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Cache like a Squirrel: Effects of Long Term Storage on Crude Fat Content of Q. Palustris and Q. Alba Acorns

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Cache Like a Squirrel: Effects of long-term storage on crude fat content of *Q. palustris* and *Q. alba* acorns

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Introduction

Acorns (*Quercus sp.*) provide a high energy, highly digestible staple for wildlife diets, especially in preparation for winter (Kirkpatrick and Pekins 2002). Acorns are not found year around and oak trees are known to have unpredictable yields (Koenig and Knops 2002). Some animals like squirrels (Sciurus sp.) cache acorns for later consumption. Similarly, rehabilitators need steady access to foods that simulate wild forage closely (Dierenfeld 1997) . However, acorns will not keep well in certain conditions (Devine 2010) resulting in mold or germination. Looking to methods used by squirrels and considering the needs of rehabilitators, our research evaluated the effects of drying and storage on the fat content of pin oak (*Q. palustris*) and white oak (*Q. alba*) acorns. We hypothesized that oven dried, freezer stored acorns would maintain the highest amount of fat content over different storage durations of 1 month and 6 months.

Methods

Collection, Preparation, and Treatment. We collected fresh white oak (Q. alba) acorns from six trees in September 2017 to October 2017, and eight pin oak (Q. palustris) trees from September 2020 to November 2020 in Bridgewater, Virginia, USA. Immediately after collection, a portion of acorns from each tree was separated for fat analysis to determine a baseline value directly from the tree. We divided the remaining acorns for three preparation groups: air drying (seven days on drying racks with an oscillating fan circulating air), oven drying (30 minutes at 121°C) , and no drying (stored immediately). A portion of dried acorns were 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), and freezer storage (in zipper lock plastic bags in a standard freezer at -18°C). We subsampled all stored acorns at one month and six months for fat analysis.

Fat Analysis. To prepare samples for fat analysis we freeze dried cracked acorns using a VirTis Benchtop Lypholyzer (SP Scientific, Stoneridge, NY, USA) for 24 hours, and ground them to pass through a 1mm screen using a Wiley mill (Thomas Wiley, NJ, USA). To avoid mold contamination and additional water retention, we returned the ground samples to the lypholizer for an additional 24 hours and sealed the samples in whirl-paks with a desiccant pouch in each. We then determined crude fat using an ether extraction method (AOAC 1990) on an Ankom XT15 Extractor (Ankom technology, Fairport, NY, USA)

Statistical analysis. To confirm overall differences between pin oak and white oak fat content we ran a t-test to after 6 months of storage only and across durations of storage. We tested the immediate effects of drying compared to straight off the tree using a one-factor ANOVA for white oak acorns only. To determine the effects of each treatment combination on both species we first calculated a percent fat response ratio comparing baseline (directly off the tree) to each treatment. We then performed a two-factor ANOVA on the response ratio for acorns stored for one month and an align rank transform ANOVA on acorns stored for six months, using a Bonferroni correction (α=0.025) to control for multiple comparisons. Finally, to determine if percent crude fat changed over time within each tree, we performed a Wilcoxon sign rank test on all trees at 1 month and 6 months. All statistical analyses were run using R Statistical Software (R Core Team 2020).

Results

Acorns collected directly from pin oak trees (N=8, $\bar{x}$=3.3) had significantly higher fat content than acorns from white oak trees (N=6, $\bar{x}$=8.8, t=2.0 p=1.3). White oak acorns were more likely to germinate during refrigeration than pin oak and refrigerator samples of both species were more likely to mold than samples stored at room temperature or in the freezer. There was no significant effect of either drying method directly after collection for white oak acorns (N=18, F=0.279, p=0.76, Figure 1) but oven drying resulted in significantly less fat loss by six months than air drying (N=32, F=6.23, p=0.019), even with a Bonferroni correction. There was no effect of drying on one month (N=60) or six month (N=59) pin oak or one month (N=50) white oak samples (all p>0.05). Storage has no effect on fat content at any time for either species (Figure 2 , N=14) nor is there an interaction between drying and storage for any samples. Although the fat response ratio differed from one month to six months (Figure 2), this was not statistically significant for white oak (V=225, p=0.886) or pin oak (V=394, p=0.165).

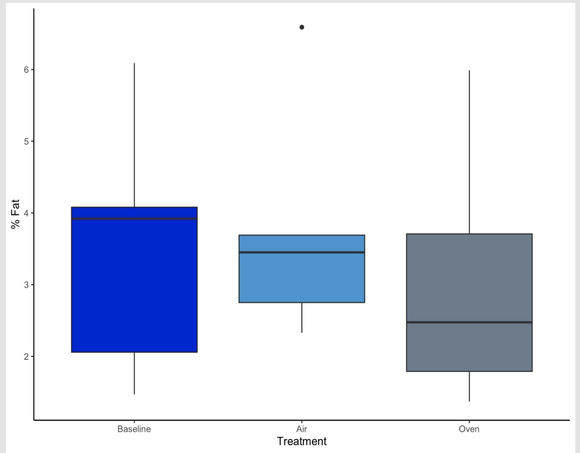

Figure 1. Crude fat content (%) of white oak (*Q. alba*) samples that were immediately processed and analyzed after collection compared to samples that were air dried with an oscillating fan for seven days or oven dried at 121°C for 30 minutes at 121º C.

