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Simultaneous extraction of oil and antioxidant compounds from oil palm fruit (*Elaeis guineensis*) by an aqueous enzymatic process

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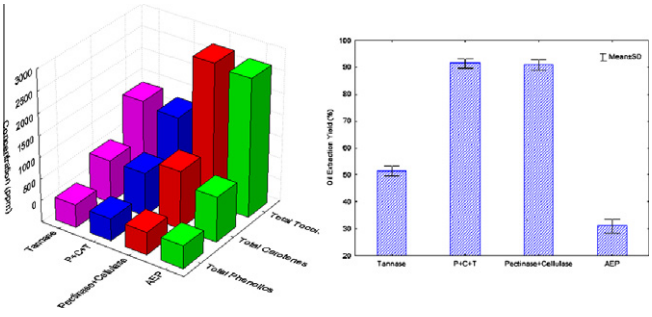
HIGHLIGHTS

- Simultaneous extraction of oil and antioxidant compounds from oil palm fruit.
- Pectinase and cellulase increased carotenes and oil recovery.
- Tannase improved the extraction of polyphenols, resulting in higher antioxidant activity.

GRAPHICAL ABSTRACT



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ABSTRACT

Oil palm (*Elaeis guineensis*) fruit was treated with enzymes to facilitate simultaneous recovery of oil and bioactive compounds. Tannase from *Paecilomyces variotii*, cellulase and pectinase were evaluated for their influence on oil recovery and antioxidant capacity (DPPH), oxidative stability (Rancimat), fatty acid profile, total phenols, total carotenoids and tocols of the oil. Maximum oil recovery (90–93% total oil) was obtained with central composite design using 4% of enzyme preparation (w/w) as 80 U of tannase, 240 U of cellulase and 178 U of pectinase, pH 4, ratio of solution to pulp of 2:1 and 30 min of incubation at 50 °C. Tannase improved the phenolic compounds extraction by 51% and pectinase plus cellulase improved carotene extraction by 153%. Samples treated with tannase showed a 27% and 53% higher antioxidant capacity for the lipophilic and hydrophilic fractions.

1. Introduction

Oil palm (*Elaeis guineensis* Jacq.) is the highest yielding edible oil crop in the world and is cultivated in 42 countries on 11 million ha worldwide (Abdul Khalil et al., 2008). Oil palm and its fractions are used in the manufacturing of cooking oil, margarines, spreads, ice creams and dairy products (Kok et al., 2011). Palm oil contains palmitic, monounsaturated oleic, polyunsaturated linoleic and stearic acids (Kok et al., 2011; Mortensen, 2005; Sampaio et al., 2011) and also minor constituents with nutritional and beneficial health properties, including tocopherols, tocotrienols, carotenoids, phytosterols, phenolic compounds and other phytonutrients (Edem, 2002; Sambanthamurthi et al., 2000).

Industrial processes for the extraction of edible oil from oil seeds generally involve solvent extraction or mechanical processing (Baryeh, 2001; Do and Sabatini, 2010); however, for oil palm extraction, a third method that can be employed is aqueous extraction (Owolarafe et al., 2007, 2008). Aqueous extraction processing (AEP) has found increasing interest due to the need for environmentally cleaner alternative technologies for oil extraction (Rosen-

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thal et al., 2001). Low oil recovery is one of the major challenges for AEP yields which can be improved when hydrolytic enzymes are applied during this process (Do and Sabatini, 2010; Lamsal and Johnson, 2007; Rosenthal et al., 1996). Greater than 90% oil extraction efficiency has been achieved for various vegetable oils (e.g., canola, soybean, peanut and coconut oils) with this approach (Lamsal and Johnson, 2007; Rosenthal et al., 1996) and the enzymatic extraction of olive oil has been reported (Aliakbarian et al., 2008; Hadj-Taieb et al., 2012; Najafian et al., 2009).

