Influence of Pecan Nut Pretreatment on the Physical Quality of Oil Bodies

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1. Introduction

In many oil-rich seeds, as well as in nuts, the lipids are stored in the form of small-sized, discrete, spherical organelles called oil bodies [1–3]. Oil bodies, also known as lipid bodies, oleosomes and spherosomes, serve as energy stores to support active metabolism periods and are composed of a neutral lipid matrix core, consisting mainly of triglycerides (TAGs), surrounded by a monolayer of phospholipid embedded with some unique integral proteins: oleosin, the major protein, stereolosin, and caleolosin [4–6]. The proteins, together with the phospholipids (PL), stabilise the oil bodies and maintain them as small discrete entities via surface charge and steric hindrance [6]. Intact oil bodies, with their noncoalescing nature, high polysaturated fatty acids and antioxidants content [7], and good oxidative stability [8], have been exploited for food and biotechnological and pharmaceutical applications [9–14].

Reports on isolating oil bodies can be traced back to at least the 1970s. Oil bodies have been isolated from a range of seeds: soybean [15], sunflower seed [16], almond [17, 18], walnut [19] Arabidopsis [20], and maize germ [21]. Characterisation of oil bodies extracted from different seed (rapeseed, sesame, cotton, flax, and maize) and nuts (peanut) has shown that the size of these organelles always falls within a narrow range (0.6–2.0 μm) with an isoelectric point from 5.7 to 6.6 [22]. Therefore, at neutral pH oil bodies tend to be negatively charged.

The process of oil body isolation can be divided into three steps: (1) the disruption of the seed matrix (via wet-grinding) to allow the release of oil bodies into an aqueous slurry; (2) filtration of the slurry to yield a “milk,” that is, a suspension of oil bodies in an aqueous medium, with reduced seed particulate material; and (3) concentration of the oil bodies into a cream through the centrifugation of the milk. Ding et al. [23] recognized that the type of homogenizer, the force applied, and the treatment time are all crucial factors for obtaining intact oil bodies, and the optimum parameters may differ from sample to sample. Also, the centrifuge speed can affect the size distribution of the oil bodies, by either being insufficient to promote the creaming of very small oil bodies or being too high, leading to oil body coalescence as also suggested by [21]. Presoaking of the seeds has been used to increase the yield of extraction of oil bodies, but the length of soaking time differs between the seeds/nut type and between
authors: for palm kernels a period of 72 h is needed [22], but
safflower seeds only need to be soaked overnight [24]; Tzen et al. [22] soaked soybeans for 1 h, while Chen et al. [25] soaked
soybeans for 18 h, and Iwanaga et al. [15] applied an overnight
soak. No justification for the selected length of soaking time is
often reported in the works leading to variations from author
to author. Furthermore, reported centrifugal forces used to
form the oil body-rich cream also differ significantly between
research groups, ranging from 5,000 RCF [24] to 20,000 RCF
[26].

Pecan nut [Carya illinoinensis] is one of the world’s most
popular tree nuts with a global production of 81,052 metric
tons shelled in 2013 (http://www.nutfruit.org/). Its total lipid
content is 72%, composed of approximately 6% total saturated
fatty acids (16:0, 18:0, and 20:0), 41% total monounsaturated
fatty acids (18:1, 20:1), and 22% total polyunsaturated fatty
acids (18:2, 18:3). Opalescent pecans, which occur when oil
is released due to oil body damage, were studied by
Wakeling et al. [27]. Their results showed that opalescence
first becomes evident in kernels after mechanical cracking
and increases further as processing continues. This indicates
that the mechanical force experienced during nut cracking is
causing some internal damage to the kernel; the presence of
free oil suggests that at least some of the oil bodies are ruptured
and oil is released, which can make its way to cut surfaces.

An insight into the structural differences between opalescent
and nonopalescent pecan nut was offered by Wakeling et al.
[28] by taking scanning electron microscopy (SEM) and
transmission electron microscopy (TEM) images of pecan
nut sections. In nonopalescent pecan nuts the oil bodies’
structure is preserved; the visualisation of broken oil bodies
in opalescent pecans suggests that the membrane of the lipid
organelles had been damaged with subsequent release of oil
leading to opalescence.

