ARTICLE

Cache like a squirrel: analysis of and recommendations for long-term storage of white oak (*Quercus alba*) and pin oak (*Q. palustris*) acorns for use in wildlife rehabilitation without fat degradation

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Abstract

Several wild animal species include acorns from white oak (*Quercus alba*), a species from the white oak section, *Quercus*, and pin oak (*Q. palustris*), a species from the red oak section, *Lobatae*, as a diet staple, particularly during preparation for winter. Acorns provide a natural, easily gathered food for wildlife recovering in rehabilitation facilities, but long-term storage can be challenging. The authors examined whether the nutritional value of acorns measured as crude fat deteriorates under temperature-controlled drying and storage methods. Fat content was assayed from 14 trees (six white oak, eight pin oak) immediately after collection (baseline) and compared to fat content after one month and six months of storage for both species. Storage at 4°C (40°F, refrigerator) resulted in the germination of some white oak acorns and mold growth in both species. Although acorns from pin oaks had significantly more fat than white oak acorns at baseline, drying, storage, and combination treatments resulted in no significant difference in fat content, as measured by effect size, for either species. The study results suggest that acorns cached by species like squirrels (*Sciurus* spp.) do not vary significantly in fat content when recovered one or six months later compared to acorns consumed directly from the trees. The authors recommend wildlife rehabilitators store acorns either in the freezer or at room temperature depending on their storage capabilities.

BIO

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Introduction

Acorns (*Quercus spp.*) are an important staple in the diet of more than 90 species of wildlife (Martin et al. 1961; McShea et al. 2007) including ruffed grouse (*Bonasa umbellus*), black bears (*Ursus americanus*), deer (*Odocoileus spp.*), and squirrels (*Sciurus spp.*). In the autumn months, animals will consume oak masts as a high energy, highly digestible food source in their preparation for winter (Martin et al. 1961; Kirkpatrick & Pekins 2002). Some species, like bears and deer, will consume high quantities of acorns according to availability, converting the high lipid content of acorns into their own fat stores for winter survival. In fact, deer can spend up to 50% of their foraging

time focused on acorn consumption during a high-mast year (McShea & Schwede 1993) with higher fat content comprising their cumulative diet than in poor-mast years (Harlow et al. 1975; Aubin et al. 2022). Other species, like squirrels and blue jays (*Cyanocitta cristata*), will scatter hoard or larder hoard acorns for long-term use throughout the winter and early spring. Squirrels make decisions based on perishability, eating acorns that degrade or germinate quickly immediately (Hadj-Chikh et al. 1996) and caching acorns that maintain fat content during storage (Wood 2005). Feeding acorns in captivity could improve rehabilitation outcomes by providing a natural, high-fat food source.

Rehabilitators and caretakers typically source foods donated from local grocery stores and community gardens for a convenient, abundant, and cost-effective food supply. In stressful captive situations like rehabilitation, wild animals need food items that simulate wild forage closely (Dierenfeld 1997; Poisson & Weiss 2016). With limited staff or volunteers, rehabilitators may struggle to provide patients with enough of their natural diet. Acorns, in particular, are easily gathered by community volunteers when mast production is high. The unpredictability of oak tree mast yields from year to year and even within year variation among trees (Koenig & Knops 2002), on the other hand, make it difficult for rehabilitators to ensure an adequate supply of acorns for patients.

Long-term storage of acorns collected during a high mast year would allow wildlife rehabilitators to stock up on acorns for use during subsequent poor mast years; however, acorns may mold and/or germinate in certain conditions (Devine et al. 2010). Even improper short-term storage of acorns within a season can result in spoilage and germination. Further, if the nutritional content of acorns degrades significantly over time, the value of labor-intensive collection and storage methods also diminishes. Previous storage studies focused on preventing acorn germination (Devine et al. 2010) for reforestation or on preserving acorn flour for human consumption (Correia et al. 2009). Little has been researched on long-term storage for captive wildlife. Our research evaluated the effects of drying and storage on the fat content of acorns from white oak (*Quercus alba*), a species from the white oak section, *Quercus*, and pin oak (*Q. palustris*), a species from the red oak section, *Lobatae*, over six months. The authors hypothesized that oven-dried, freezer-stored acorns would maintain the highest amount of fat content over different storage durations of one month and six months. The results of the study are discussed and recommendations are provided for wildlife rehabilitators, zoos, and other captive facilities.