The aqueous enzyme-assisted extraction process removes phospholipids, which eliminates the degumming step from the process and reduces the overall cost of the final product (Christensen, 1991; Latif and Anwar, 2011). The extraction efficiency and quality of the oils depend on the combination of the applied enzymes (Lamsal et al., 2006). The enzymes for oil aqueous enzymatic extraction that are most frequently reported in the literature are protease, α-amylase, cellulase and pectinase (Rosenthal et al., 1996). Extraction of oil palm from the flesh of the fruit (mesocarp) using the enzymatic process has been reported recently by Rathi et al. (2012). The present study employed a new enzyme blend composed of commercial pectinase and cellulase, and non-commercial tannase which was included because of its ability to hydrolyse polyphenols releasing gallic acid and increasing the antioxidant compounds in the product.

It was evaluated the performance of the enzymes with respect to oil recovery based on oil recovery, chemical composition and quality of the obtained oil. The parameters determined were total polyphenol content, oxidative stability, fatty acid profiles, and total carotenoid, tocotrienol and tocopherol contents.

2. Methodology

2.1. Palm fruit treatment

The oil palm (*E. guineensis*) fruits were supplied by Agropalma Industries (Tailândia, Pará, Brazil). The fresh fruits were steam treated at 125 °C for 5 min). This process loosens the fruits that are still attached to the bunch. Fruits of uniform size (15.29 ± 3.0 g) and with no visible defects were packed and stored under refrigeration at −18 °C.

2.2. Enzymes

The blends of hydrolytic enzymes that were applied in the enzymatic palm oil extract were composed of Celluclast 1.5 L® (Novozymes, Paraná, Brazil) with 1800 U/g of CMCase activity, Pectinase Multieffect FE® (Genencor Int. Rochester, NY) with 1338 U/g of PG activity and tannase produced by a wild strain of *Paecilomyces variotii* (Battestin and Macedo, 2007) with 600 U/g activity.

2.3. Total lipid content

The total amount of oil palm was determined by solvent extraction (Bligh and Dyer, 1959). The solvent extraction of palm samples yielded 59.32 g oil/100 g palm pulp, which was considered to be a 100% yield for measuring the oil by aqueous extraction.

2.4. The enzyme-assisted process design

The palm mesocarp fruit was separated manually from the kernel and crushed in a food processor. Ten grams of the palm pulp was dissolved in different volumes of buffer solution (0.1 M citrate–phosphate) with enzymes in a 125-mL glass reactor in thermostatic controlled water heater. The extraction was carried at 50 °C, with constant shaking at 200 rpm for 1 h. After incubation, the enzymes were inactivated at 95 °C for 5 min and the reaction mixture was centrifuged at 2012*g* for 15 min.

To optimize the formulation of the enzymatic blend and the oil recovery; a central composite design (CCD) (2^3 full factorial design) with three replicates at the central point, combined with response surface methodology (RSM) was employed. The independent variables of the process were the enzyme concentration (% w/w) [Enzyme], buffer solution/mass sample ratio (mL/g) and solution pH (pH). The enzyme blend was composed of equal parts of each enzyme (pectinase: cellulase: tannase; 1:1:1) as a standard mixture. Each variable was examined at a high level (coded +1) and a low level (coded −1), which corresponded to the basal level ±50%, respectively. The center points were the trials under the basal level conditions (coded 0). Table 1 shows the variable combinations plus three replicates at the center point and levels in detail.

2.5. Comparison between aqueous and aqueous enzymatic oil extraction from palm mesocarp fruit

For aqueous oil extraction (AOE), 10 g of the palm pulp was dissolved in 20 mL (Dilution = 2) of buffer (0.1 M citrate phosphate, pH 4.0) in a 125-mL glass reactor in thermostatic controlled water heater. The extraction was carried at 50 °C with constant shaking at 200 rpm for 1hour. The upper oil phase was collected after centrifugation at 2012*g* for 15 min (Beckman J2-21, Beckman-Coulter, Inc., Fullerton, CA, USA), weighed and stored at −20 °C. The oil recovery was expressed in terms of the mass percentage of the samples and was calculated as according to Eq. (1):

$$\text{RECOVERY}(\%) = \frac{W_e}{W_t} \times 100 \quad (1)$$

where: W_e is the mass of palm oil extracted from the sample (g) and W_t is the mass of total oil in the sample (g). W_t was determined by solvent extraction (Bligh and Dyer, 1959).