Despite having developed a structural understanding of
the differences between the types of pecans, to date no paper
has been published on the recovery and characterisation
of pecan nut oil bodies. Therefore, the aim of this work
was to develop a method to extract oil bodies from pecan
nuts. The parameters investigated were the effect of nuts
pre-soaking and centrifugation force on the final size of
oil bodies. Furthermore, due to the reported sensitivity
of pecan nut oil bodies to mechanical forces, our work
aimed at characterising oil bodies extracted from opalescent
and nonopalescent pecan nuts, where the former are nuts
which have experienced mechanical damage. The particle size
of recovered oil body emulsions was measured, and light
microscopy images were obtained to evaluate the quality of
the oil bodies.

2. Materials and Methods

2.1. Materials. Pecan nuts with shells were purchased from
Whole Food Online and were used as a raw material for
the aqueous extraction of oil bodies. The pecan nut kernels were
obtained using a hand cracker to remove the shell. Care was
taken to avoid mechanical damage to oil bodies during nut
shelling; occurring mechanical damage was manifested as an
opalescent effect on exposed surface.

All chemicals used in this study were of analytical grade
or above purity and sourced from Sigma Chemical Company
(St. Louis, MO) unless otherwise stated. Deionised water
was obtained using a Nanopure Water System (Nanopure Infinity,
Barnstead International, IA) and used to prepare all solutions
and emulsions.

2.2. Oil Body Extraction from Nonopalescent Pecan Nut. Oil
bodies were isolated using an aqueous extraction method
employing a grinding medium of 0.1 M Tris-HCl pH 8.0
containing 1 mM EDTA and a washing buffer of 1 mM
NaHCO₃ containing 1 mM EDTA pH 8.0. Soaking time
and centrifugal force were altered according to individual
experimental parameters (Section Effect of Nut Soaking Time
and Effect of Centrifugal Force).

The nonopalescent pecan nut kernels (bright white inte-
rior kernels) were initially broken into small pieces by hand
and then soaked in deionised water (1:10 w/v) at 4°C for a
length of time dependent on the experimental parameters.
Sodium azide (0.02 M, 0.05% volume of soaking water) was
added to avoid microbial spoilage. The soaked pecan nuts
were then ground in the grinding medium (1:10 w/v) with two
drops of 0.02 M sodium azide) in a Kenwood blender (BL315)
at full power for 2 minutes. After blending, the slurry was
filtered through three layers of cheesecloth. The filtrate was
then centrifuged (Beckman J2-21 centrifuge, fixed rotor JA-
10) for 20 minutes at 4°C. The centrifugal force was varied
with experiments. The resulting upper layer was collected as
the crude oil bodies (COBs) for analysis. The COBs were then
washed twice with washing buffer (1:4 w/w) followed by two
rinses with deionised water (1:4 w/w), through resuspension
(shake gently first and then vortex for 2 minutes at the low
level) and centrifugation at 2885 RCF (JOUAN CR3i 4 x
280 mL multifunction centrifuge) for 20 minutes at 4°C to
remove extraneous proteins and contaminants. The resulting
upper layer was collected as the washed oil bodies (WOBs)
for analysis.

2.3. Effect of Nut Soaking Time. In order to evaluate the effect
of soaking time on the size of the recovered oil bodies, pecan
nut pieces were soaked in deionised water for 0 h, 2 h, 6 h,
12 h, 24 h, and 72 h at 4°C. After blending and filtering, the
filtrate was centrifuged at 5500 RCF for 20 minutes at 4°C.

