A SIMPLE FREEZE-DRYING TECHNIQUE TO PRESERVE MACROFUNGI

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Introduction

At the Western Australian Museum where I work, I have been working on a simple, inexpensive method to preserve macrofungi in a way that retains their life-like form, and which I am confident can be carried out in a laboratory or even in the home as long as care is taken. This involves the use of a freezer, silica gel beads, and (optionally) a bath of petroleum wax that has been heated to around 130°C.

Freeze-drying units used by museums and industry are very expensive; however, the use of silica gel presents an extremely cheap alternative. Silica gel is often used to dry flowers at room temperature, although web searching revealed nothing about using gel with macrofungi or with any specimens under freezer conditions. I should acknowledge the preparator staff from Museum Victoria who described to me how to use heated wax to impregnate fungi that were freeze-dried conventionally. I had seen some excellent specimens on display when I visited in May 2003.

Materials

- Freezer space. Note: the freezer used is around -32°C, which is colder than the average home freezer of -20 to -16°C.
- Silica gel beads (see the note on page 32 for further details).
- Foam or wood bases with pre-drilled holes to hold fungi upright.
- Fine wire of various gauges, cut into short lengths, and pliers.
- Containers. These must be dry with a good seal to reduce external moisture contaminating the gel.

Optional: for wax impregnation.

- Microcrystalline wax and a saucepan. Paraffin wax (candle wax) for adding to microcrystalline wax (see the note on page 31 for further details).
- Battery operated probe thermometer capable of reading up to 150°C.

Methods

Freeze-drying specimens

Start by using small, robust fungi in good condition. Do not try delicate ones, e.g. *Mycena* spp., *Panaeolus* spp. and *Coprinus* spp. Collect fungi carefully so they are not bruised or otherwise damaged. Set up fresh specimens (preferably in the field) by carefully pushing a fine wire or pin up the stem and securing the end of the wire into a foam or wooden base with pre-drilled holes. Seal in a container, making sure the fungi are not in contact with the sides or each other. Take notes and photographs, and additional herbaria specimens if required. Transport carefully and, as soon as possible, place the container in the freezer. It may be possible to collect fungi with the substrate on which they are growing, although this increases the quantity of moisture that needs to be removed.

The silica gel needs freezing too. The brand used has a blue indicator that changes to pink as moisture is absorbed. For reuse it can be dried out either in a microwave set on high for 20 seconds or in a saucepan on the stove, stirring and reheating as needed. Care should be taken not to breathe dust from the gel, particularly if using a chipped gel. Test for moisture by placing a clear lid over the gel—if any steam condenses then reheat again. When completely dehydrated, immediately seal in a container (for containers with a poor seal, place inside a sealable plastic bag). When the gel has

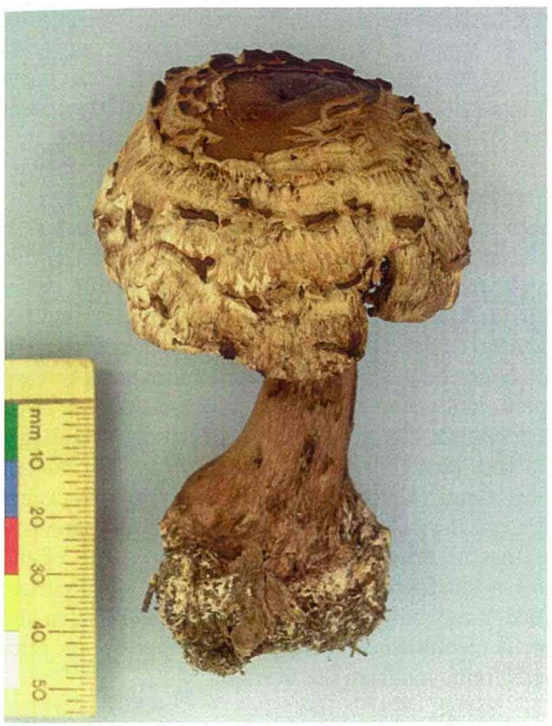

Figure 1. *Lepiota* sp. (parasol mushroom)—not wax dipped.

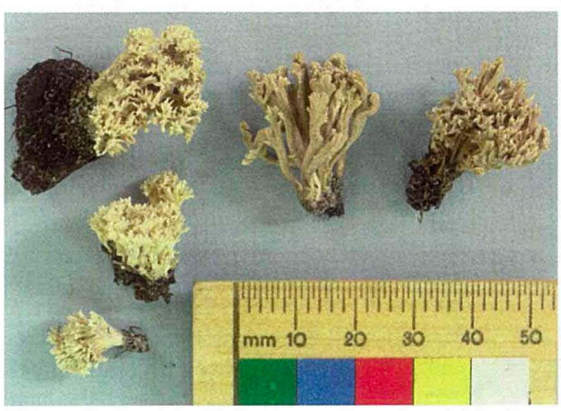

Figure 2. Coral fungi—not wax dipped.