The aqueous enzymatic oil extraction (AEOE) was performed under the best process conditions determined in the CCD study. The pH of extraction was 4.0, the ratio of buffer/substrate was 2 v/w and the enzyme concentration was 4%. To evaluate the performance of tannase and the other enzymes separately, 3 different combinations of enzyme blends were tested: pectinase + cellulase (PC); pectinase + cellulase + tannase (PCT); and tannase (T).

2.6. Determination of total carotenes, tocopherols and tocotrienols

The analyses of carotenes, tocopherols and tocotrienols were carried out simultaneously by chromatographic analyses, as described previously (Silva et al., 2011), with a Shimadzu HPLC, series LC-20AT (Japan) equipped with a quaternary pump, auto sampler (SIL-20A), degasser, SPD-M20A spectrophotometric detector (Photo Diode Array detector–PDA) that was set at 292 and 455 nm and RF-10AXL fluorescence detector that was set at 290 nm for excitation and 330 nm for emission. Chromatographic separation of the compounds was achieved at 30 °C with a normal-phase Lichrospher column (Merck, 250 × 4.6 mm id; 5 μm particle size) with a guard column (10 × 4.6 mm) purchased from Merck (Germany). The concentration gradient used was as follows: 0–7 min 99.5% hexane and 0.5% isopropanol; 7–9 min linear gradient of 0.5–1% isopropanol; 9–20 min 99.0% hexane and 1.0% isopropanol; 20–25 min reconditioning of the column with 0.5% isopropanol isocratic for 10 min. The chromatographic run time for each analysis was 35 min. Samples were dissolved in hexane, and aliquots of 20 μL were injected into the HPLC system.

Table 1
Central composite design (CCD) experimental matrix with predicted and observed results.

Independent variables				Response variable	
Run	*X*1 (%Enzyme)	*X*2 (Dilution)	*X*3 (pH)	*Y* observed % oil recovery	*Y* predicted % oil recovery
1	2	3	4.7	62.12	61.24
2	4	3	4.7	81.15	82.35
3	2	5	4.7	40.66	44.36
4	4	5	4.7	75.39	70.61
5	2	3	6.3	44.67	48.04
6	4	3	6.3	58.63	53.52
7	2	5	6.3	13.26	10.65
8	4	5	6.3	21.81	21.27
9	1.3	4	5.5	35.6	32.79
10	4.6	4	5.5	54.62	59.44
11	3	2.3	5.5	76.09	76.26
12	3	5.6	5.5	33.15	34.99
13	3	4	4	71.9	71.68
14	3	4	7	16.92	19.15
15	3	4	5.5	50.43	49.73
16	3	4	5.5	50.61	49.73
17	3	4	5.5	48.51	49.73

2.7. Analysis of fatty acid composition

The fatty acid composition was determined by conversion to fatty acid methyl esters (FAMEs) based on the method of Rodrigues et al. (2010) and the esters were detected by a gas chromatograph (Varian model CP 3380) equipped with a flame ionization detector and a CP-Sil 88 capillary column (length 60 m, internal diameter 0.25 mm, film thickness 0.25 μm; Varian Inc., USA). The operating conditions were as follows: helium as the carrier gas, a flow rate of 0.9 mL/min, FID detector temperature of 250 °C, an injector temperature (split ratio 1:100) at 245 °C and an injection volume of 1 μL. The temperature program for the column was 4 min at 80 °C and a subsequent increase to 220 °C at 4 °C/min. The individual fatty acid peaks were identified by comparison of the retention times with those of known mixtures of standard fatty acids (Nucheck-prep, Inc., USA) run under the same operating conditions. The retention time and the area of each peak were computed with Varian Star 3.4.1. software. The results were expressed as relative percentages of total fatty acids.

