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Enhancing the recovery of tiger nut (*Cyperus esculentus)* oil by mechanical pressing: moisture content, particle size, high pressure and enzymatic pre-treatment effects

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Abbreviated running title

High Pressure and Enzyme Pre-treatment on Tiger nut Oil Recovery by Pressing

ABSTRACT: Tiger nut (*Cyperus esculentus*) tuber contains oil that is high in monounsaturated fatty acids, and this oil makes up about 23% of the tuber. The study aimed at evaluating the impact of several factors and enzymatic pre-treatment on the recovery of pressed tiger nut oil. Smaller particles were more favourable for pressing. High pressure pre-treatment did not increase oil recovery but enzymatic treatment did. The highest yield obtained by enzymatic treatment prior to mechanical extraction was 33 % on a dry defatted basis, which represents a recovery of 90 % of the oil. Tiger nut oil consists mainly of oleic acid; its acid and peroxide values reflect the high stability of the oil.

Keywords: Tiger nut oil, pressing, particle size, enzymes, polyphenol

1. INTRODUCTION

Tiger nut or yellow nutsedge (*Cyperus esculentus)* is a perennial herb from rhizomes with hard tubers at its endings. Its use dates back to ancient Egypt (Yeboah, Mitei, Ngila, Wessjohann, & Schmidt, 2012), while today they are cultivated in Africa, Spain, and North America. Tiger nuts are often consumed raw, roasted or ground to make beverages. There are various applications of the plant which includes the use of its extract in the cosmetic industry and production of gluten free flour.

The oil fraction of tiger nut tuber is comparable to olive oil in its fatty acid profile and is dominated mostly with oleic acid (Linssen, Kielman, Cozijnsen, & Pilnik, 1988). It is considered to be a superior edible oil due to its stability and nutritional quality. Tiger nut oil (TNO) contains a high proportion of unsaturated fatty acids, vitamin E and phenolic compounds. Its phytosterol content, especially stigmasterol and campesterol, is higher than that of olive oil, which allows the two oils to be differentiated (Sánchez-Zapata, Fernández-López, & Angel Pérez-Alvarez, 2012). Other properties of TNO have also been investigated such as its potential for biodiesel (Ali Rehab & El Anany, 2012).

Currently, tiger nut oil is extracted and sold as cold pressed oil**.** For research purposes, TNO is extracted either using a laboratory press or solvent extraction with n-hexane (Ali Rehab & El Anany, 2012; Yeboah, Mitei, Ngila, Wessjohann, & Schmidt, 2012). Despite the higher recovery of oil achieved with solvent extraction (over 95%) as reported by Rosenthal and Niranjan (1996), there remains apprehensions regarding sustainable availability of petroleum based solvents, as well as the contribution of these solvents to the emission of volatile organic compounds. To overcome this problem, other methods of oil extraction have been investigated and re-visited along with pre-treatment effects on the yield of the extracted oil. Examples include employing enzymes and applying high hydrostatic pressure treatment. Enzymes are used to degrade cellular wall components such as cellulose, and pectin and this

facilitates oil release from the cells. They are commonly used in aqueous extraction processes where they have been found to significantly increase oil recoveries from oil seeds. Peanuts, soybeans, pumpkin, and horse radish seeds are some materials that have benefitted from the use of enzymes in aqueous extraction (Mat Yusoff, Gordon, & Niranjan, 2015). Only a few studies have implemented use of enzymes prior to mechanical oil extraction despite the potential benefits it may offer. With mechanical presses, there is no difficulty of de-emulsification that arises with aqueous extraction and this eliminates an additional processing step.

Mechanical separation of oil from oil seeds can be done either using expellers (screw press) or hydraulic presses. The high quality oil obtained is one of the reasons why these are continually being used especially as there is an increasing niche market for novel oils. In some rural areas, it also remains the sole method of oil extraction. But it still is an inefficient process. Depending on the equipment used, authors have conducted studies to increase oil recoveries and optimize the process by varying operational variables like temperature, applied pressure and time (Adeeko & Ajibola, 1990; Ajibola, Eniyemo, Fasina, & Adeeko, 1990). Sample preparation must also be taken into account, as pre-treatments such as the application of extrusion, and enzymes are employed (Nelson, Wijeratne, Yeh, Wei, & Wei, 1987; Smith, Agrawal, Sarkar, & Singh, 1993). Generally these treatment lead to increases in oil yields because they tend to either soften and/or destroy cellular structure thus aiding the extraction. When hydraulic presses are used, important parameters that have been observed to influence oil yields are moisture content of sample, temperature, maximum applied pressure, and particle size.

Extraction of oil from tiger nut has not been researched extensively and has very few reported studies. A majority of growers of the tuber reside in African countries and they stand to benefit from more research in this area especially with the multiple uses of the oil.

Potentially, it could substitute for the more expensive imported olive oil in these countries. In addition, the oil could be employed to improve the diets of consumers in penurious areas. Consumption of a blend of coconut and tiger nut oil for instance has been shown to control the total plasma cholesterol levels in albino rats and also reduce their LDL- cholesterol levels (Ali Rehab & El Anany, 2012).