2.4. Percentage of Weight Change of Pecan Nuts during
Soaking. To quantify the water absorbed, the mass of pecan
nuts was measured at different time points. To calculate the
percentage weight change, we use the following equation:

\[
\text{Weight Change (\%)} = \left( \frac{\text{weight of soaked nuts} - \text{weight of dry nuts}}{\text{weight of dry nuts}} \right) \times 100 \%
\]

2.5. Conductivity of Soaking Water. The conductivity of soaking
water at 4°C was directly measured using a conductivity
2.6. Effect of Centrifugal Force. To evaluate the effect of the centrifugal force on oil bodies size, the pecan nut kernels were soaked in deionised water for 24 hours. After blending and filtering, the filtrate (i.e., the “milk”) was transferred into four centrifuge tubes and centrifuged separately using different centrifugal forces: 3000 RCF, 5500 RCF, 7500 RCF, and 10000 RCF, for 20 minutes at 4°C.

2.7. Emulsion Preparation for Physical Properties Characterisation. The oil bodies emulsions were prepared by mixing 1 g (wet weight) of COB or WOB cream with 9 g of deionised water using the Wheaton Potter Elvehjem Tissue Grinder (Safe-Grind 55 mL, Fisher Scientific). The emulsions were then used directly for light microscopy visualisation and particle size analysis.

2.8. Oil Body Extraction from Opalescent Pecan Nut. Opalescent pecan nut kernels, characterised by a milky iridescence [27], were broken into small pieces by hand and then soaked in deionised water for 24 h (1:10 w/v) at 4°C. After blending and filtering, the filtrate was then centrifuged at 5500 RCF for 20 minutes at 4°C to recover the COBs. Washing to obtain the WOBs was done as referred in Oil Body Extraction from nonopalescent pecan nut.

2.9. Light Microscopy Visualisation of Oil Bodies. The microstructure of all oil body emulsions was investigated using light microscopy (EVOS inverted microscope, UK). Oil body emulsions were gently shaken before measurement to ensure homogeneity. A small drop of oil body emulsion was then placed on a glass slide, covered with a cover slide, and imaged immediately at a magnification of 40x.

2.10. Particle Size Analysis of Oil Body Emulsions. The volume weighted mean diameter ($D_{4,3}$) and the surface weighted mean diameter ($D_{3,2}$) are commonly used to measure particle sizes. $D_{4,3}$ is used throughout the paper as this parameter is sensitive to the presence of large particles, whereas $D_{3,2}$ is more sensitive to the presence of smaller particles [29].

The particle size distribution of oil body emulsions was measured using a laser diffraction particle size analyser (LS 13 320, Beckman Coulter, High Wycombe, UK). One drop of samples was mixed into 125 mL dispersing liquid and circulated inside the Universal Liquid Module. The diffraction data was analysed using the Fraunhofer diffraction model. The distribution curve and volume-mean diameter ($D_{4,3}$) were measured; readings were taken in triplicate for each sample and averaged.

2.11. Statistical Analysis. Extraction experiments were performed in triplicate. Statistical analysis to evaluate significant differences was performed by one-way ANOVA ($P < 0.05$).
change of soaked pecan nuts increased dramatically from measurements over time (Figure 5). The percentage weight bodies’ size was gained by water uptake and conductivity of the majority of large droplets in washed oil body emulsions absence of the peak at approximately 20 of little amount of free oil. This hypothesis is supported by the destabilisation and loss of those large droplets in the form of net water uptake is zero. If membrane disruption and subsequent conductivity increase are caused by enzymatic activity, then conductivity may be an indirect measure of water-induced enzyme activation in the seed. The risk associated with prolonged soaking times is that the activated enzymes may start destabilising the oil bodies to produce the energy needed on germination. Nevertheless, in this study the size of oil bodies is unaffected by prolonged soaking time (72 h).

The results from the presoaking experiment strongly suggest that softer nuts yield fewer damaged oil bodies. With increasing soaking time, the plasticisation of the nuts should increase, and consequently the mechanical forces experienced by the oil bodies during grinding should decrease. This would ultimately result in less damaged oil bodies with a narrower size distribution as observed for soaking times above 24 h. In order to understand in more depth the effect of nut plasticisation on oil bodies’ size, it would be important to measure the mechanical properties of pecan nuts during soaking. An attempt was made to study pecans mechanical properties; nevertheless, the irregular shape of pecan nuts presents a challenge to these measurements and these aspects may be investigated in the future.