2.8. Determination of the total phenolic content (TPC)

The TPC of the oil extracts and subfractions (1 g of oil extract in 80% methanol) was quantified using Folin–Ciocalteu reagent (Singleton and Rossi, 1965), with minor modifications. A 300-μL aliquot of extract solution was mixed with 5 mL of Folin–Ciocalteu reagent (10% in distilled water). After 5 min, 4 mL of sodium carbonate (7.5% in distilled water) was added. The samples were incubated for 2 h at room temperature in the dark. The absorbance was measured at 765 nm. A standard curve was prepared with gallic acid solutions of know concentrations. The results were expressed as mg gallic acid equivalents (GAE) per kg of extract.

2.9. Measurement of oxidative stability

The oxidative stability of the oils was evaluated with a Metrohm 743 Rancimat instrument (Brinkman, Herisau, Switzerland), which measures the rate of oxidation under accelerated conditions. The tests were performed with 3 g oil samples at a temperature of 130 °C and an flow of air 20 L/h. The oxidative stability was expressed as the induction time for oxidation of the oils.

2.10. Total antioxidant capacity measurement

The potential antioxidant activity of the oil samples was assessed on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, as previously described (Tuberoso et al., 2007). The solutions were prepared and stored in the dark before analysis. Measurements were performed in triplicate. The *in vitro* antioxidant activity of the oil (total fraction) and the hydrophilic and lipophilic fractions were determined. The separation of the two fractions was performed as follows: 1 g of oil was dissolved in 2 mL of 80% methanol, the solution was agitated for 30 min at room temperature and centrifuged at 700*g* for 10 min to separate the methanol phase (hydrophilic fraction), while ethyl acetate was added to the remaining solution (lipophilic fraction). The total fraction is the dilution of oil in ethyl acetate without fractionation. Antiradical activity was expressed as μmol of Trolox equivalent/mg of the fraction oil sample, and the absorbance was measured at 517 nm.

2.11. Statistical analysis

All tests were carried out in triplicate. Values are expressed as arithmetic mean. Statistical significance of the differences between the groups was analyzed by the Tukey test. Differences were considered to be significant when $P < 0.05$. The central composite design data were compared using an analysis of variance (ANOVA) with a 5% significance level ($P < 0.05$) in the statistical software STATISTICA 8.0 (Stat Soft Inc., Tulsa, Oklahoma, USA).

3. Results and discussion

3.1. Central composite design for the evaluation of enzymatic process

To optimize the key parameters of the enzyme-assisted process for oil palm extraction, a CCD study was conducted. Table 1 shows the experimental matrix with the experimental results and the predictions made by the model for oil recovery.

The quadratic model for the maximum oil extraction yield, after the elimination of statistically insignificant terms ($P > 0.05$), was as follows:

$$Y = 7.93X1 - 12.28X2 + 2.09(X2)^2 - 15.63X3 - 1.53(X3)^2 - 3.91X1X3 - 5.13X2X3 + 49.73$$

The ANOVA that is presented in Table 2 showed that the model was significant. The Fisher *F*-test ($F_{test} = 41.66 > F_{tab9;7;0.05} = 3.67$) was approximately 10 times higher than the F_{tab}, and the $P < 0.00003$ demonstrated that this regression was statistically significant at the 95% confidence level. In addition, the R^2 (multiple correlation coefficient) value of the obtained regression equation

Table 2
Analysis of variance of polynomial model from CCD design.