There is a paucity of research reports on techniques for extraction of tiger nut oil or optimising the yield of oil. However a study of the effects of extraction parameters on oil yield when using supercritical CO_2 was recently reported (Lasekan & Abdulkarim, 2012). There are also a few studies on enzymatic aqueous oil extraction. In terms of pre-treatments, high pressure is not commonly used prior to oil extraction. It is commercially used in pasteurization and food preservation as the high pressures applied inactivate microbes, spores, and spoilage inducing enzymes (Ly-Nguyen, Van Loey, Smout, ErenÖzcan, Fachin, Verlent, et al., 2003). It has though been used along with enzymatic aqueous extraction of soybean flakes (Uhm & Yoon, 2011). This study aims to investigate the impact of moisture content in the tubers, particle size, high hydrostatic pressure and enzymatic pre-treatment prior to oil extraction by mechanical pressing on the recovery of tiger nut oil. Oil quality parameters are reported for the pressed oil (without enzymatic treatment).

2. MATERIALS AND METHODS

2.1 Samples

Dried brown tiger nuts from Spain, purchased from Real Foods, Edinburgh UK were used for all experiments.

2.2 Chemicals

Fatty acid methyl ester standards, α-tocopherol standard, gallic acid, methanol, alcohol oxidase (from *Pichia pastoris*), purpald (4-amino-3-hydrazino-5-mercapto-1, 2, 4-triazole), phosphate buffer, safranin and sodium hydroxide were purchased from Sigma-Aldrich (Dorset, UK). All chemicals were of analytical grade.

2.3 Sample preparation and treatments

The initial moisture content of tiger nuts was measured using an infrared moisture analyser (Sartorius, Surrey UK) and this was given on a wet basis (wb). They were then soaked in distilled water at room temperature for 6 h, followed by drying in a conventional oven at 70 ^{o}C for 13 h. A preliminary study was carried out to obtain a drying curve and used to determine the time taken to achieve an initial moisture content of 4% (wb) for all samples. The samples with the desired moisture content were prepared by adding calculated amounts of distilled water using equation 1 (Mwithiga & Sifuna, 2006) and mixed thoroughly. The weighed tubers were placed in polyethylene bags and kept in a refrigerator for a minimum of 48 h to establish uniform moisture content. Prior to oil extraction, they were withdrawn and left for 2 h at room temperature to equilibrate.

$$Q = \frac{w_i(m_f - m_i)}{(100 - m_f)} \qquad \text{(Equation 1)}$$

where Q = mass of water to be added in grams, w_i is the initial mass of the sample in grams, m_i and m_f are the initial and desired final moisture contents in percentages (wb), respectively.

157 The prepared samples prior to pressing were ground in a coffee mill and sieved with three
158 ASTM testing meshes to produce average particle sizes of ≤1.16 mm, ≤0.841 mm and ≤0.5
159 mm.

160 **2.3.1 High Pressure Processing**

161 Tiger nuts were dried for 1.5 h or until the moisture content was between 6.5-8 %. They were
162 then ground, and sieved to a particle size of ≤0.85 mm. 30 g of the samples were vacuum
163 sealed in polyethylene bags and placed in a pressure vessel (Stanstead Fluid Power, Ltd) to be
164 subjected to pressures of 50, 300, 500 and 700 MPa (15 min holding time, 40 ^{o}C). Whole
165 tubers in 0.5 M citric acid were also high pressure treated. A preliminary study found no
166 interaction between pressure, temperature and time on the yield. A mixture of water and 1, 2-
167 propanediol (70:30, v/v) served as the pressure transmitting fluid. The adiabatic temperature
168 rise during the pressure treatment was 3.3 ^{o}C per 100 MPa.

169 **2.3.2 Enzymatic treatment**

170 Protease (from *Bacillus licheniformis*), α-amylase (*Bacillus licheniformis*) and Viscozyme L
171 (hemi cellulolytic enzyme mixture from *Aspergillus*) were purchased from Sigma-Aldrich,
172 UK. A combination of all three enzymes was used (1:1:1). Enzymes and their hydrolysis
173 conditions were based on specifications given by suppliers and experiments done in our
174 laboratory. Whole tiger nut tubers were soaked in distilled water for 6 h, ground and sieved to
175 a particle size of ≤0.425 mm. Enzymes of varying total weights (0.15g, 0.30g, 0.45g) were
176 added to 50 ml of distilled water, 30 g of ground tiger nut sample, and pH was adjusted to 8
177 using 0.5 M NaOH. Incubation was carried out for 6 h at 40 ^{o}C in a water bath with a linear
178 agitation speed of 180 strokes per min. After incubation, the mixture was dried in a vacuum
179 oven till the moisture content was between 6.5 - 8 %. Temperature in the oven was 55 ^{o}C
180 while the maximum pressure reached was 700 mm Hg. Following drying, oil was extracted
181 by pressing.

2.4 Mechanical Pressing of Tiger nut oil

Tiger nut oil was obtained by double pressing 30 g of ground tiger nuts with a hydraulic laboratory press (Specac, Ltd UK). A maximum pressure of 38 MPa was exerted due to the limitation of the strength of the nylon sieve material used. The samples were placed in the sieve and then in a metal chamber. The total time for pressing was between 40-50 s. Hexane was used to collect the expressed oil and recovered in a rotary evaporator. The amount of oil extracted was measured gravimetrically and stored in an amber glass bottle for analysis.

Hexane extraction was carried out to determine the total extractable oil in tiger nuts and to measure total oil recovery. The total oil content was measured gravimetrically from 10 g of ground tiger nuts extracted with 150 ml hexane for 6 h in a Soxhlet unit. Hexane was recovered in a rotary evaporator. Residual solvent was removed in an oven at 105 ^{o}C for 15 min and the residue was cooled in a desiccator. Throughout the work, oil recovery is synonymous with oil yield.