3.2. Effect of Centrifugal Force on Physical Quality of Pecan Nut Oil Bodies. The purpose of this series of experiments was to determine the influence of centrifugal force on the physical stability of pecan nut oil bodies. The recovery forces studied were 3000 RCF, 5500 RCF, 7500 RCF, and 10,000 RCF, and all pecan nuts were presoaked for 24 h to minimise damage experiences during grinding.

The average diameter ($D_{4,3}$) of COBs was approximately 3.3 $\mu m$ when recovery forces of 3000 RCF, 5500 RCF, and 7500 RCF were used (Figure 6), which is similar to the size of pecan nut oil bodies in vivo, as estimated through SEM/TEM images of pecan nuts from literature [28]. When the recovery force was increased to 10,000 RCF, significant differences ($P < 0.05$) were observed as $D_{4,3}$ of COBs increased to 4.8 $\mu m$ (Figure 6). This suggests that, due to high centrifugal force, oil bodies may aggregate and eventually coalesce during the centrifugation process. The particle size distributions of COBs (Figure 7(a)) also indicate the appearance of larger droplets when the 10,000 RCF recovery force was used: the

common range reported in literature for oil bodies extracted from oil-rich seeds (0.5–2.5 $\mu m$) [22, 30].

The average $D_{4,3}$ of washed oil bodies (WOBs) was comparable across all oil body emulsions independently from the length of soaking (Figure 1). The size distribution curves for WOBs (Figure 6(b)) appear narrower compared to those obtained for the COBs, which indicates that the washing step is removing some of the larger droplets. We hypothesise that the largest droplets (above 15 $\mu m$, Figures 3(a) and 3(b)) may be coalesced oil bodies formed on grinding and weakly stabilised by interfacially active materials (proteins, micron size particle material, and phospholipids) released and/or produced on pecans milling. This interfacial material could be easily removed on COBs washing, which would result in the destabilisation and loss of those large droplets in the form of little amount of free oil. This hypothesis is supported by the absence of the peak at approximately 20 $\mu m$ (Figure 2(b)) and of the majority of large droplets in washed oil body emulsions produced following 0 h of soaking (Figure 4).

A better understanding of the effect of soaking on oil bodies’ size was gained by water uptake and conductivity measurements over time (Figure 5). The percentage weight change of soaked pecan nuts increased dramatically from 2 h to 6 h and from 22% to 33% and continued to increase from 6 h until 24 h, although to a smaller extent (from 33% to 37%). After 24 h no significant increase occurred. This result suggests that if the soaking time is below 24 h, the nuts still have the capability to absorb water and might still be hard on grinding.

Another approach to evaluate the evolution of pecan nut over soaking is by measurement of the electrical conductivity of the soaking water. The conductivity test is acknowledged as suitable method to evaluate the loss of cell membrane integrity through measuring the concentration of electrolytes released by seeds during imbibition [31–33]. The deterioration of the cell membranes during presoaking may facilitate the release of oil bodies from the nut tissue. In line with the data of water mass uptake (Figure 5), water conductivity continues to increase until 24 h of soaking, that is, until the net water uptake is zero. If membrane disruption and subsequent conductivity increase are caused by enzymatic activity, then conductivity may be an indirect measure of water-induced enzyme activation in the seed. The risk associated with prolonged soaking times is that the activated enzymes may start destabilising the oil bodies to produce the energy needed on germination. Nevertheless, in this study the size of oil bodies is unaffected by prolonged soaking time (72 h).

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The average diameter ($D_{4,3}$) of COBs was approximately 3.3 $\mu m$ when recovery forces of 3000 RCF, 5500 RCF, and 7500 RCF were used (Figure 6), which is similar to the size of pecan nut oil bodies in vivo, as estimated through SEM/TEM images of pecan nuts from literature [28]. When the recovery force was increased to 10,000 RCF, significant differences ($P < 0.05$) were observed as $D_{4,3}$ of COBs increased to 4.8 $\mu m$ (Figure 6). This suggests that, due to high centrifugal force, oil bodies may aggregate and eventually coalesce during the centrifugation process. The particle size distributions of COBs (Figure 7(a)) also indicate the appearance of larger droplets when the 10,000 RCF recovery force was used: the
Figure 3: Light micrographs of crude oil body (COBs) emulsions of pecan nuts soaked for 0 h (a and b) and 24 h (c and d). The scale bars represent 100 μm.