Source of variation	Sum of squares	Degrees of freedom	Mean squares	F_{test}	F_{tab}	p-Value
Regression*	6728.5	9	747.61	41.66	3.67	0.00003
Residual	125.61	7	17.94			
Lack of fit	122.91	5	24.58	18.14	19.29	0.0531
Pure error	2.71	2	1.35			
Total	6854.2	16				

*Significant at 5% of significance level.
Coefficient of determination: $R^2 = 0.98$.

was 0.98 (a value >0.75 indicates aptness of the model), which means that the model can explain 98% of the variation in the response.

The results from the CCD showed that the best conditions for the enzymatic oil palm recovery occurred under the conditions of trial 2, which recovered 81.15% of the total content of the oil in the pulp under the following operational conditions: enzyme concentration of 4%, dilution rate of 3 (mL/g) and pH 4.7. The response surfaces and contour curves are shown in Fig. 1(a–c). Analysis of the contour plot indicates that a higher oil recovery yield can be obtained when the system is operated with even lower values of enzyme concentration than those of the experimental conditions of trial 2. The process would result in greater than 80% oil recovery. All the variables were significant in order of pH > dilution > enzyme concentration.

Fig. 1a illustrates the interaction between enzyme concentration and dilution, which were not statistically significant. The oil recovery was higher when high enzyme concentrations and low dilution levels were employed. Fig. 1b represents the interaction between enzyme concentrations and pH and it indicates that the enzyme concentration only has a positive effect on oil recovery when used in acidic pH. Fig. 1c illustrates the interaction between pH and dilution and it indicates that oil recovery increases when a low ratio of dilution is used. However, this effect appears only in acidic pH.

The conditions that were chosen for the rest of the oil recovery study were an enzyme concentration of 4%, a dilution rate of 2 (v/w) and pH 4 for maximum oil recovery, with variations in the enzymatic composition of the blends tested. A time course study,