Pressing after enzymatic treatment was carried out in 30 min. Controls consisted of pressing for 30 min without enzymatic pre-treatment.

2.5 Cell evaluation

2.5.1 Methanol content in tissues

A spectrophotometric method was used to determine methanol content (Gonzalez, Jernstedt, Slaughter, & Barrett, 2010). Pectin methyl esterase activity was assayed by determination of the amount of methanol present in the tissues. Methanol is enzymatically oxidized to formaldehyde with alcohol oxidase and calorimetrically determined with Purpald (4- amino-3-hydrazinio-5-mercapto-1,2, 4 triazole). Ground tiger nut sample (1g) was vortexed with 50 % trichloracetic acid (TCA) and distilled water in the ratio 1:2:1. The mixture was centrifuged and the oxidation was begun by adding 0.25 ml of the vortexed mixture to 0.9 ml of 100 mM phosphate buffer (pH 7.5), 0.75 ml supernatant, 0.5 ml distilled water, and 1 ul

alcohol oxidase (27 U/mg protein, 42 mg protein/ml). The samples were incubated in a water bath at 30 ^{o}C for 10 min after which 2 ml of 5 mg/ml Purpald in 0.5 M NaOH was added and the mixture was left for an additional 30 min. At the end of this period, 6 ml of distilled water was added and the absorbance at 550 nm was measured.

2.5.2 Confocal Light Scanning Microscopy

The staining method reported by Sineiro, Domínguez, Núñez and Lema (1998) was adopted and modified. Ground tiger nut samples were mounted on glass slides using Evo-Stik rapid set adhesive. Cell walls were stained in Safranin solution for 1 min (10 g safranin in 155 ml 95 % ethanol and 145 ml distilled water; this was diluted 1:1 with 50 % ethanol). Sections were rinsed afterwards with distilled water and observed under a Leica SP2 Inverted Confocal Microscope (Carl Zeiss) operating in confocal mode. A Leica 10x/0.3 HC PL Fluotar dry lens (Carl Zeiss) was used. An Argon laser (488 nm, 496 nm and 514 nm excitation) provided the incident light and emission bandwidth set from 525 to 606 nm. The obtained images were 1024 × 1024 pixels in size and were scanned at various zoom factors to obtained desired magnifications.

2.6 Oil Analysis

Non-treated pressed oil was used in all oil analysis.

2.6.1 Fatty Acid Content

The pressed oil was analysed for fatty acid composition by Gas Chromatography (Agilent HP 6890 fitted with FID). Fatty acid methyl esters were prepared by saponification as described in the International Union of Pure and Applied Chemistry method 2.301 (Dieffenbacher & Pocklington, 1992). The esters were analysed using fused silica capillary column Varian CP-Sil 88 (50 m × 0.25 mm × 0.20 µm). The injector temperature was 250 ^{o}C; detection temperature was 260 ^{o}C and oven temperature was initially 100 ^{o}C, held for 3 min and

ramped to 240 ^{o}C at 4 ^{o}C per min. The carrier gas was hydrogen at a flow rate of 0.8 ml/min. The fatty acids were identified by comparing retention times with those of standards.

2.6.2 Acid and Peroxide Values

Acid value (AV) and peroxide values were determined according to Cd 3d-63 and Cd 8b-90 AOCS official methods respectively (Firestone, 1998).

2.6.3 Tocopherol Content

For tocopherol extraction and analysis, the procedure described by Costa, Ballus, Teixeira-Filho and Godoy (2010) was followed. Analysis was performed with a HPLC-UV system (Agilent 1200, Manchester, UK) using a Nucleosil C-18-100 reverse phase column (25 cm × 4.6 mm i.d.) with a particle size of 5 µm (Macherey-Nagel, Duren, Germany). Dilute concentrations of α–tocopherol standard were prepared by dissolving in methanol. Tocopherol was identified by comparing the retention times with those of the standards and comparing the absorption spectra obtained by the DAD. An external calibration was used for quantification.

2.6.4 Total Phenolic Compound Analysis

The extraction of phenols was carried out using liquid-liquid extraction with methanol as solvent. The procedure reported by Baiano, Gambacorta, Terracone, Previtali, Lamacchia, and La Notte (2009) was followed. 2 ml of methanol/water (70:30, v/v) and 2 ml of hexane were added to 5 g of tiger nut oil and vortexed for 10 min. The organic phase and the aqueous phase were separated by centrifugation (6000 rpm, 4 ^{o}C, 10 min). The aqueous phase containing the phenolics was collected and centrifugation was repeated (13000 rpm, room temperature, 4 min). Finally, the aqueous phase was collected with a pipette for analysis. Total phenolic content was quantified using a spectrophotometric method (Stanković, 2011). The hydroalcoholic extract or blank methanol sample (0.5 ml) was mixed with 10% Folin-Ciocalteu reagent (2.5 ml) dissolved in water and 7.5% Na_2CO_3 (2.5 ml). The mixtures were

incubated at 45 ^{o}C for 45 min and the absorbance was measured using a spectrophotometer at 765 nm. A standard curve was prepared using standard diluted solutions of gallic acid in methanol. Total phenolic content is expressed as milligrams of gallic acid equivalents (GAE) per kg of oil.

2.7 Statistical Analysis

All analysis was done in triplicate and the mean values are presented. Statistical analysis was carried out by ANOVA using SPSS Version 20 Statistical software (SPSS Inc, Chicago, USA). Significance was defined at $p<0.05$.