Figure 4: Light micrographs of washed oil body (WOBs) emulsions of pecan nuts soaked for 0 h (a and b) and 24 h (c and d). The scale bars represent 100 μm.
size distribution curve produced using the highest recovery force is broader than the distribution curves obtained at the other three centrifugal forces. Furthermore, for samples recovered at 10,000 RCF another mode appeared at 25 μm (Figure 7(a)), which indicates the presence of large droplets as also shown in Figure 8(b), where many large oil bodies and oil bodies aggregates are observed. When the recovery force was lower than 10,000 RCF, the oil bodies appear as small and discrete droplets (Figure 8(a)).

10,000 RCF is a commonly reported recovery force in literature for oil bodies separation [3, 34, 35]. However, results obtained in this study show that the pecan nut oil bodies were damaged at this force. One possible explanation for the damage caused by the highest RCF force might be that the membrane tension of pecan nut oil bodies is low due to their relatively large size: since membrane tension decreases with increasing particle size [36], the pecan nut oil bodies resistance to an externally applied force is likely to be lower than that of oil bodies extracted from other lipid sources.

3.3. Oil Body Extraction from Opalescent Pecan Nuts. Opalescent pecan nuts are a type of pecan nut where at least some of the oil bodies have been broken inside the nut and, due to the oil release, the kernel exhibits a brown or caramel colour compared with the white interior of nonopalescent pecan nuts (Figure 9). The appearance of opalescence in nuts we provided in our work is in line with the current (fairly limited) literature on nuts, where the phenomenon of opalescence is considered a result simply of mechanical damage occurring at various processing stages. However, it is very likely that mechanical damage of the OBs, even if occurred at a limited extent, may cause the triggering of native enzymes and their activity would further promote OBs degradation leading to oil leak and opalescence. The opalescent pecan nuts (selected out from shelled pecan nuts) were presoaked for 24 h and the oil bodies recovered using 5500 RCF. These conditions were selected since they limit the mechanical damage of the oil bodies. $D_{4,3}$ values of both COBs (5.7 μm) and WOBs (5.2 μm) extracted from opalescent pecan nuts are larger than those for the nonopalescent pecan nuts ($D_{4,3}$ of 3.2 μm and 3.3 μm for COBs and WOBs, resp.) (Figure 10). The particle size distributions of opalescent pecan nuts were wider than that of the oil bodies from nonopalescent nuts and were clearly shifted to the right (Figure 11). Many large droplets were visible in light microscopy images for both the COBs
Figure 8: Light micrographs of crude oil body (COBs) emulsions of pecan nuts recovered using (a) 5500 RCF and (b) 10000 RCF. The arrows indicate aggregations. The scale bars represent 100 μm.

Figure 9: Image showing the contrast between (top) opalescent and (bottom) nonopalescent pecan nuts.

Figure 10: Average particle sizes of both crude oil body (COBs) and washed oil body (WOBs) emulsions of opalescent and nonopalescent pecan nuts.

Figure 11: Particle size distributions of crude oil body (COBs) and washed oil body (WOBs) emulsions of opalescent and nonopalescent pecan nuts.

Figure 12: Average particle size diameters (D4,3) of COBs and WOBs of opalescent pecan nuts. The presence of large droplets in COBs suggests that mechanical damage of oil bodies had occurred prior to wet-milling producing the opalescence effect. This result is in agreement with results from Wakeling et al. [27, 28], who demonstrated that the opalescence results from the release of oil from broken oil bodies. On washing no change in the size distribution and appearance of oil bodies can be observed (Figures 11 and 12). This result remains unexplained. Considering that the large oil bodies observed for opalescent pecans are able to endure the washing step, it could be hypothesised that these oil bodies have a different surface chemistry compared to the large droplets formed on grinding of nonopalescent nuts after limited soaking (less than 6 h).