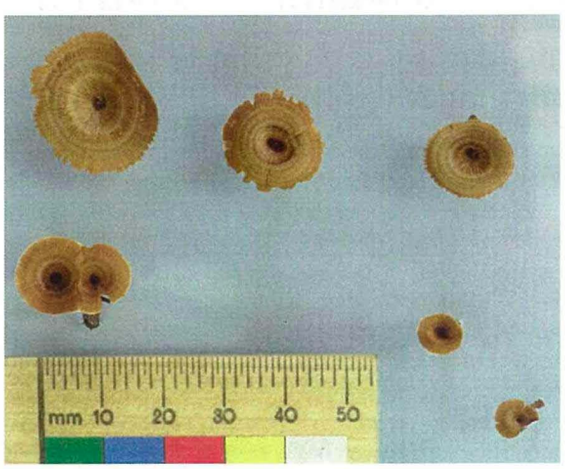

Figure 3. *Coltricia cinnamomea*—not wax dipped.

Figure 4. *Laccaria* sp.—wax dipped 130-135˚C.

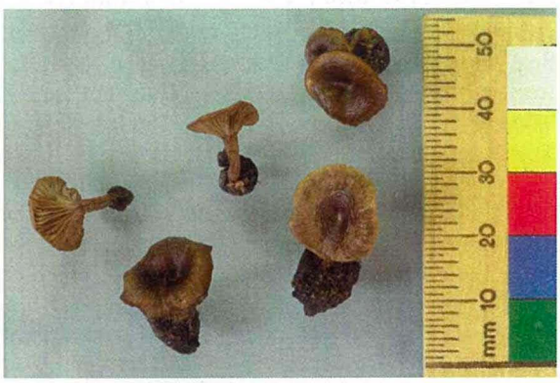

Figure 5. *Omphalina* sp. (brown belly buttons)—wax dipped, 130–136˚C.

Figure 6. *Crucibulum* sp. (bird's nest fungus)—not wax dipped.

cooled, place the container in the freezer.

Once the specimens and gel are fully frozen—it is best to allow 24 hours—the gel can be poured around the fungi so they are totally surrounded. This allows the gradual transfer of moisture from the fungi to the gel. The end result should be totally dry fungi, in the shape and form they were in when growing. Because the fungi are frozen, they are not prone to damage on contact with the gel. Gel should

not be poured onto fresh fungi, unless they are by nature very hard specimens. It is recommended specimens are numbered and labelled, and records taken of methods for future reference.

Remove the frozen specimens and gel from the freezer, but keep the door open and stay within the cold air as close as possible. It is necessary to work very quickly because defrosting will increase the likelihood of the fungi being marked or damaged by the gel. Everything should be at hand and one specimen or container should be done at a time. Chest freezers are probably better than refrigerator freezer compartments, being larger and easier to use, and because it is possible to work over the open lid in the cold. A walk-in freezer would be ideal. Carefully pour the gel around the fungi until they are covered. Seal and immediately return to the freezer. Seal excess gel and refreeze for later use. The freezer used was set at -32°C, so I am not sure if using a standard freezer (-20 to -16°C) will give satisfactory results.

Check the following day or when convenient. The gel immediately surrounding the fungi should be pink. Pour this off and replace it with frozen blue gel. If there is no fresh gel to hand or there are few pink beads then this can be mixed and poured back around the fungi. Repeat this procedure daily, or when convenient. Small fungi should be fully dry within a few changes. Specimens can be left for longer periods before being removed from the gel; however, it is not known whether over-drying will cause brittleness. Excessive humidity, especially when accompanied by heat, such as in the tropical north, may present problems for dried fungi that are not subsequently treated with wax. While Perth has a humid climate at times, the dried and wax-treated specimens have been kept in pest-free air-conditioned environments.

It can be useful to include a small piece of frozen damp sponge as a moisture indicator—thus warming the sponge will indicate the level in the sponge and hence the specimens. A one gram piece of sponge dampened with three grams of water dries out in around seven days as long as the gel is replaced or mixed two or three times.

Contamination of the gel with spores is likely, and specimens kept for microscopic work that have been dried in re-used gel should be noted.

If the dried fungi are not to be wax-impregnated, they may be vulnerable to damage by pests. An alternative to using chemicals is periodic freezing for around six days.

SAFETY NOTE

Silica gel chips are not recommended, as harmful silica dust may be released. While beads appear to be dust free, care should be taken. Wear a dust mask if in doubt, or if available use a laminar flow unit (e.g. when drying gel for reuse).

Wax impregnation

This involves quickly dipping dried specimens in microcrystalline wax melted at 130°C ± 5°C. This treatment will preserve fungi for many years provided they are not handled or exposed to excessive heat. On the other hand, treated fungi will likely not be available for microscopic work or easy spore collection. The brief exposure to high temperature might also cause microscopic damage. Another change is in the colour. Wax will darken most fungi (although in some cases, freeze-drying lightens the colour of specimens) and so there will be loss of brighter colours. However, some of the brown mushrooms will look quite natural. Because dried fungi may reabsorb some moisture from the air; it recommended that they be dipped soon after being removed from the freezer.