3. RESULTS AND DISCUSSION

3.1 Effects of Moisture Content and Particle Size

The total extractable oil in the tiger nut tuber was 23.1% (w/w) or 35.5 % on a dry defatted basis (d.d.b) taking into account the initial moisture content of the samples. Thus it is a low oil bearing material, similar to soybean (18-20%) (Nelson, Wijeratne, Yeh, Wei, & Wei, 1987). The lipid content falls within the range of 22.8-32.8 % reported in literature (Sánchez-Zapata, Fernández-López, & Angel Pérez-Alvarez, 2012) .

Reducing the particle size was necessary to increase the oil recovery (Figure 1). These results were in agreement with those obtained for ground melon seeds (Ajibola, Eniyemo, Fasina, & Adeeko, 1990) and in contrast to results obtained for peanuts. Finely ground melon seed particles (1.10 mm) were found to give higher oil yields compared to coarsely ground particles (1.85 mm). In contrast, peanut oil yield was increased when the particle size was increased (Adeeko & Ajibola, 1990). Particle size is known to play a role in oil extraction processes such as solvent and aqueous extraction (A Rosenthal, Pyle, & Niranjan, 1998). In these techniques the smaller the particle size, the higher the oil recovery because of an increase in surface area allowing for more contact between the solvents and the oleaginous

material. The pressing operation has been described as being analogous to a capillary filtration process, and the Hagen-Poiseuille equation below expresses this (Sorin-Stefan, Ionescu, Voicu, Ungureanu, & Vladut, 2013)

$$V\ (m3) = \frac{\pi.p.d.t}{128.\eta.l} \qquad \text{(Equation 2)}$$

Where V (m^3) - volume of separated liquid (passing through capillaries); p (N/m^2) – apparent pressure; d (m) – diameter of capillary channel; η (Pa s) – dynamic viscosity of liquid; l (m) – length of capillary channel; t (s) – time of applied pressure.

From equation 2, the volume of oil that gets released is proportional to the pore diameter and inversely proportional to the length of the capillary channel. The pore diameter and capillary channel length can be increased and decreased respectively with greater cellular destruction. This may explain the higher yields obtained with smaller particles.

Despite the fact that moisture content is a key controlling factor in mechanical oil extraction, oil yield was not significantly affected by moisture content of the samples. However, the maximum oil yields (17-18%, d.d.b) for each particle size used, were observed to occur between 6.8-8% moisture. Different oilseeds exhibit different behaviour with varying moisture levels. It was reported for walnuts and peanuts, that an increase in moisture content from 2.4 % to 7% increased oil extraction yield from 61% to 84 % while in some materials like sesame, optimum moisture content exists. Although these extraction processes were carried out in a continuous process, similar observations were noted for hydraulic presses (Savoire, Lanoisellé, & Vorobiev, 2013). In the case of tiger nuts, even though the impact of moisture level on extraction yield was found to be statistically insignificant, subsequent pressing experiments were carried within the observed favourable moisture level range. Other factors that influence oil yield include temperature and pressure. In this study, existing constraints prevented a manipulation of these factors; pressure due to the strength of the

sieving material used and temperature due to the lack of temperature control of the hydraulic press.

3.2 Effect of High Pressure Processing

Samples pre-treated with high pressure showed no significant increase in oil recovery regardless of the pressure employed (Figure 2). The high pressures (50-700 MPa) did not cause any further destruction of the parenchyma cells which had already suffered some disintegration due to the grinding process. Confocal images of control and high pressure treated samples (300-700 MPa) revealed similar cellular damage in all samples (Figure 3), thus supporting the hypothesis that HPP does not induce any damage to cell walls. Safranin was used to stain cell walls and is also known to stain lipids which explain the multiple drops in the images. Focusing on different regions of the cells showed areas with intact cell walls and some cell damage. Two microscope slides per treated sample were viewed under the confocal laser microscope.

According to Jung, Maurer & Johnson, (2009), application of high pressures (200 MPa and 500 MPa) did not result in any significant increase in oil yield following both aqueous and enzyme assisted aqueous extraction. It was suggested that high pressure treatment did not promote any cell rupture in cotyledon cells of sunflower seeds. This observation can also be used to explain the lack of an effect on tiger nut tubers. Tiger nut tubers have a tough texture, even tougher than potatoes and this characteristic was attributed to the cross linking of diferulic acid with arabinoxylans in the parenchyma cells of the tubers (Parker, Ng, Smith, & Waldron, 2000). High pressure alone is not sufficient to induce cell separation as it is only able to break weak non covalent bonds (Jung, Maurer, & Johnson, 2009). An initial hypothesis that the lack of effect of HPP on the oil yield was due to pectin methyl esterase (PME) activity often present in plant cells was considered. PME demethylates pectin molecules, releasing pectin with free carboxyl groups and methanol. Pectin precipitates in the

presence of calcium forming strong bond linkages that preserves plant tissues when HPP is applied. Studies have shown HPP as an alternative to thermal treatment to maintain membrane integrity of tissues (De Roeck, Sila, Duvetter, Van Loey, & Hendrickx, 2008; Gonzalez, Jernstedt, Slaughter, & Barrett, 2010). If this was the case, the addition of an acid may be able to combat the effects of PME, and this was investigated.

3.3 Effect of Citric Acid and Methanol Content in Cell tissues

The tubers were placed in 0.5 M citric acid during the application of high pressure (700 MPa). Citric acid was used to inhibit pectin-calcium linkages that may have formed if PME activity was present in tiger nut tissues. An increase in plant cells hardness or toughening when calcium is present has been attributed to the precipitation of pectin. Despite the addition of citric acid, tiger nut oil recovery did not increase. This suggests that pectin-calcium linkages are not responsible for the lack of cell destruction by HPP.