It has been reported in literature that pecans grown in the United States developed less severe opalescence than those grown in Australia because these pecans had lower levels of calcium, coincident with higher levels of oil [27]. Pecan composition varies depending on location, climate condition, horticultural practices, cultivar, season, and maturity level [37]; hence, it is important to establish the optimum soaking.
time and centrifugal force when different pecan nut cultivars are being used for oil bodies extraction.

4. Conclusions

This work is the first to describe a method for the extraction and characterisation of pecan nut oil bodies. It was demonstrated that presoaking affects the physical properties of pecan nut oil bodies. Through the soaking experiment we found that there was a decrease in volume-mean diameter ($D_{4,3}$) of COBs with an increase in presoaking time suggesting that the plasticisation during soaking in water reduces oil bodies damage following grinding. The smallest oil bodies were extracted from nuts presoaked for 24 h and 72 h, and the size of the isolated lipid organelles was similar to that of pecan nut oil bodies in vivo. We also show that the washing step is removing the large droplets and giving narrower distribution curves compared to the COBs only when mechanical damage of the oil bodies has occurred. Evaluation of the effect of the centrifugal force showed that using 10,000 RCF recovery force induces damaging of the oil bodies with an increase of $D_{4,3}$ of COBs from 3.3 to 4.8 μm. Finally, we have provided a quantitative difference of the COBs and WOBs size recovered from opalescent and nonopalescent pecan nuts: oil bodies extracted from opalescent pecans are larger than those recovered from nonopalescent nuts, confirming that the oil bodies inside the opalescent pecan nut are damaged prior to wet-milling. From all of our findings, we can conclude that a 24 h soaking time coupled with a recovery force of 5500 RCF allows for the recovery of intact pecan nut oil bodies.

Additional Points

Intact oil bodies have been exploited for food and biotechnological and pharmaceutical applications because of their non-coalescing nature, high polyunsaturated fatty acids, antioxidants content, and good oxidative stability. The oil content of pecan nuts is around 70% and rich in unsaturated fatty acids. The method of isolating intact pecan nut oil bodies can promote the application of pecan nut oil bodies and the discussed recovery factors include soaking time and recovery force will give a reference for the oil body isolation from other materials.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

P. Jolivet, E. Roux, S. D'Andrea et al., "Protein composition of...

D. J. Lacey, N. Wellner, F. Beaudoin, J. A. Napier, and P. R. J. T. C. Tzen, Y.-Z. Cao, P. Laurent, C. Ratnayake, and A. H. C. Y. Chen, L. Zhao, Y. Cao, X. Kong, and Y. Hua, "Oleosins (24..."

I. D. Fisk, D. A. White, M. Lad, and D. A. Gray, "Oxidative...


P. Jolivet, C. Deruyffelaere, C. Boulard et al., "Deciphering the structural organization of the oil bodies in the Brassica napus seed as a mean to improve the oil extraction yield;" *Industrial Crops and Products*, vol. 44, pp. 549–557, 2013.


C. V. Nikiforidis and V. Kiosseoglou, "Aqueous extraction of oil bodies from maize germ (Zea mays) and characterization of the resulting natural oil-in-water emulsion;" *Journal of Agricultural and Food Chemistry*, vol. 57, no. 12, pp. 5591–5596, 2009.


Y. Chen, L. Zhao, Y. Cao, X. Kong, and Y. Hua, "Oleosins (24 and 18 kDa) are hydrolyzed not only in extracted soybean oil bodies but also in soybean germination;" *Journal of Agricultural and Food Chemistry*, vol. 62, no. 4, pp. 956–965, 2014.

C. V. Nikiforidis, V. Kiosseoglou, and E. Scholten, "Oil bodies: an insight on their microstructure — maize germ vs sunflower seed;" *Food Research International*, vol. 52, no. 1, pp. 136–141, 2013.


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