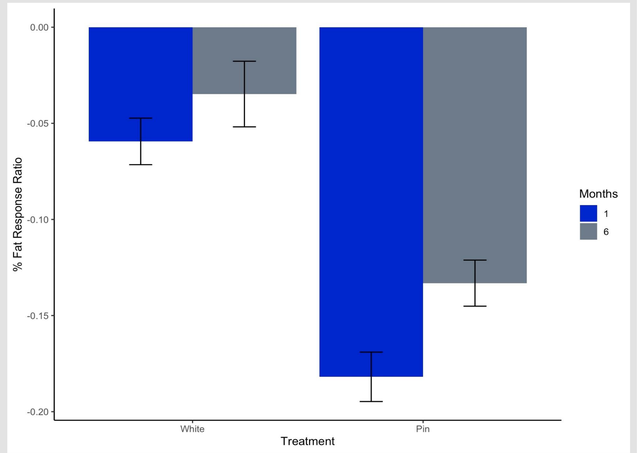

Figure 2. Crude fat response ratio (%) across eight white oak trees (*Q. alba*) acorn samples and six pin oak trees (Q. Palustris) acorn samples stored for one and six months. The response ratio was calculated by comparing the baseline percent fat concentration directly after collection from the tree to the percent fat concentration after storage one month and six months. Error bars represent standard error.

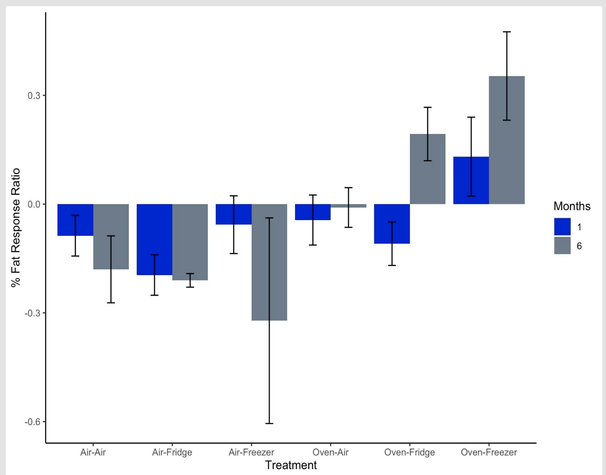

Figure 3. Crude fat response ratio (%) across six white oak trees (*Q. alba*) after drying and storing acorn samples for one month and 6 months. Acorns were air dried with a fan current for seven days or were placed in the oven at 121ºC for 30 minutes. Subsamples of each tree were analyzed after one month and 6 months of storage at room temperature, in a refrigerator, or in a freezer. Error bars represent standard error.

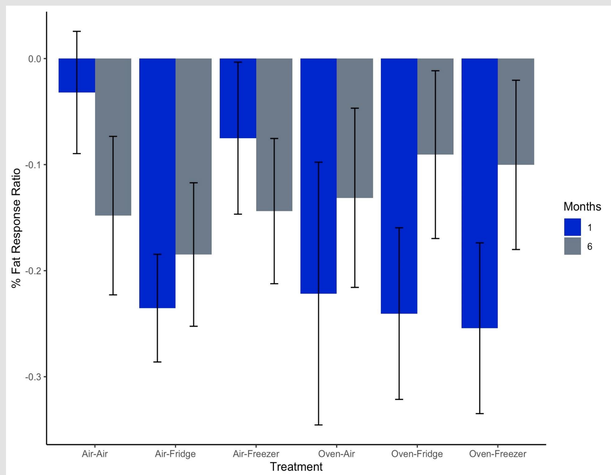

Figure 4. Crude fat response ratio (%) across eight pin oak trees (*Q. palustris*) after drying and storing acorn samples for one month and 6 months. Acorns were air dried with a fan current for seven days or were placed in the oven at 121º C for 30 minutes. Subsamples of each tree were analyzed after one month and 6 months of storage at room temperature, in a refrigerator, or in a freezer. Error bars represent standard error.

Discussion

Aligning with published values (Kirkpatrick and Pekins 2002) our pin oak acorns had a higher crude fat content than our white oak acorns and supports observations that squirrels store more red oak (of which pin oak is included) than white oak acorns (Wood 2005). The wide variability within a species both between trees and between acorn subsamples within a tree, however, suggests that handling of acorns does not consistently or predictably affect nutritional degradation. Because there is no way to repeatedly test the same exact acorns, subsampling was our only option. We did find a significant difference in drying method after white acorns were stored for six months, regardless of storage method (Figure 3). This result could mean one of two things. First, because pin oak acorns remain dormant until the spring after falling (Fox 1982) we would not expect to see any changes in nutritional content till after six months of dormancy. White oak acorns, on the other hand, begin germination immediately after dropping from the tree (Fox 1982). It is possible that oven drying slows the germination process enough to see effects by six months. More likely, however, the variability among trees and acorns resulted in an effect of drying by chance. The acorns dried using the oven and stored in the refrigerator and freezer show a positive net gain in fat after six months (Figure 3) suggesting that these subsamples had higher fat content to begin with compared to the baseline subsamples.

Recommendations. Given the unpredictable and variable nature of acorns, wildlife rehabilitators that 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. Freezing acorns will not affect the fat content of the acorns but will prevent mold growth and germination. If freezer space is not available, we suggest hanging acorns in a well ventilated area using onion bags. We do not recommend long-term storage of either species in the refrigerator because of the high potential of mold growth and white oak germination in the stagnant, humid air of a refrigerator. Rehabilitators can dry either species in an oven at 121ºC for 30 minutes before storage if they wish to avoid the emergence of weevil parasites (McShea and Healy 2002) when storing at room temperature, but this is not necessary when freezing acorns.

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