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REVIEW

The Effect of Freeze-drying and its Implications for Botanical Medicine: A Review

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Botanical samples are often freeze-dried (lyophilized) for use in research studies, and a variety of freeze-dried botanicals are marketed to the public. In both instances, there is an underlying assumption that freeze-drying properly preserves the medicinal qualities of plants, and is superior to other preservation methods. In fact, little systematic research has been done to verify this assumption. A review of the existing research, done primarily by the food and spice industry, indicates that freeze-drying has unanticipated and significant effects on the constituent profiles of medicinal plants that puts into question whether freeze-drying necessarily is the best method to preserve botanical medicines.

This research review finds there is insufficient information to conclude that freeze-drying has negative effects on the medicinal qualities of plants. But, because existing research indicates that freeze-drying imperfectly preserves important classes of medicinal compounds (such as volatiles, phenolics and carotenoids), may increase the mutation rate in unicellular organisms and may diminish some medicinal plant actions, researchers and practitioners should carefully consider how the use of freeze-dried material may affect pharmacological and clinical study results.

Keywords: lyophilization; phytotherapy; volatiles; phenolics; clinical effect; review.

INTRODUCTION

Botanical samples are often freeze-dried (lyophilized) before use in research studies, and a variety of different freeze-dried botanicals are marketed to the public. In both instances, there is an underlying assumption that freeze-drying properly preserves the medicinal qualities of plants, and is superior to other drying methods. In fact, little systematic research has been done to verify these assumptions. An analysis of the existing research, done primarily by the food and spice industry, indicates that freeze-drying has unanticipated and significant effects on the constituent profiles of medicinal plants that put into question whether freeze-drying necessarily is the best method to preserve botanical medicines.

There is insufficient information to conclude that freeze-drying has negative effects on the medicinal qualities of plants. But, because freeze-drying imperfectly preserves important classes of medicinal compounds (e.g. phenolics, volatiles) and because it may diminish some medicinal plant actions, researchers should carefully consider how the use of freeze-dried material may affect their study results.

Freeze-dried material is very hydrophilic yet there is no information on whether this characteristic affects the shelf-life of capsules after the consumer packaging is opened and its contents are exposed to oxygen and humidity. It is possible that these products may degrade rapidly, and prescribing practitioners and consumers should consider the possibility that a freeze-dried product might not retain its medicinal qualities for any substantial length of time after the container has been opened.

The lack of systematic research into the effects of freeze-drying on botanical medicines makes it impossible to draw firm conclusions about its effects. The existing studies had varying objectives and used various techniques in their analyses which may have affected the results in a variety of ways. This review is thus a starting point, not an ending point, for an evaluation of how freeze-drying may affect botanical medicines.

VOLATILES

Most drying methods are known to affect volatiles but freeze-drying generally has the most pronounced effect and consistently fails to preserve fully the volatile profile of the fresh plant. On occasion, total volatiles were not changed but, as a rule, freeze-drying changed the relative concentrations of volatile compounds, usually failing to preserve the volatiles that give the studied plant its unique aroma characteristics. This may not always be undesirable. For instance, all drying methods increase the characteristic minty odour of spearmint which may be a plus for some uses, such as in herbal teas. The review of the effects of freeze-drying on volatile compounds is limited by the fact that, in many cases, freeze-drying was only compared with hot oven-drying and did not include ambient air-drying. The freeze-dried product was rarely compared with an extraction of fresh

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plant although there are indications that volatile plant constituents are more effectively preserved where drying is not part of the process.

One study concluded that all drying methods increased the eugenol content of bay leaf (*Laurus nobilis*) of the order of 60%, but freeze-drying alone substantially reduced the amounts of certain monoterpenes (1,8-cineole, linalool and geraniol) (Diaz-Maroto *et al.*, 2002). Freeze-drying increased eugenol, elimicin and the sesqueterpenes spathulenol and beta-eudesmol. The authors concluded that air- or oven-drying at 45 °C are better at preserving the sensory characteristics of bay leaf than freeze-drying.