Methods

Collection, preparation, and treatment

Fresh acorns were collected from six white oak trees from September 2017 to October 2017, an above average mast year (Martin 2023), and eight pin oak trees from September 2020 to November 2020, a below average mast year (Martin 2023) in Bridgewater, Virginia, USA. Acorns were collected by student volunteers at Bridgewater College and trees were selected based on proximity to the campus (within 2-mile radius). Only solitary trees were sampled to avoid error when assigning acorns to a tree if multiple oak trees were dropping acorns in close proximity to each other. Acorns were collected from the ground of each tree within three to five days of dropping, or directly from trees when ripe. Volunteers filled a one-gallon storage bag with acorns gathered from all sides of the tree using an acorn roller, a rake, or by hand. Acorns from the gallon bag were separated into a fully crossed experimental design to test the effects of drying and storage on percent fat (Fig. 1). Acorns were inspected for general condition before subsampling; any damaged or already germinating acorns were removed, but acorns with exit holes from acorn weevils (Coleoptera: Curculionidae) were not. Immediately after collection, a subsample (approximately 4–6 acorns to ensure enough dried and ground material for nutritional analysis) of acorns from each tree ($N_{white} = 6$, $N_{pin} = 8$) was separated for fat analysis to determine a "baseline" value; that is, the fat content of acorns collected soon after dropping or pulled directly from the tree when ripe. The remaining acorns were divided into three equal preparation groups: air drying (seven days on drying racks with an oscillating fan circulating air), oven drying (30 minutes at 121°C [250°F]), and no drying (stored immediately) (Fig. 1). After drying, a subsample of dried acorns was separated for fat analysis to determine the effects of drying, and the remaining acorns divided into three storage options: air storage (hanging in mesh onion bags at room temperature), refrigerator storage (in zipper-lock plastic bags in a standard refrigerator at 4°C [40°F]), and freezer storage (in zipper-lock plastic bags in a standard freezer at −18°C [0°F], $N_{white} = 6$, $N_{pin} = 8$) (Fig. 1). All stored acorns that did not germinate or develop mold (see below) were subsampled at one month ($N_{white} = 52$, $N_{pin} = 60$) and six months ($N_{white} = 44$, $N_{pin} = 60$) for fat analysis. Because there is no way to test the same acorns repeatedly, all subsampling (4–6 acorns selected haphazardly after each treatment) was the best option to determine treatment effects.
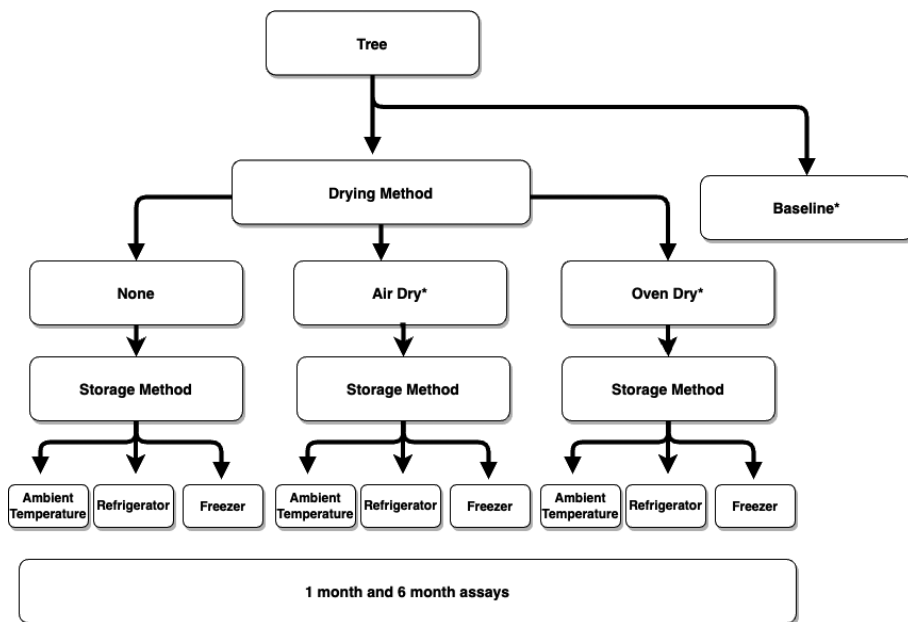
Figure 1 Diagram of fully crossed experimental design. Each tree from both species was subjected to each drying treatment, which was crossed with each storage treatment, resulting in 10 total treatments per tree. Each crossed treatment was analyzed for fat content for one month of storage and six months of storage and compared to baseline. Asterisks indicate additional post-treatment fat analyses.