Parker, Ng, Smith & Waldron, (2000) attributed the toughness of cell walls of tiger nut tuber to phenolic acids, particularly diferulic acid. The toughness was recognised to be greater than that of both raw potatoes and Chinese water chestnut. These acids were suspected to form stable bonds between polysaccharides in the cell wall. It was also recommended that cell separation in tiger nut tubers may be achieved by using hot dilute acid (100 ^{o}C). High temperature was avoided because it would cause starch present to gelatinize and also affect oil quality.

The methanol content was investigated by a colorimetric method. An increase in absorbance indicates an increase in amount of methanol released. This would suggest an increase in PME activity. From Figure 4, there were no significant differences between control and all high pressure treated samples except 700 MPa. The absorbance value of the 700 MPa treated sample was observed to be lower. The action of grinding already plays a role in some cellular destruction, which may have led to PME release from within the cells and hence the observed

355 measured absorbance in control and pressure treated samples. The reduced absorbance
356 suggests a decrease in PME activity due to the high pressure. Enzymes tend to be resilient at
357 high pressures and PME is no exception. In carrots, PME was observed to have high
358 stabilities even at high pressures up to 825MPa (Ly-Nguyen, et al., 2003). In another study, a
359 high temperature, high pressure process was not able to solubilize cell walls but a low
360 pressure/high temperature combination did achieve this (0.1 MPa, 80 ^{o}C) (De Roeck, Sila,
361 Duvetter, Van Loey, & Hendrickx, 2008). PME of tiger nut may require thermal treatment for
362 complete inactivation but this was not needed for the purpose of this study.

363 **3.4 Effect of Enzymatic Pre-treatment**

364 The degrading action of enzymes significantly increased the pressed oil recovered. Tiger nut
365 is known to have a relatively high starch content of tiger nut of about 23. Cellulose also
366 makes up a large fraction of its crude fiber. The occurrence of these cell components in tiger
367 nuts would explain why α-amylase, protease and cellulolytic enzyme mixture enhanced oil
368 recovery. A confocal image with Safranin stained cell walls confirmed greater cellular
369 damage as a result of enzymatic treatment (Figure 3). An enzyme to substrate ratio of 1% was
370 found to achieve the highest oil recovery of 90%. Products from the degraded materials may
371 prevent enzymes from reaching their substrates and any additional enzyme was not
372 beneficial. The recovered oil was much higher than some reported recoveries obtained from
373 other materials via pressing such as values for soybeans (64%), or rosehip (74 %) (Concha,
374 Soto, Chamy, & Zúñiga, 2004; Smith, Agrawal, Sarkar, & Singh, 1993). In other studies
375 where higher oil recoveries up to 98 % were obtained, thermal treatments as well as longer
376 pressing times may have further improved the oil extraction. In addition, increasing pressing
377 time from 40-50 s to 30 min contributed to an 11% increase in oil yield without any
378 enzymatic pre-treatment.

379

3.5 Fatty Acid Composition

The most abundant saturated fatty acids in pressed tiger nut oil are palmitic (13.5 %) and stearic acid (6.3 %) while the major unsaturated fatty acid is oleic acid (67.4 %). The fatty acid composition is given in Table 1. Traces of myristic, gondoic, linolenic, and arachidic acids were also detected. The concentration of oleic acid is in agreement with previous studies and similar to that of olive oil (Linssen, Kielman, Cozijnsen, & Pilnik, 1988). Eteshola & Oraedu, (1996) found a rather high proportion of myristic acid (28.1 %) with a much lower oleic acid content (44.8 %). This discrepancy in values may be due to a difference in the origin of the tiger nut tubers, genetic history, the age of the tissue analysed and temperature and oxygen tension, since these variables can alter the lipid content of oilseeds (Eteshola & Oraedu, 1996). Aside from this minor difference, the composition of fatty acids is similar to those reported in a number of studies and similar to the fatty acid profiles of olive, hazelnut, macadamia and avocado oil (Sánchez-Zapata, Fernández-López, & Angel Pérez-Alvarez, 2012). As fatty acid composition is a determinant of the quality of edible oils, the high concentration of monounsaturated fatty acids (MUFA) makes it desirable due to its good shelf life and potential health benefits. The carbon double bonds in fatty acids are prone to oxidation, producing aldehydes, ketones and hydrocarbons that cause odours and flavours linked with rancidity. Hence, oxidative stability increases with decreased levels of unsaturated fatty acids, most especially PUFA (Moore & Knauft, 1989). This has been observed for olive oil and the lower PUFA content in tiger nut oil gives it the same advantage. MUFAs are much more stable and less prone to peroxidation due to their chemical structure compared to PUFAs. The above mentioned health benefits of olive oil are at least partly due to the MUFA content. Tiger nut oil can be substituted for olive oil in areas where the tuber is grown locally.

3.6 Quality Indices

The acid and peroxide values were found to be 1.2 mg KOH/ g oil and 2.1 mEq/ kg oil respectively. Acid value quantifies the concentration of free fatty acids and is an important indicator of oil quality. The low acid value obtained indicates the low level of free fatty acid in pressed tiger nut oil and thus reflects its high quality. Ali Rehab & El Anany, (2012) reported an even lower acid value of 0.31 in pressed tiger nut oil. Free fatty acids in oil occur as a result of hydrolysis which requires moisture to develop but the non-enzymic reaction only occurs at high temperatures. Lipase in the tubers may have increased the hydrolysis reaction leading to the release of free fatty acid during grinding and extraction.