Freeze-drying resulted in substantial losses of oxygenated monoterpenes and sesquiterpenes in spearmint. Of the methods tested, oven-drying at 45 °C and air-drying preserved the greatest amount of volatile compounds as well as the sensory characteristics of the plant although all drying methods decreased the herbaceous and floral notes while increasing the plant's minty odour (Diaz-Maroto and Cabezudo, 2003).

Air-drying preserved more parsley (*Petroselinum crispum* L.) volatiles compared with oven-drying at 45 °C and freeze-drying. The volatiles lost using the latter methods are those monoterpenes with the greatest effect on parsley aroma, *p*-mentha-1,3,8-triene and apiole (Diaz-Maroto *et al.*, 2002). Another study confirmed that air-drying preserved more of the herbaceous aroma characteristics of fresh parsley than freeze- and oven-drying (Diaz-Maroto *et al.*, 2003).

Freeze- and air-drying significantly reduced the volatile components of *Antriscus sylvestris* (L.) Hoffm compared with fresh plant (Bos *et al.*, 2002).

Freeze-drying resulted in losses of several flavour compounds in blueberries (*Vaccinium spp.*), including 1,8-cineole that gives blueberries their characteristic aroma (Feng *et al.*, 1999).

Freeze-drying preserved more of the volatile aroma compounds of dill (*Anethum graveolens*) than hot air-drying but both methods failed to capture over 80% of the most important aroma component in dill (e.g. benzofuranoid). Over 50% of the total volatiles in the dried and freeze-dried samples consisted of secondary aroma compounds (mostly phytadienes) formed during processing. In the final analysis, the freeze-dried sample preserved only 25% of the total primary aroma compounds found in fresh dill (Huopalahti *et al.*, 1985).

Oven-drying at 30 °C did not greatly affect the volatiles in sage (*Salvia officinalis* L.) and thyme (*Thymus vulgaris* L.) but drying at 60 °C caused a marked loss of volatiles (Venskutonis, 1997). Freeze-drying preserved total volatiles in sage relatively well but increased the absolute thymol content by 33%, indicating a change in the volatile profile of the plant. Both oven- and freeze-drying increased the percentage of unknown volatiles in the headspace†, presumably degradation products. Freeze-drying increased the thymol content of Mexican oregano (*Lippia berlandieri* Schauer); other terpenoids were not quantified (Yousif *et al.*, 2000).

In summary, freeze-drying often alters the volatile profile of plants and will sometimes significantly increase the amount of secondary and/or degradation volatiles. No conclusions about the ability of freeze-drying to preserve the medicinal qualities of volatile-rich plants can be drawn from this research. However, volatiles are active plant constituents, and freeze-drying may not be the optimum method of preserving such plants for clinical research or patient use. Further research comparing various methods of preserving volatiles is definitely needed. Ideally, this research would include a comparison of various drying methods (including freeze-drying) with hydroethanolic or other fresh plant extracts to determine which method best preserves the volatile profiles of medicinal plants, and ultimately compares how the clinical activity of the plant is affected by the choice of preservation method.

† The 'headspace' is the gas space in a chromatography vial above the sample. Headspace analysis is the analysis of the components present in that gas.

CAROTENOIDS

There are insufficient data on the effect of freeze-drying on carotenoids. Ethanol extracts of tomato skins contained more lycopene than freeze-dried skins (Inakuma *et al.*, 1998). Frozen or boiled soybeans had a higher lutein and beta-carotene content than freeze-dried beans (Simonne *et al.*, 2000). Boiled soybeans had a significantly higher content of daidzin, genistin and genistein than frozen and freeze-dried soybeans. Freeze-drying preserved more carotenoids from daylily (*Hemerocallis disticha*) flowers than air-drying (Tai and Chen, 2000). Finally, one study compared oven-drying with freeze-drying on the formononetin content of red clover. Oven-drying reduced formononetin content while freeze-drying did not (Jones, 1979). Oven-drying did not, however, affect formononetin glucoside content. Freeze-drying preserved more carotenoids in eight Malaysian medicinal plants (*Apium graveolens, Averrhoa bilimbi, Hydroctyle asiatica, Mentha arvensis, Psidium guajava, Sauropus androgynous, Solanum nigrum, Polygonum minus*) than oven-drying at 50 °C for 9 h or at 70 °C for 1 h.