Nutritional analysis

Widely accepted fat analysis procedures developed for forages, seeds, and oils (Undersander et al. 1993) were used and were validated by Ankom Technology for the Ankom[XT15] Extractor (Ankom Technology, Fairport, NY, USA).

To prepare samples for fat analysis, acorns were cracked and freeze-dried using a VirTis® Benchtop Lyophilizer (SP Scientific, Stoneridge, NY, USA) for 24 hours. The use of a lyophilizer preserves the chemical and structural integrity of macromolecules in a sample at the time of drying until nutritional assays can be completed. The subsamples were ground through a 1 mm screen using a Wiley® mill (Thomas Wiley, NJ, USA). To avoid mold contamination and additional water retention, the ground samples were returned to the lyophilizer for an additional 24 hours and sealed in Whirl-pak® bags with a desiccant pouch in each. Crude fat content was determined using an ether extraction method (Komarek et al. 2004) on the Ankom[XT15] Extractor. Any subsamples that germinated or developed mold were removed from analysis because the authors were not able to account for nutritional differences caused by a developing cotyledon or mold.

Statistical analysis

Overall fat content of acorns immediately collected from white and pin oaks was compared using a Welch two sample t-test in R (R Core Team 2021). The immediate effect of drying on fat content was then tested using a repeated measures ANOVA for white and red oak acorns, treating the tree that acorns were collected from as the common identifier ("rstatix" package, Kassambara 2022). To compare acorn fat content between trees, a treatment effect size was calculated by taking the natural log of the fat content of acorns after treatments divided by the baseline fat content from a given tree. The effect of drying and storage on mean acorn fat content was compared after one month using a two-factor ANOVA. Effect sizes for six-month acorns were non-normally distributed, even after log-transformation, and were, therefore, analyzed using a non-parametric, aligned rank transformed ANOVA ("art" package, Kay et al. 2021). Finally, to determine whether percent crude fat changed over time within each tree, a paired Wilcoxon sign rank test comparing one-month and six-month treatment effect sizes ("stats" package) was performed. To correct for multiple comparisons, Wilcoxon tests were performed using Bonferroni adjusted alpha levels of 0.025 per test (0.05/2).

Results

Baseline acorns collected directly from pin oak trees had significantly higher percent fat content ($N = 8$, $\bar{x} = 10.14$) than acorns from white oak trees ($N = 6$, $\bar{x} = 3.54$, $t = -4.1$, $p = 0.002$, Fig. 2). The whiskers around the boxes, which represent the middle 50% of the data, demonstrate wide
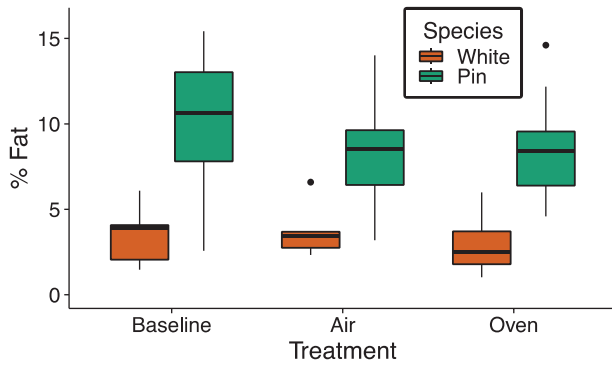
Figure 2 Crude fat content (%) of white oak (*Quercus alba*) and pin oak (*Q. palustris*) samples that were immediately processed and analyzed after collection ("Baseline") compared to samples that were air dried with an oscillating fan for seven days ("Air") or oven dried at 121°C (250°F) for 30 minutes ("Oven"). Boxes represent the middle 50% of the data, the central line indicates the median, and the whiskers show the range of the data. The dot above the air treatment represents an outlier.