The peroxide value of tiger nut oil is lower than the value determined by Yeboah, Mitei, Ngila, Wessjohann and Schmidt (2012) of 5.54 mEq/kg which was deemed reasonable as it was in accordance with Codex recommended values for virgin olive oil. Peroxide value measures the concentration of hydroperoxides, which are the intermediate products during oxidation in oil and so is used to detect the early stages of rancidity. It gives an indication of the development of oxidative rancidity in oils. The low value of 2.1 mEq/kg found in this study shows that oxidation had not progressed to a significant extent in this sample of tiger nut oil.

3.7 Total Phenolic Content

Tiger nut oil polyphenol content was 17.9 mg GAE per kg oil. This is lower than the value found by Ali Rehab and El Anany (2012) who obtained their nuts from Egypt which might explain the differences. Pellegrini, Visioli, Buratti and Brighenti (2001) reported on the polyphenol content in refined, virgin and extra virgin oils as 0.4, 1.4-2.4 and 7.3-26.5 mg GAE/ 100 g oil respectively. Soybean, sunflower and corn oils have been found to contain 6-8, 0.3-0.4 and less than 0.1 mg/ 100 g oil respectively (Valavanidis, Nisiotou, Papageorgiou, Kremli, Satravelas, Zinieris, et al., 2004). Compared with these values, tiger nut oil has

similar polyphenol content to virgin olive oil and much lower than soybean and extra virgin olive oil. The phenolic content of oils is important in assessing its antioxidant activity. These bioactive compounds play a protective role in the degradation of tocopherols during cooking processes and storage (Marfil, Giménez, Martínez, Bouzas, Rufián‐Henares, Mesías, et al., 2011). Polyphenol content and oxidative stability have been found to have a linear correlation in virgin olive oil during storage at 60 ^{o}C and polyphenol content was proposed as an indicator of olive oil quality (Gutfinger, 1981). On the basis of the high MUFA content and the moderate polyphenol content, one can expect the oxidative stability of tiger nut oil to be comparable to that of olive oil.

3.8 Tocopherol Content

The quantification of α-tocopherol is given in Table 2. It shows that tiger nut oil contained 145.7 μg/g. β-tocopherol was not quantified but was identified to be present in the oil. The total tocopherol content of tiger nut oil is thus expected to be higher than 145.7 μg/g. Yeboah, Mitei, Ngila, Wessjohann and Schmidt (2012) reported an α-tocopherol content of 86.7 μg/g in solvent extracted tiger nut oil. Tocopherol content is affected by mode of oil extraction. Organic solvents are able to penetrate the cells of the oil-containing plant material dissolving more non-polar compounds. For a crude oil, the tocopherol value obtained is higher than some olive oil values of 100-250 mg/kg, but there is a high variability in the amount of tocopherols reported (Boskou, 2008). The high tocopherol content also contributes to the stability of the oil as tocopherols acts as antioxidants. α -Tocopherol is more stable than β–tocopherol. A good correlation between tocopherol and PUFA content has also been described, suggesting that tocopherols are important in protecting them against oxidation (Quiles, Ramírez-Tortosa, Gómez, Huertas, & Mataix, 2002).

4. CONCLUSION

Small particle sizes with 6.88 to 8% moisture content were found to give higher oil recoveries. High pressure did not improve the extractability of oil and this may be due to the presence of diferulic bonds present in the cell walls. Enzyme pre-treatment on the other hand allowed for a 90 % oil recovery. The triacylglycerol profile of tiger nut oil predominantly consists of oleic acid and **78.6** % of the oil is unsaturated fatty acid. It can thus be used as a source of these beneficial fatty acids. The acid and peroxide values indicate its high stability and these were confirmed by the high polyphenol and tocopherol content. Polyphenols and tocopherols both have antioxidant capabilities, protecting oil from oxidative rancidity and prolonging its shelf life. Higher temperature and pressure were proposed to further increase the oil extracted from tiger nuts.

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Figure and Table Captions

Figure 1: Effect of moisture content and particle size on oil recovery (mean)* from tiger nuts

*Standard error = 1.51

Figure 2: Effect of High Pressure on Oil Recovery (mean)*

*Standard error = 0.03

Figure 3: Confocal images showing intact cell walls of control, and high pressure treated tiger nuts (A-D), and damaged cell walls of enzyme treated tiger nuts (E) *white arrows indicate cells walls.

Figure 4: Absorbance values (mean)* reflecting methanol content in tiger nut tissues