No firm conclusions can be drawn from these varied studies. It appears that freeze-drying can have a negative effect on carotenoid preservation but may nonetheless be preferable to air-drying and oven-drying of botanicals where carotenoid extraction is an issue. Where preservation of maximum carotenoid content is desired, other methods of preserving plants including ethanol extracts of fresh plant should be studied in comparison with freeze-drying.

POLYPHENOLS

Freeze-drying appears to be a good method for preserving large-molecular-weight condensed tannins. Its effect on other polyphenols is variable. The studies do not always compare the same drying methods so it is difficult to draw firm conclusions. However, it appears that ethanol extracts of the fresh plant will best preserve phenolics and that freeze-drying will often have an undesirable effect on those compounds.

Both freeze- and air-drying preserved tannins well while oven-drying caused a profound inactivation of condensed tannins in *Eulalia villosa* (Needs) Thunb

(Du Toit and Wolfson, 1996). Freeze-dried willow (*Salix spp*.) leaves had lower concentrations of phenolic glycosides but higher concentrations of condensed tannins compared with air-dried leaves; vacuum-dried leaves had high concentrations of both (Orians, 1995). Salicortin and 2-cinnamoyl salicortin dropped in freeze-dried leaves while their degradation product, salicin, was high. Salicin was present in air-dried leaves but not in vacuum-dried leaves. Freeze-drying preserved the highest percentage of condensed tannins from *Sericea lespedeza* compared with sun-cured, oven-dried and fresh-frozen leaves (Terrill *et al.*, 1990). Thus, it is clear that tannin concentrations vary with drying method.

Freeze-dried leaves of quaking aspen (*Populus tremuloides*) did not differ significantly from vacuum-dried leaves in terms of levels of phenolic glycosides and condensed tannins (Lindroth and Koss, 1996). However, this study only looked at these two methods of drying. Another study recommended caution in the interpretation of phenolic glycoside assays because freeze- and air-drying of *Populus* and *Salix* leaves produced dramatic changes in the total and relative concentrations of specific phenolic glycosides compared with fresh leaves (Lindroth and Pajutee, 1987). Extraction in aqueous and alcohol media for extended (24 h) periods also caused changes in glycoside concentrations with alterations in phenolic glycoside concentrations, interconversions among glycosides, and production of artifactual glycosides resulting from hydrolytic reactions. The authors state that these effects are best, but not entirely, avoided by the use of fresh plant material, cold, non-aqueous extraction solvents, and short extraction times.

The drying method did not dramatically change the relative phenolic profile of birch (*Betula pendula* Roth) leaf, but the ethanol extraction of fresh plant best preserved the phenolic concentration of the crude plant (Keinanen and Julkunen, 1996). Of the various drying methods tested, freeze-drying of a slowly frozen (as opposed to flash-frozen) leaf preserved most of the phenolics. The authors concluded that an analysis of the fresh plant would be preferable for quantitative research purposes, and that the choice of preservation method should be based on the particular research goals of any study. Freeze-dried red wine preserved about 70% of the four main polyphenols present in red wine (gallic acid, catechin, epicatechin and quercetin) (van Golde *et al.*, 2004). In grape pomace peel, oven-drying at 60 °C and freeze-drying resulted in about the same amounts of total extractable polyphenols, condensed tannins, colour and antioxidant activity (Laurrauri *et al.*, 1997). Oven-drying at higher temperatures resulted in considerable losses of polyphenols, condensed tannins and antioxidant activity. Freeze-dried marion berries, strawberries and corn consistently had a higher level of total phenolic content compared with those air-dried (Asami *et al.*, 2003).