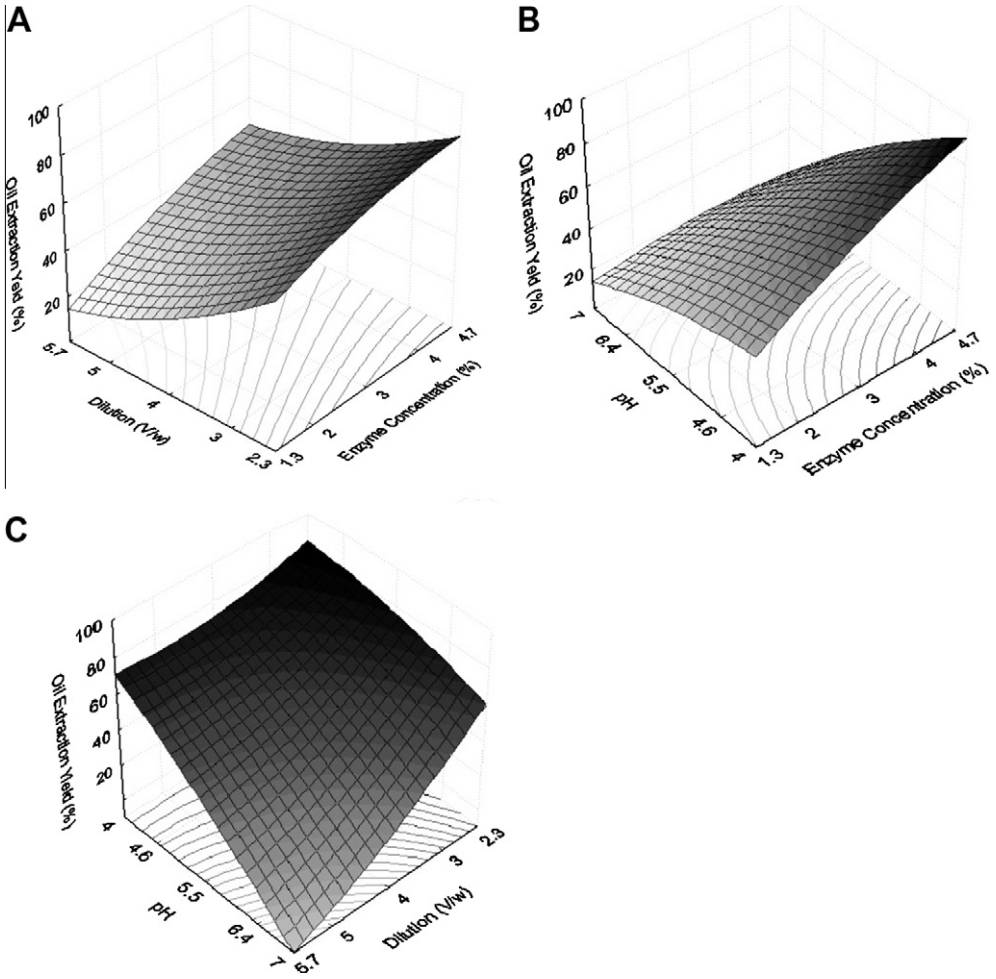

Fig. 1. Response surfaces from enzymatic oil extraction statistical modeling. (a) Effect of Enzyme concentration and Dilution. (b) Enzyme concentration and pH. (c) pH and Dilution.

Table 3
Oil recovery from enzymatic and control processes.

Extraction process	Oil recovery (%) (mean ± standard deviation)
AOE	31 ± 2.59
Pectinase/cellulase	90.96 ± 1.96[a]
Pectinase/cellulase/tannase	91.52 ± 1.69[a]
Tannase	52.41 ± 1.95

Means followed by the same letter are not different according to the Tukey test at the 5% level of significance.
All results presented as mean ± standard deviation.

using the best reaction conditions, showed that after 30 min maximum oil recovery was achieved (data not shown).

Najafian et al. (2009), using the enzyme Pectinex Ultra SP-L (Novozymes) for the extraction of olive oil, found that the enzyme concentration was significant and could increase the extraction of oil and polyphenols. In the present work, it was observed that the oil recovery was directly proportional to the enzyme concentration over the mass of pulp palm oil used. However, at high levels of dilution (up to 4), the enzyme concentration had little effect on the oil yield. Increasing the enzyme concentration and decreasing the ratio of solution/sample (dilution) favored the accessibility of the enzyme to the cell walls and was more effective at low levels of dilution.

3.2. Oil recovery in aqueous and enzyme-assisted processes

The oil recoveries by the different methods are shown in Table 3. A comparison of the oil extraction by the AOE method and the results of the three AEOE processes reveals that the enzymatic treatment was significant for oil extraction. The AOE extraction had a yield of 31%, whereas the tests that used pectinase/cellulase and pectinase/tannase/cellulase had yields of approximately 90%. The results obtained for the AEOE extraction methods are statistically equivalent to the oil recovery obtained with thermal aqueous extraction when the AOE extraction was performed at 90 °C (data not shown). The enzymatic treatment was able to replace the high temperatures of the aqueous process of extraction.

Rosenthal et al. (2001) evaluated the effects of enzyme concentration (w/w), ratio of buffer/mass (Dilution), material particle size (mm) and hydrolysis time for the extraction yield of soybean oil and protein hydrolysates. Analysis of the dilution variable had a significant positive effect and revealed a direct relationship with the oil yield that resulted in more than an 80% yield of extraction with this process. In the present work, it was observed that dilution had an inverse effect on oil recovery.

Based on the yields shown in Table 3, it is possible to conclude that the mix of pectinase and cellulase is efficient despite the presence of tannase. The AEOE process that was tested demonstrated a commercial potential with high yields of oil palm recovery in an aqueous process at low temperatures.