Standard error = 0.14

Table 1: Fatty Acid Composition

Table 2: Quality indices, tocopherol and total phenols in tiger nut oil

FIGURES

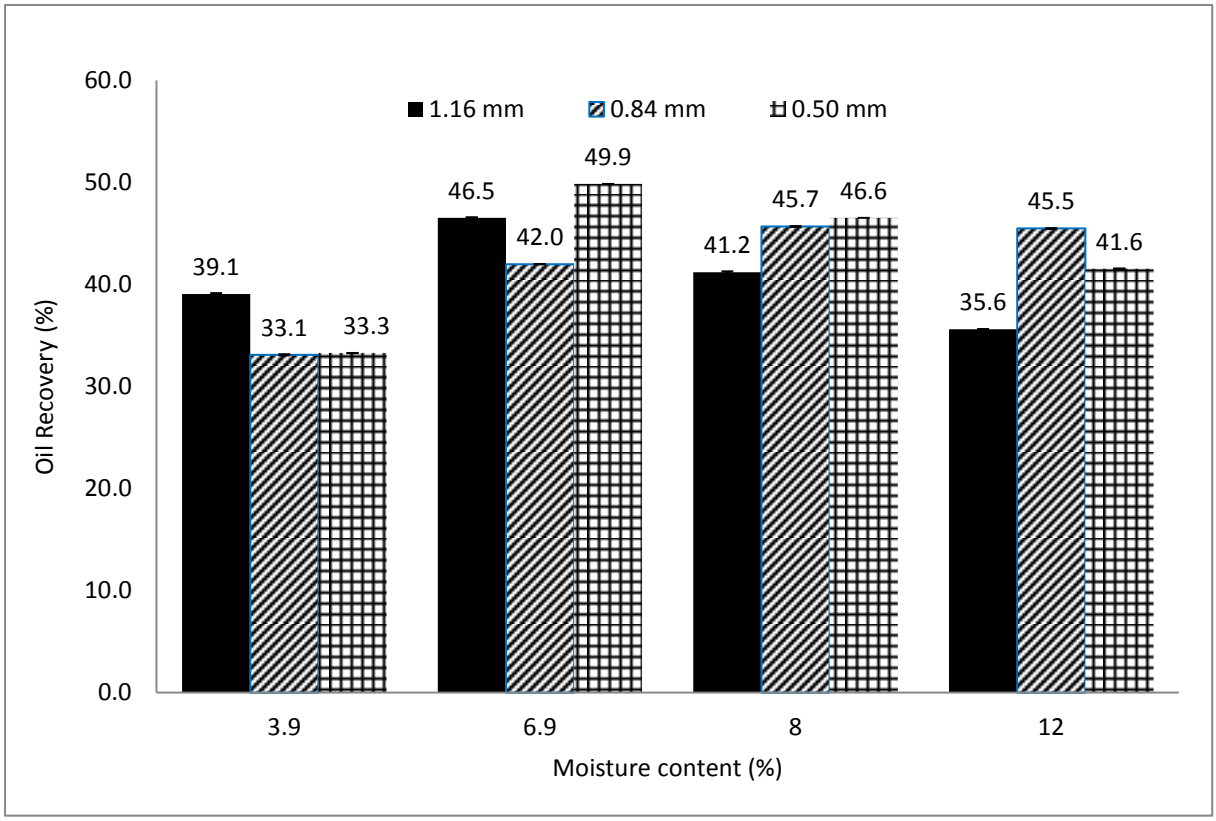

Figure 1

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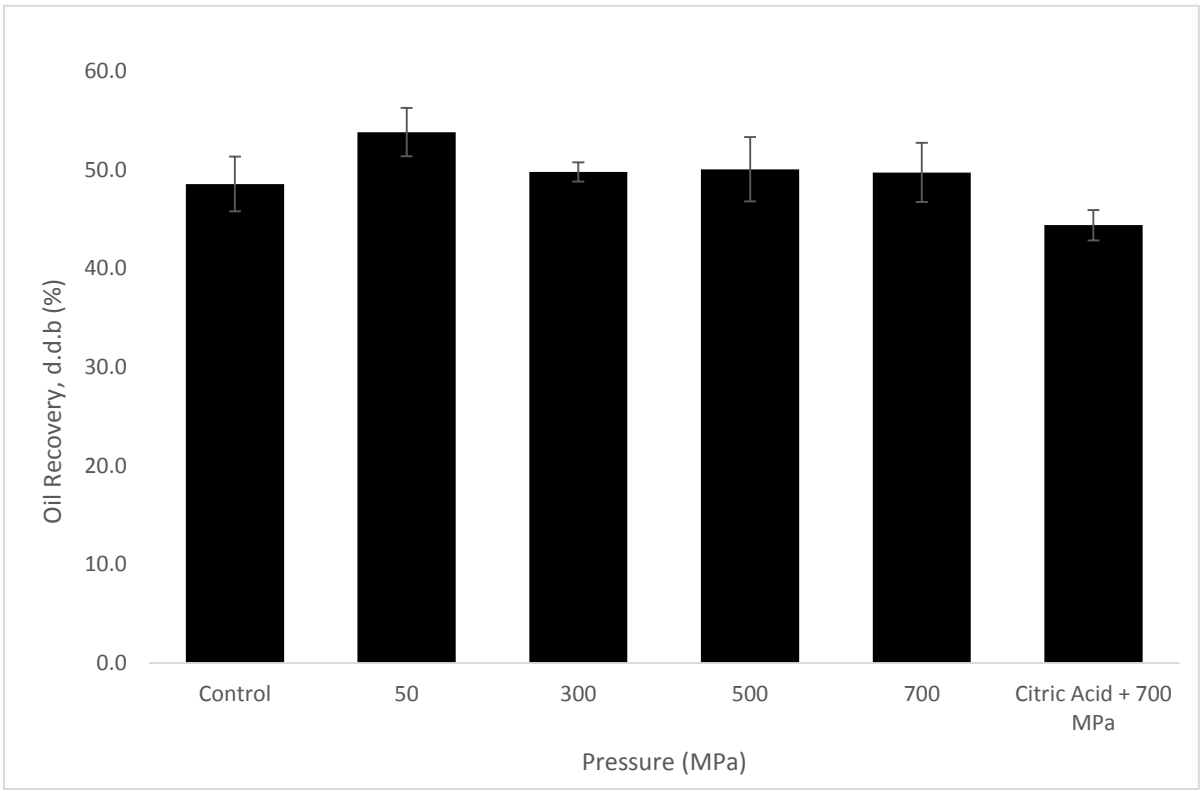

