	Glucose (mol m^{-3} plant water)	Fructose (mol m^{-3} plant water)	Sucrose (mol m^{-3} plant water)	Σ Glc, Frc, 2xSuc
Microwave	49.26±4.65 (9.4)	67.68±2.65 (3.9)	10.89±1.27 (11.7)	140.01±4.61 (3.3)
Freeze drying	44 69±3.21 (7.2)	66.43±1.18 (1.8)	7.58±0.53 (7.0)	124.98±4.48 (3.6)
Microwave sap	44.03±2.81 (6.4)	67.74±6.40 (9 4)	19.65±2.55 (13.0)	151.07±14.08 (9.3)
Freeze-thawing sap	72.61±4.05 (5.6)	100.14±8.71 (8.7)	3.45±0.38 (11.0)	179.65±12.15 (6.8)
Test sample	133.15±9.15 (6.9)	113.78±3.01 (5.3)	87.47±2.77 (3.2)	421.88±11.72 (2.8)

samples of *Ananas comosus* were for sure the most problematic samples dealt with. There were no such pronounced differences in sucrose content in the phloem and buds of *Acer pseudoplatanus* dried by microwave or freeze-drying (Table 3). In the case of the phloem higher values were obtained in microwaved samples than in freeze-dried ones, while the situation was vice versa in the buds (Table 3). Quebrachitol content agreed quite well, which demonstrated that cyclitols are very stable compounds, whereas sucrose is much more liable to break down.

This also became evident when leaves of *Sambucus nigra* were dried in the two different compartments available in the freeze dryer used(Table 4), where the temperature at the condenser part was about −52 °C, while the drying bell jar was exposed to room temperature. There was no temperature indication for this part of the instruments, but certainly the temperature for the samples was higher there than −52 °C.

This higher temperature during the drying process decreased sucrose content and enhanced glucose and fructose compared to the samples dried at −52 °C (Table 4). The increase in glucose and fructose was less than what would have been equivalent to the reduction in sucrose of the samples dried in the drying bell jar (Table 4). Nevertheless, the coefficient of variation in the latter samples was much lower than in those dried in the condenser compartment (Table 4).

In the case of the amino acids the two plant materials under investigation gave a quite different picture. In buds of *Acer pseudoplatanus* the different drying methods led to very similar values for all amino acids and ASN, the (overall high) SD was always higher for the microwaved samples (except for lysine, Table 5). In *Ananas comosus* leaf samples ASP, GLU and ASN differed to a high extent, while ALA agreed quite well. In this set of samples SD was lower in the microwaved ones. Total nitrogen content was not affected by the drying procedure (Table 5).

Good agreement between the drying methods was also achieved for glycinebetaine content in leaf samples of *Avicennia marina* (Table 6).

Discussion

In most of the cases presented here the results of the two drying methods agreed very well: glycinebetaine in *Avicennia germinans* leaves (Table 6), amino acids and neutral fraction in *Acer pseudoplatanus* buds (Tables 5,

Table 3. *Quebrachitol and sucrose content in phloem and buds of* Acer pseudoplatanus *dried by microwave or freeze-drying*

Values represent means ±SD of five replicates. The coefficients of variation (in %) are indicated in parentheses.

Method	Quebrachitol (mmol kg^{-1} dry matter)		Sucrose (mmol kg^{-1} dry matter)	
	Phloem	Buds	Phloem	Buds
Microwave	14.68±1.12 (7.6)	116.28±12.79 (11.0)	47.88±1.61 (3.3)	50.98±5 79 (11.4)
Freeze-drying	11.98±1.19 (9.9)	119.76±14.31 (12.0)	36.90±6.87 (18.6)	57.76±5.19 (9.0)

Table 4. *Sugar content in leaf samples of* Sambucus nigra *dried either in the condenser compartment (-52°C) or the drying bell jar of a CHRIST Alpha 1–4 freeze-dryer*

Values represent means ±SD of five replicates. The coefficients of variation (in %) are indicated in parentheses.

Method	Glucose (mmol kg^{-1} dry matter)	Fructose (mmol kg^{-1} dry matter)	Sucrose (mmol kg^{-1} dry matter)	ΣGlc, Frc, 2 × Suc
Condenser compartment (−52 °C)	108.19±24.88 (23.0)	190.23±39.06 (20.5)	303.88±51.02 (16.8)	906.18±163.99 (18.1)
Drying bell jar	158.67±10.96 (6.9)	227.64±12.67 (5.6)	222.09±19.45(8.8)	866.46±175.88 (8.8)

Table 5. *Amino acid content of buds of* Acer pseudoplatanus *and* Ananas comosus *leaf samples and total nitrogen of* A. comosus *leaf samples dried by microwave or freeze-drying (mean ±SD, n=5)*

Method	ASP	GLU	SER	ASN	GABA	ALA	PRO	LYS	Total N
				(mmol kg^{-1} dry matter)					(% dry matter)
Acer pseudoplatanus									
Microwave	2.25±0.91	2.35±1.02	5.95±1.90	15.74±4.22	9.05±2.53	4.58±1.63	11.64±3.15	3.06±0 40	n.d.
Freeze-drying	2.16±0.63	2.91±0.81	6 11±0.96	15 90±1.58	7.54±0.53	4.78±0.70	10.90±1 65	3.06±0.51	n.d.
Ananas commosus									
Microwave	2 56±0.33	1 50±0.09		9.57±2.03		1.49±0.13			1.27±0.01
Freeze-drying	4.25±0.68	4.75±1.04		17.06±2 46		1.84±0.34			1.25±0.03

n.d., Not determined.