Dehydration in any form resulted in losses of oil polyphenols in *Euphorbia esula* (Wiatr, 1984). Air- and warm oven-drying gave the highest oil yields. Freeze drying and frozen storage resulted in the retention of over 97% of the pigment (betacyanin) of *Amaranthus* samples (Cai and Corke, 2001). Natural air drying preserved 52.8%, solar drying 63.5%, and air oven drying 61.9%–81% of the pigment.

Phenolics are important medicinal compounds. The potential effect of freeze-drying on these constituents should be considered when planning and analysing research studies on the medicinal properties of plants.

EFFECTS ON OTHER CONSTITUENTS

Cichoric acid is very sensitive to moisture content during storage. Freeze-drying *Echinacea purpurea* led to a greater retention of cichoric acid than did air drying; the two drying methods preserved caftaric acid equally (Kim *et al.*, 2000). Both cichoric and caftaric acids are phenylpropanoids. The oily phenylpropanoid derivative gingerol has also been studied. Freeze-drying of ginger (*Zingiber officinale*) did not decrease the levels of the pungent principle, gingerol (Zhang *et al.*, 1994).

Freeze-drying preserved more alkamides (alkylamides) in *Echinacea purpurea* roots than did air-drying but the two methods equally preserved alkamide content in its leaves (Kim *et al.*, 2000). The alkamide content in air-dried *Echinacea angustifolia* roots was higher than in fresh. However, this may be a result of water entrainment during the carbon dioxide extraction rather than an actual change in content during drying. Freeze-drying and air-drying at 50 °C resulted in similar alkamide contents (Sun *et al.*, 2002).

Freeze-drying preserved more camptothecin from *Camptotheca acuminata* than air- or oven-drying (Liu *et al.*, 1998). Camptothecin is a pyrroloquinoline alkaloid with antineoplastic properties.

Slow drying caused reduced recovery of total taxanes from an ornamental *Taxus sp*. (Elsohly *et al.*, 1997). Air- and freeze-drying preserved taxol and cephalomannine levels but reduced somewhat 10-deacetyltaxol and 10-deacetylbaccatin III. These compounds are all diterpenoid, antineoplastic taxanes.

All drying methods preserved the seven major ginsenoside saponins from *Panax japonicus* (var repens) (Reshetniak *et al.*, 2003). Freeze-drying did not affect the ratios of individual ginsenosides while air-drying decreased these compounds in the calli but not in the leaf.

Freeze-drying preserved more sideroxylonals (dimeric phloroglucinols) from *Eucalyptus* leaves than did air-drying (Wallis *et al.*, 2003). Dried leaf lost 22% of these compounds when stored at room temperature for 20 months.

Freeze-drying reduced triacylglycerol levels and changed the relative percentages of unsaturated fatty acids in the oyster *Crassostrea gigas* (Dustan *et al.*, 1993). It did not change the amounts of polar lipids, total sterols, free fatty acids or hydrocarbons in oysters. Freeze-drying of the seaweed *Sargassum hemiphyllum* (Turn) C. preserved more total amino acids, total polyunsaturated fatty acids and vitamin C than air- or oven-drying (Chan *et al.*, 1997). Oven-drying resulted in the greatest nutritional loss but preserved the highest mineral content. Freeze-drying preserved more vitamin C, chlorophyll, riboflavin and niacin from eight medicinal plants (*Apium graveolens, Averrhoa bilimbi, Centella asiatica, Mentha arvensis, Psidium guajava, Sauropus androgynous, Solanum nigrum, Polygonum minus*) in a Malaysian study than oven-drying at 50 °C for 9 h or at 70 °C for 1 h.

Drying (ambient 25 °C, oven 50 °C, oven 105 °C and freeze-drying) did not change the relative composition of oils from *Euphorbia esula* but dehydration in any form resulted in losses of oil, hydrocarbons and polyphenols (Wiatr, 1984). Air- and warm oven-drying gave the highest oil yields that did not significantly differ from fresh tissue. No difference was found in the extracted oils from oven- and freeze-dried samples of *Echinacea pallida*, *E. angustifolia* and *E. purpurea* roots (Smith-Jochum and Davis, 1991).