Table 1 Effects on percent fat content of white oak (*Quercus alba*) acorns and pin oak (*Quercus palustris*) acorns after drying and storing acorn samples for one month and six months. Acorns were air-dried with a fan current for seven days or were placed in the oven at 121°C (250°F) for 30 minutes. Subsamples of each tree were analyzed after one month and six months of storage at room temperature in a refrigerator or in a freezer. Effect size was calculated as the natural log of the fat content of acorns after treatments divided by the baseline fat content from a given tree. One-month results were analyzed using a two-factor ANOVA but the non-normally distributed six-month results were analyzed using aligned rank-transformed ANOVA.

Explanatory Variable	One month			Six months		
	df	F	p	df	F	p
White oak						
Drying method	1	1.21	0.28	1	2.36	0.14
Storage method	2	0.04	0.96	2	0.29	0.75
Drying x Storage	2	0.06	0.94	2	0.38	0.69
Within	46	-	-	38	-	-
Total	51	-	-	43	-	-
Pin oak						
Drying method	1	0.35	0.56	1	0.04	0.85
Storage method	2	0.07	0.93	2	0.01	0.99
Drying x Storage	2	0.10	0.91	2	0.01	0.99
Within	54	-	-	53	-	-
Total	59	-	-	58	-	-

variation in fat content within and between tree subsamples. Oven drying and air drying with a fan immediately after picking resulted in no statistical change in percent fat content compared to baseline for white oak acorns ($N_{baseline}$ = 6, N_{oven} = 6, N_{air} = 5, F = 1.47, p = 0.29) or pin oak acorns ($N_{baseline}$ = 8, N_{oven} = 8, N_{air} = 8, F = 0.88, p = 0.40, Fig. 2).

Of all subsamples, 6.3% of white oak acorns either germinated or developed mold during refrigeration storage before nutritional analysis could be completed. Similarly, 7.6% of pin oak acorn subsamples developed mold during refrigeration before nutritional analysis.

Drying method, storage method, and the interaction between the two demonstrated no significant difference (Table 1), for white oak acorns (Fig. 3A) or pin oak acorns (Fig. 3B) after one and six months.

White oak acorns (Fig. 3A) showed a positive effect size (i.e., more fat after one month of storage than baseline) for some treatments. Pin oak acorns (Fig. 3B) all lost fat but, in some cases, showed less fat compared to baseline after one month than after six months. However, there was no statistical difference in effect sizes between one and six months of storage for either white oak (N_1 = 45, N_6 = 45, V = 264, p = 0.53, α = 0.025) or pin oak acorns (N_1 = 36, N_6 = 36, V = 341, p = 0.047, α = 0.025, Fig. 4). When ignoring drying method and pooling all samples together across all storage methods, white oak acorns had 2.4% more and 8.0% less fat than baseline after one and six months of storage respectively, indicating a 436% decrease in fat content over the span of five months. Pin oak acorns had 19.5 and 12.2% less fat than baseline after one and six months of storage respectively, representing a 37.5% increase in fat content over five months.

Discussion

As expected, pin oak acorns had a higher crude fat content than our white oak acorns, aligning with published values (Kirkpatrick & Pekins 2002). Drying acorns shortly after collection had no discernible effect on the fat content compared to fresh acorns (baseline) for either species. There were no significant changes in percent fat content from the storage method or the interaction between storage and drying methods as indicated by effect size. These results suggest that drying has no short-term or long-term effects on white oak acorns or pin oak acorns at one month or six months compared to baseline. The wide variability between trees and among subsamples within trees suggests that handling of acorns does not consistently, nor predictably, impact fat content.