Table 6. *Glycinebetaine content in leaf samples of* Avicennia germinans *dried by microwave or freeze-drying.*

Values represent means ±SD of five replicates. The coefficients of variation (in %) are indicated in parantheses.

Method	Glycinebetaine (mmol kg^{-1} dry matter)
Microwave	259.4±19.2 (7.4)
Freeze-drying	265.2±20.4 7.8)

3), glucose and fructose in leaf samples of *Ananas comosus* (Table 2) and quebrachitol in *Acer pseudoplatanus* phloem (Table 3). The slightly higher values of sucrose in the microwaved samples of *Acer pseudoplatanus* phloem and *Ananas comosus* leaves were in accordance with the findings of Smart *et al.* (1994), who also found higher sucrose contents in microwaved samples compared to freeze-dried ones in wheat. As pointed out above, the difference in sucrose did not make up for an equivalent increase in glucose and fructose (Tables 2, 4). Thus other processes than hydrolysis of sucrose had been involved. The variability may be due to the fact that enzymes in the samples are inactivated in the microwave, but not in the freeze-dryer. Thus, apart of the drying process differences may occur during the storage, handling and processing of the samples afterwards (Hendrix and Peelen, 1987). This might also explain why the highest amounts of sucrose in *Ananas comosus* leaf samples were found in the microwaved tissue saps. This way of preparation took the shortest time.

However, in the same set of samples the microwave-dried material agreed very well with the two tissue sap preparations in respect to organic acids, but differed substantially from the freeze-dried ones (Table 1), which were much lower. In the case of sucrose one is inclined to assume that the higher values are those which more reflect the *in vivo* situation, since sucrose is very susceptible to decay and hydrolysis; on the other hand breakdown of starch would result in maltose. This is not so clear for organic acids, which are exchanged with various other metabolic pools. An increase could be explained by a deamination of amides, whereas a decrease might be caused by enhanced respiration. In the *Ananas comosus* samples under investigation the difference in the organic acids (Table 1) was even higher than that for the amino acids (Table 5) between microwave- and freeze-dried samples.

However, for the amino acids as well, it is difficult to decide which are the values more in accordance with the original situation in the plant material. On the one hand it is feasible that amino acids are deaminated by the drying procedure and thus measured concentrations are decreased. On the other hand protein breakdown might take place, since most of the catabolic enzymes are known to be very stable and in many cases favoured by low pH. This latter fact might explain that differences in amino acid concentration occurred especially in the *Ananas comosus* samples of low pH and not in the bud samples of *Acer pseudoplatanus*.

However, total nitrogen agreed well in the pineapple leaf samples, whereas a major loss of amino acids should have influenced those numbers (Table 5). Our findings in respect to total nitrogen were also in accordance with a recent report on rice leaves, where microwave drying was recommended for a quick estimation of nitrogen by a micro-Kjeldahl procedure (Peng *et al.*, 1994).

It may be questioned if freeze-drying is really the 'absolute reference' for drying procedures and if either higher or lower quantities of a certain kind of low molecular weight compounds are those closer to the real situation. For instance, samples of *Nicotiana tabacum* contained higher amounts of alkaloids in the microwaved samples than in the freeze-dried ones (Jaquin-Dubrenil *et al.*, 1989).

Another essential finding in the latter paper is that no qualitative changes were introduced by the two different drying methods. This also holds true for all of our investigations. Earlier reports in the literature mentioned that amino acids may be changed from their L- into the D-form by microwave treatment. Since our and several other common procedures for amino acid determination do not distinguish between D- and L- forms of amino acids this can be regarded of minor importance. Of course it is of consequence for the nutritional value, however, a recent paper has shown that at least for milk microwave

heating did not cause formation of D-amino-acids (Zagon *et al.*, 1994).

In conclusion it may be stated that microwave drying presents a good way to preserve plant material for analysis of organic compounds. 'Problems', that means significant differences between microwave and freeze-dried samples, occurred only in the case of the morning samples of *Ananas comosus*, which were the most problematic ones because of their low pH and high water content. Our future research will focus on the reasons for the differences in the results of the two drying methods in this case. However, as long as the plant material to be investigated is of normal water content and moderate pH there is no doubt that microwave drying is a useful alternative to freeze-drying.

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