3.3. Oil composition

Table 4 shows the values for tocopherol, tocotrienol, carotenes and total phenolics (TPC) in the palm oils obtained by the different methods of extraction. Despite the relatively large standard deviations, it was possible to conclude that the differences in the values of tocopherol and tocotrienol concentrations in the oil from the AOE process and the AEOE process that used cellulase and pectinase are nonexistent or small. The application of tannase in the oil extraction process seems to have a negative influence on the concentration of tocopherols and tocotrienols in the oil. This trend was also observed in preliminary tests to quantify the tocopherol fractions (alpha, gamma and delta tocopherol) of the oil samples obtained from the different extraction methods tested; the tocopherol concentrations of the oils extracted in the presence of tannase were lower for all of the fractions (data not published). Further studies will be necessary to elucidate the cause of this finding. It is interesting to emphasize that no previous reports on the tocopherol contents of AEOE palm mesocarp oils are available.

Puah et al. (2007), studied the tocopherol concentration of palm oil during physical refining and observed a mean value of 1273 ± 18 ppm in crude palm oil, 1134 ± 20 ppm after degumming, 1095 ± 18 ppm after bleaching and 1029 ± 18 ppm after deodorization.

The carotene concentration in the oils (Table 4) was three times higher in the AEOE (cellulase/pectinase) extraction than in the AOE extraction oil. The carotene concentration in the oils extracted in the presence of tannase was similar to that in the AOE oil. The presence of tannase in the enzymatic mix reduced the capacity for carotene extraction in the oil.

The presence of tannase in the AEOE process of extraction led to a significant increase in the polyphenol content over that of the AOE processed oil (Table 4). The other enzyme combinations in the AEOE tests resulted in lower concentrations of total polyphenol than those in the AOE samples. These results were expected and confirm the hydrolytic action of tannase on phenolic substrates of the palm oil matrix.

Szydłowska-Czerniak et al. (2011) evaluated the total phenolic, antioxidant capacity and total carotenoid content of oil palm samples after non-enzymatic refining. The total phenolic compounds ranged from about 41 to 124 mg GA/kg and carotenoids from 1.8 to 458 mg/kg oil samples. Thus, the total carotenoid content obtained by enzymatic extraction in the present experiments was similar and the total phenolic compounds was higher.

There were no significant differences in the concentrations of the fatty acids in all of the oil samples from the different extraction processes (Table 5).

3.4. Quality parameters of the palm oil

There was no significant difference in oxidative stabilities of the tested samples. The AOE sample achieved 6.2 ± 1.66 h; PC 5.35 ± 1.74 h; PCT 5.81 ± 0.55 h and Tannase 5.52 ± 2.66 h.

Table 4
Lipophilic and hydrophilic antioxidants present in oil samples from different treatments.

Treatment	Total tocopherol (mg/kg, mean ± SD)	Total tocotrienol (mg/kg, mean ± SD)	Total carotene (β-carotene) (mg/kg, mean ± SD)	TPC (galic acid equivalent) (mg/kg, mean ± SD)
AOE	325.27 ± 76.65[a]	2360.89 ± 1532.04[a]	463.45 ± 19.61[a]	21.43 ± 0.58[a]
Pectinase/cellulase	251.11 ± 9.1[a,b]	2130.73 ± 738.18[a]	1268.69 ± 222.81	17.43 ± 1.53[ca]
Pectinase/cellulase/ Tannase	200.54 ± 5.94[b]	1044.28 ± 59.77[a]	488.91 ± 18.24[a]	14.76 ± 1.00[c]
Tannase	204.26 ± 4.16[b]	1140.54 ± 74.31[a]	499.74 ± 14.14[a]	26.43 ± 0.58

Means followed by the same letter in the same column are not different according to the Tukey test at the 5% level of significance.
All results presented as mean ± standard deviation.

Table 5
Fatty acid profiles of aqueous enzymatic oil extraction (AEOE) and aqueous oil extraction (AOE) samples.