Although stored samples of both species did lose fat, the high variability among subsamples (as indicated by the large error bars) likely obscured any effects of storage method and the interaction between storage and drying method. The higher fat content of oven-dried white oak acorns compared to air drying after one month of storage was peculiar; white oak acorns begin germination after dropping from the tree (Fox 1982; Devine et al. 2010) and
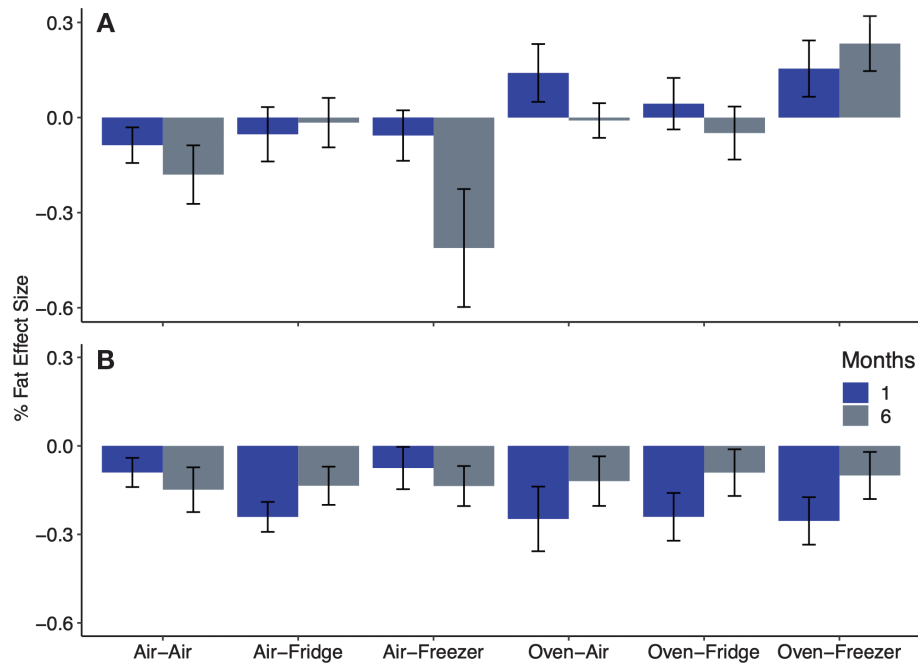
Figure 3 Crude fat effect size (%) for (A) white oak trees (*Quercus alba*, N_1 = 36, N_6 = 31) and (B) pin oak trees (*Quercus palustris*, N_1 = 45, N_6 = 48) after drying and storing acorn samples for one month and six months compared to amount of fat immediately after collection. Acorns were air dried ("Air") with a fan current for seven days or were placed in the oven ("Oven") at 121°C (250°F) for 30 minutes. Subsamples of each tree were analyzed after one month and six months of storage at room temperature ("Air"), in a refrigerator ("Fridge") or a freezer ("Freezer"). Error bars represent standard error and average effect size were calculated using subsamples that did not mold or germinate over six months (three to six white oak subsamples and four to eight pin oak subsamples).
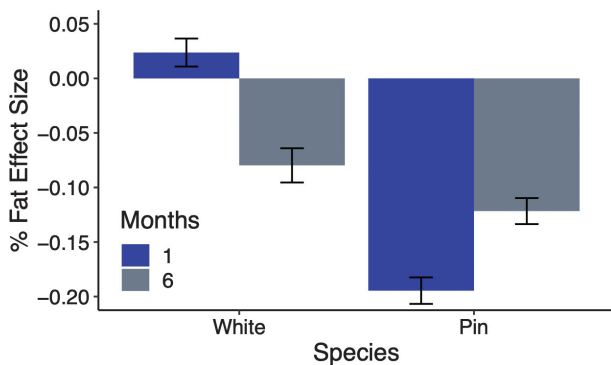
Figure 4 Crude fat effect size (%) across all drying and storage methods for acorns from white oak trees (*Quercus alba*) and pin oak trees (*Quercus palustris*), stored for one month (*Q. alba* N = 36, *Q. palustris* N = 45) and six months (*Q. alba* N = 31, *Q. palustris* N = 48) pooled across all drying methods. The effect size was calculated by comparing the fat content after storage for one month and six months to the baseline percent fat content directly after collection from the tree. Error bars represent standard error.