The temperature of the wax, at around 130°C, is important. A probe type thermometer can be secured in place with a wire cradle. If the wax is too hot, the fungi will sizzle. Wax that is too cool will not soak in sufficiently leaving an obvious coating on the surface or between the gills.

The wax tests carried out were of two mixes:

a) 60% soft microcrystalline (*Mobilwax 2305* - superceded by *Mobilwax 2405*) * : 40% macrocrystalline wax (paraffin or candle wax) **.
b) 80% soft (*Mobilwax 2305*) : 20% hard microcrystalline wax (*Mobilwax 190Y* – see end of report for further details). The soft wax is translucent pale yellow, the hard wax pale beige, and the paraffin wax is translucent white.

The wax needs to be kept stirred to maintain a reasonably even temperature. Securely hold the stem's support wire with forceps and hold the specimen above the surface of the wax to warm it before dipping. Dip for one to three seconds. Immediately drain excess wax on a tissue by gently touching and dabbing. This will reduce any accumulation on the surface and between the gills, etc. It will be best to have several specimens, to allow experimentation with slight variations of temperature and dipping times. It is also useful to leave some undipped for comparison, for microscopic work if needed, or to confirm identification, etc. I have kept a small number of

fungi un-dipped and, after nearly three years in a secure container under air-conditioning, the successful specimens still look good. Ideally, if new fresh specimens can be obtained, preserved fungi could be compared with fresh.

The use of acrylic lacquers on freeze-dried fungi instead of wax is an alternative treatment, particularly as colours can be restored in some cases. Restoring colours using automotive lacquers and an artists' air brush on low pressure (>10psi) and/or spraying with acrylic lacquer is being investigated.

NOTE

* Microcrystalline wax is a petroleum wax, containing branched and cyclic saturated hydrocarbons, as well as normal alkanes from de-oiled residual bright stock lube oil streams. Microcrystalline waxes have a crystalline structure much smaller than totally natural waxes and have a very high resistance to moisture, alcohol, acids and fingerprints.

Species	Common name	Freeze-dry results	Wax-impregnation results	Comments
?Aluerina spp.	fleshy cup fungi	very good	very good	some loss of colour when dipped
?Bovista spp.	puffball	very good	not tried	-
Clitocybe spp.	-	poor-good	poor-good	some wrinkling of caps
Coltricia cinnamomea	tough cinnamon fungus	very good	poor	wax-dipping destroys surface sheen
Crucibulum spp.	birds nest fungi	very good	good	appearance better un-dipped
Hydnum spp.	tooth fungi	good	OK	some shrinkage noticeable, accumulation of wax between teeth
Inocybe spp.	-	very good	not tried	-
Laccaria spp.	-	very good	very good	small specimens
Lepiota spp.	parasol mushroom	very good	not tried	the largest specimen attempted: 10 cm high by 8 cm across. However, had noticeably darkened after three years
Omphalina spp.	brown belly buttons	very good	very good	-
Ramaria spp.	coral fungi	very good	not tried	-
Stropharia spp.	-	poor-good	poor-good	-
Tubaria spp.	-	poor-good	not tried	some shrinkage noticeable

For further information I can be contacted at kirsten.tullis@museum.wa.gov.au.

**Macrocrystalline wax (paraffin wax) is also a petroleum wax made from de-oiled slack wax, which is derived by de-waxing base distillate lube oil streams of mainly straight chain alkanes. Paraffin wax is brittle and has a low melting point between 46 and 71 degrees C. It is highly resistant to moisture. Due to its low cost, paraffin wax is frequently added to other wax blends.
(source
http://www.arbortech.com.au/articles/036.html by S. Russell.)

Results

Approximately 20 species were tried, with mixed results. The best generally were achieved with firmer or less fleshy species. A few fleshy and more fragile species, some not included in the table because they were not identified, exhibited shrinkage. Apart from the *Lepiota* spp. most specimens were less than 30 mm × 20 mm. See Figures 1–6.

NOTE

Silica gel beads are available for $13/kilo + GST from Kirkside Products—21a Roberts St West, Osborne Park, W.A. Ph (08) 9242 2990.

Waxes: Microcrystalline *Mobilwax 2405* was, in mid-2005, available from Statewide Oil Distributors in Welshpool, W.A. Ph (08) 9350 6777—allow two weeks if none in stock. However, the smallest quantity available is in 24 kg boxes (~$140 + GST). This is a soft wax and needs to be hardened by adding either hard microcrystalline wax (we had some old stock of *Mobilwax 190Y* which appears to be no longer available) or paraffin wax. Paraffin wax is a macrocrystalline wax commonly used for candle-making, is cheaper and is most likely available from craft shops but has a very low melting point and is too brittle to use alone. The use of a medium-grade microcrystalline wax is an obvious alternative; however, blending soft and hard waxes to suit the climate gives more flexibility. Fungi dipped in a blend that is very soft may be affected by higher ambient temperatures and, because harder waxes (microcrystalline and paraffin) are brittle especially at low temperatures, fungi dipped in too hard a wax may crack or break if handled.