Fatty Acids (%)	PC	PCT	Tannase	AOE
C12:0	0.02 ± 0.001[a]	0.02 ± 0.003[a]	0.02 ± 0.001[a]	0.02 ± 0.001[a]
C14:0	0.82 ± 0.07[b]	0.72 ± 0.09[b]	0.63 ± 0.13[b]	0.87 ± 0.10[b]
C16:0	46.78 ± 1.23[c]	48.94 ± 1.79[c]	46.16 ± 1.08[c]	46.02 ± 1.29[c]
C16:1	0.16 ± 0.04[d]	0.18 ± 0.07[d]	0.17 ± 0.03[d]	0.15 ± 0.01[d]
C17:0	0.08 ± 0.01[e]	0.08 ± 0.02[e]	0.08 ± 0.01[e]	0.09 ± 0.007[e]
C18:0	4.39 ± 0.44[f]	3.67 ± 0.41[f]	4.42 ± 0.75[f]	4.73 ± 0.38[f]
C18:1	37.61 ± 1.72[g]	35.71 ± 1.17[g]	38.59 ± 1.42[g]	38.21 ± 1.76[g]
C18:2	9.61 ± 0.35[h]	10.14 ± 0.27[h]	9.58 ± 0.36[h]	9.35 ± 0.41[h]
C18:3	0.32 ± 0.07[i]	0.31 ± 0.04[i]	0.31 ± 0.07[i]	0.35 ± 0.05[i]
C20:0	0.21 ± 0.05[j]	0.21 ± 0.07[j]	0.18 ± 0.02[j]	0.21 ± 0.03[j]

All results presented as mean ± standard deviation.
Means followed by the same letter in the same row are not different according to the Tukey test at the 5% level of significance.
PC – Pectinase + Cellulase; PCT – Pectinase + Cellulase + Tannase.

Table 6
Antioxidant capacity (DPPH) of palm oil fractions.

Treatment	Whole oil (μmol Trolox/g oil) mean ± SD	Hydrophilic fraction (HF) (μmol Trolox/g oil) mean ± SD	Lipophilic fraction (LF) (μmol Trolox/g oil) mean ± SD
AOE	756.8 ± 13[a]	266.1 ± 28[a]	644.6 ± 47[a]
Pectinase/cellulase	728.5 ± 20[a]	377.8 ± 66[a]	584.10 ± 51[a]
Pectinase/cellulase/ tannase	854.1 ± 18[b]	456.1 ± 54[a]	991.8 ± 217[b]
Tannase	820.7 ± 10[b]	754.5 ± 18[b]	791.8 ± 73[ab]

Means followed by the same letter in the same column are not different according to the Tukey test at the 5% level of significance.
All results presented as mean ± standard deviation.

The results for the DPPH assays are presented on Table 6. These results represent the antioxidant capacities of the lipophilic and hydrophilic fractions and show relatively large standard deviations as is common natural samples (Hanson et al., 2004; Ou et al., 2002).

Enzymatic treatment during the oil extraction process probably increased the concentration, or the availability, of the antioxidant compounds in the whole oil, the hydrophilic fraction and the lipophilic fraction, when tannase was used as the only enzyme in the process. Tannase alone, increased the total phenolic content and antioxidant capacity in all the fractions tested (Tables 4 and 6).

The enzymatic blend with tannase yielded higher antioxidant capacity results for the lipophilic and whole oil samples, which clearly indicates the positive contribution of this particular enzyme to increase of biological property of the oil palm.

Szydłowska-Czerniak et al. (2011) verified that carotenes from refined oil palm had a higher positive correlation with DPPH antioxidant capacity. In the present work, carotenes had a negative correlation with total oil fraction DPPH.

4. Conclusion

A new application for tannase was tested, and its performance, when blended with cellulase and pectinase, in oil and antioxidants extraction was evaluated. The aqueous enzyme-assisted process compared to the control showed a synergistic effect of the enzymatic blend for a high extraction yield. The enzymatic process simultaneously improved the quality of oil samples by increasing the content of bioactive compounds, such as phenolics and carotenoids. The use of tannase led to an increase in total phenolic compounds in the lipophilic and hydrophilic fractions, resulting in and a higher antioxidant capacity of the oil. This process is an environmentally friendly means for high quality palm oil extraction.

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