would presumably begin using fat stores immediately for seedling growth. It is possible that oven drying slows the germination process enough that the short-term difference in fat content is not seen. Pin oak acorns, unlike white oak

acorns, remain dormant until the spring after falling (Fox 1982; Steele & Smallwood 2002; Steele 2021) making it possible that changes would not be seen in fat content until after six months. However, given the non-significant difference in effect size for both species, it is more likely that inherent variability among subsamples precludes any significant differences caused by drying or storage. For example, the net gain in fat after storing white acorns for one month after oven drying probably indicates the individual acorns of the subsamples had higher fat content to begin with compared to the baseline subsample of the same tree rather than an indication of increasing fat content over time. More testing on more trees of both species would be necessary to confirm the lack of short-term or long-term drying effects; however, the authors believe such confirmation is not necessary given the results of storage methods.

During storage, some samples of both species showed signs of acorn weevils (Coleoptera: Curculionidae), as indicated by the presence of the weevils themselves or exit holes. Weevil infestation can affect germination rates (Lombardo & McCarthy 2009), but this does not necessarily mean animals will avoid infested acorns. Blue jays will consume infested acorns, though significantly

less often than non-infested acorns (Dixon et al. 1997). Squirrels will eat the infested acorns immediately and store non-infested acorns for later consumption (Steele et al. 1996; Steele 2021). The presence of weevils was noted only anecdotally. Future research could explore the effects of weevils on fat content, but given the variation in fat content across all acorns of all trees, regardless of treatment, a significant impact on acorn nutrition would not be expected when fed in ample quantities. Weevil presence could provide an opportunistic source of protein to patients.

Conclusion and recommendations

Given the unpredictable and variable nature of acorns, wildlife rehabilitators who wish to store acorns as part of a natural diet for patients should follow a procedure that is most convenient and feasible for each facility with the following recommendations. Freezing is the best option for long-term storage; there is no significant effect of freezing on fat content; freezing kills parasites and prevents mold growth and germination. If freezer space is not available or limited, hanging acorns in a well-ventilated area using receptacles like onion bags that allow airflow is suggested.

Although squirrels prefer the higher fat content of red oaks (of which pin oak is included), they will store more red oak than white oak acorns (Wood 2005) because of differences in dormancy and germination. The authors suggest mimicking squirrel caching behavior (Steele et al. 1996) by feeding white oak acorns immediately before they germinate and storing pin oak acorns when storage space is limited. Further, the higher fat content of pin oak acorns may be more valuable than the white oak acorns for long-term care. Alternatively, rehabilitators can excise the embryos of white oak acorns, like squirrels often do, using a knife (Fox 1982) to prevent germination before storing at room temperature. This is labor-intensive but an option when only white oak acorns are available. Long-term storage in the refrigerator is not recommended because of the high risk of mold growth of either species in the stagnant, humid air and the germination risk of white oak acorns. Before storing or feeding acorns, some collectors will employ a float test to indicate the quality of acorns and determine the presence of parasites. However, the results of these float tests are unreliable (Morina et al. 2017). Moreover, float tests are labor-intensive (Gribko & Jones 1995), and unnecessarily result in the disposal of many usable acorns collected by volunteers. If rehabilitators want to avoid the emergence of acorn weevils, they can dry either acorn species in an oven at 121°C (250°F) for 30 minutes before storage or rely on freezer

storage only. Nevertheless, the authors do not think this is necessary; the presence of weevils may provide additional protein to patients, and because weevils cannot survive in artificial environments, clean-up of emerging weevils is not labor-intensive.

Disclosure statement

The authors report no conflict of interest.

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