Freeze-drying of quaking aspen leaves caused a small decline in nitrogen and soluble protein while vacuum drying reduced starch concentrations by 38% (Lindroth and Koss, 1996). Freeze- and air-drying preserved the same levels of protein in sea buckthorn leaves (*Hippophae rhamnoides*) (Li and Wardle, 2003). However, the protein actin lost its ability to polymerize on rehydration whether air- or freeze-dried and the authors stated that the drying process itself caused structural damage that led to a loss of activity after rehydration (Allison *et al.*, 1998).

These studies combine to show that freeze-drying affects different plants and different constituents in a variety of ways. They underscore the need for more systematic studies to assist in determining the preferred preservation method for various plant constituents in botanical research studies.

MISCELLANEOUS PHARMACOLOGICAL EFFECTS

There have been no systematic studies of how freeze-drying affects the medicinal properties of plants, and it is extremely difficult to tease out the potential pharmacological effects that freeze-drying may have. What follows is neither a complete nor thorough analysis of what existing studies may divulge on this topic. Instead, this review simply shows that freeze-drying affects the activity of botanicals and underscores that the choice of the botanical preparation needs to be considered carefully when researching the medicinal effects of botanicals or analysing the results of clinical and pharmacological studies.

A pilot clinical trial demonstrated that freeze-drying can effectively preserve the medicinal effects of a plant. In this study, a 600 mg dose of freeze-dried, powdered *Urtica dioica* (stinging nettle) leaf one or more times a day was more effective than placebo at reducing symptoms of allergic rhinitis (Mittman, 1990). Unfortunately no statistical analysis was provided to support this conclusion or to determine statistical significance.

We did not locate any studies showing the relative clinical efficacy of freeze-dried compared with other types of preparations. However, some animal and *in vitro* studies have compared freeze-dried extracts with other extracts. The ethanol, dichloromethane and freeze-dried aqueous extracts of *Cassia occidentalis* root bark, *Morinda morindoides* leaves and whole plants of *Phyllanthus niuri* were administered to mice with malaria (*Plasmodium berghei* Anka) (Tona *et al*., 2001). At 200 mg/kg the ethanol and dichloromethane extracts of *M. morindoides* and *P. niuri* suppressed over 60% of parasitaemia and *C. occidentalis* suppressed 30%. The freeze-dried aqueous extract was less active than the ethanol extract. These results are likely due to differences between water and ethanol as solvents rather than to the negative effects of freeze-drying.

Freeze-drying only captured around 70% of the polyphenols of red wine; nonetheless, the resulting preparation retained its antioxidant activity *in vitro* in human blood (van Golde *et al.*, 2004). Unfortunately the relative antioxidant effects of the alcohol and the freeze-dried preparations were not quantified.

Quercetin glycosides predominated in freeze-dried onion while quercetin aglycones predominated in hot air-dried onion. Hot air-dried onion had a strong antiproliferative effect in leukaemia cell lines while that of freeze-dried was only moderate (Fu, 2004). It is possible that heating liberated the aglycones from their glycosides, and that aglycones are the more active compounds. Human stomach acid would likely be able to hydrolyse glycosides in a similar fashion.

Air-dried rosemary (*Rosmarinus officinalis*) and summer savory (*Satureja hortensis* L.) had a greater antioxidant effect when added to salad dressing than did freeze-dried methanol extract of these herbs (Laurrauri *et al.*, 1997). This result may have been due to the failure of methanol to extract efficiently the active plant components or it may have been due to a failure of freeze-drying to preserve constituents in the methanol extract. Freeze-dried grape pomace peel had the same antioxidant activity as oven-dried at 60 °C but the study did not test air-dried preparations (Laurrauri *et al.*, 1997). Freeze-drying preserved more vitamin C and enhanced the total antioxidant activity of asparagus, especially compared with heated air-drying methods (Nindo *et al.*, 2003). Freeze-dried water hyacinth leaves had a higher antioxidant activity *in vitro* than did sun-dried and oven-dried at 40° and 60 °C. Oven-dried at 60 °C had the lowest antioxidant activity (Bodo *et al.*, 2004).