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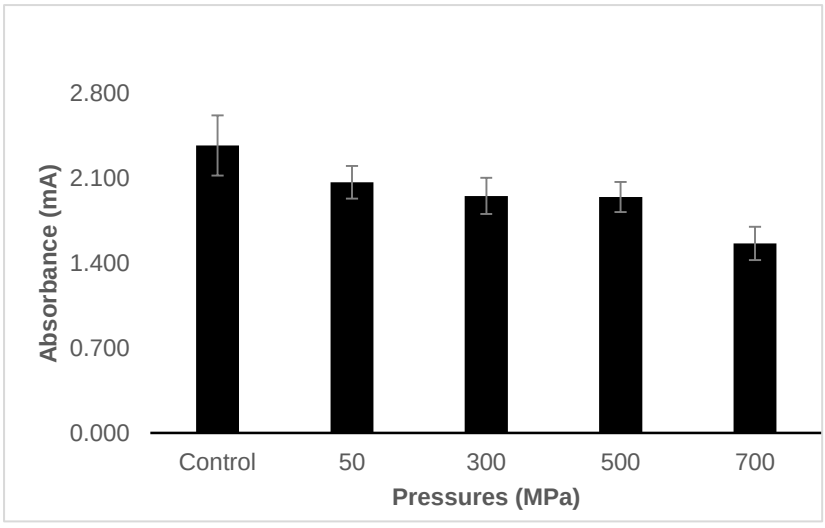

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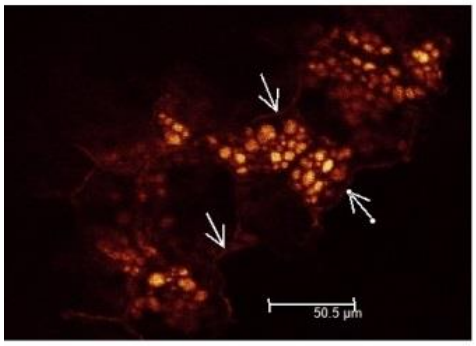
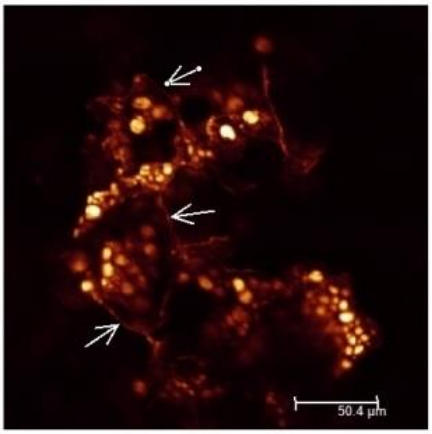
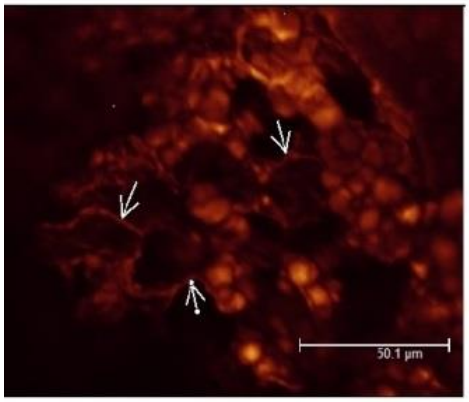


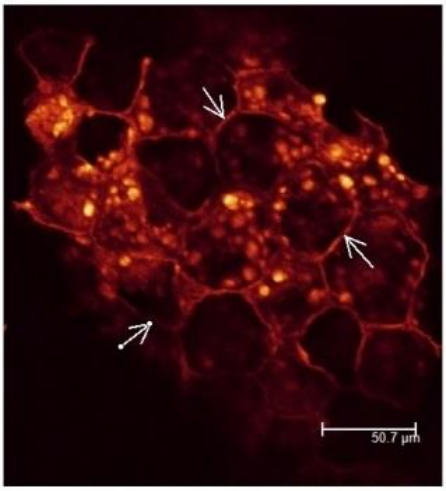
A: Control



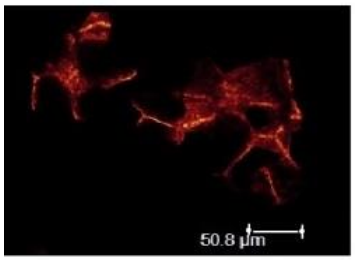
B: 300 MPa



C: 500 MPa



D: 700 MPa



E: Enzyme treated

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628 **Figure 3**

TABLES

Table 1

Fatty Acid	Tiger nut oil %
C14:0	0.10 ± 0.00
C16:0	13.5 ± 0.00
C16:1	0.3 ± 0.00
C18:0	6.3 ± 0.03
C18:1	67.4 ± 0.07
C18:2	10.7 ± 0.05
C18:3n6	0.1 ± 0.00
C20:0	0.7 ± 0.01
C20:1	0.1± 0.00
C24:0	0.2 ± 0.01
Unknown	0.4 ± 0.02

Table 2

AV (mg KOH/ g)	PV (mEq/ kg)	Total Phenols (mg GAE/ kg oil)	α-tocopherol(μg/g)
1.2 ± 0.00	2.1 ± 0.02	17.9 ± 0.04	145.7 ± 2.34