Pulverized decocted cat's claw (*Uncaria tomentosa*) was a more effective scavenger of [alpha]-[alpha]-diphenyl-[beta]-picrylhydrazyl (DPPH) than freeze-dried plant, but freeze-dried plant suppressed a higher percentage of tumour necrosis factor alpha (TNF-a) than did the pulverized decoction (Sandoval *et al.*, 2000). Interestingly, in a clinical study of freeze-dried cat's claw in osteoarthritis, cat's claw displayed both an ability to quench DPPH radicals and to inhibit TNF-a. However, the latter was registered at much lower concentrations. The authors suggested that cat's claw's ability to treat osteoarthritis effectively was likely due to the plant's ability to inhibit TNF-a (Piscoya *et al.*, 2001).

Drought-stressed plants produce more free radicals than do more pampered plants. Freeze-dried preparations of drought-stressed plants contained increased levels of free radicals generated by such stress while freezing failed to preserve the additional free radicals. The study showed that freeze-drying did not itself generate the observed increase in free radicals (Pirker *et al.*, 2002).

Freeze-dried banana pulp had a more marked cholesterol-lowering effect in rats than did pulp oven-dried at 65 °C. This effect was due to changes in the soluble and insoluble fibre content in the resulting pulp (Horigome *et al.*, 1992).

Freeze-drying slightly decreased the hepatoprotective effects of fresh vegetable juice compared with boiling or freezing (Gazzani *et al*., 1998).

Research suggests that freeze-drying causes significant changes in bacteria. *Daphia magna* fed on freeze-dried chlorella exhibited reduced fecundity and neonate size compared with *Daphia* fed fresh chlorella (Naylor *et al.*, 1993). Freeze-drying caused mutations in *Escherichia coli* and, upon rehydration, resulted in an increased mutation rate (Tanaka *et al.*, 1979). While these results may not apply directly to plants, they raise serious concerns that must be examined given the widespread use of freeze-dried probiotics in alternative medicine.

On the other hand, freeze-drying may effectively reduce microbial contamination of botanicals. Freeze-drying reduced the survival count of molds and aerobic spore-formers compared with air-drying of a variety of herbs (Malmsten *et al.*, 1991). This is significant as a recent study showed widespread mycotoxin contamination of some herb/spice samples (Patel *et al.*, 1996).

OTHER CONSIDERATIONS

There are a variety of techniques used in freeze-drying. These techniques and their pros and cons are not considered here. All, however, emphasize that reducing the moisture content in the freeze-dried product is extremely important. Freeze-dried products are hydrophilic and rapidly reabsorb moisture. This aspect of freeze-drying may be of considerable consequence to users of freeze-dried botanicals: Once a freeze-dried product is purchased and opened, what happens to that product? Does it rapidly absorb moisture and begin to degrade? Does the medicinal effect of the product change significantly? What is the actual shelf life of a freeze-dried product from the consumer's perspective?

We have located no studies on this important topic. Nor have we noted any comments on this aspect of freeze-drying in longer term clinical studies using freeze-dried products.

CONCLUSION

Freeze-drying has some unanticipated and potentially significant effects on constituent profiles and the medicinal action of plants. Nonetheless, freeze-dried plant materials are frequently used both in pharmacological and clinical trials without consideration of how the choice of plant preparation might affect outcomes. There is an unwarranted and unexamined assumption in botanical research that freeze-drying properly and optimally preserves the plant's constituents. Studies indicate that these assumptions may be erroneous. As a result, further systematic research is needed on the effects of freeze-drying compared with other preparation methods, including alcohol extractions of fresh plant material, to allow a more accurate testing of the medicinal effects of botanicals.

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