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The effect of pectinase pretreatment on the extraction of essential oil from the seed kernels of Nutmeg (*Myristica fragrans*)

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Abstract

In this study, the seed kernels of Nutmeg (*Myristica fragrans*) were pretreated with partially purified pectinase (PPP) prior to hydrodistillation to enhance the extraction of essential oil. The yield of the essential oil obtained after pretreatment with PPP was 4.70%. GC-MS analysis of the essential oils obtained after pretreatments with PPP showed a total of 33 constituents classified into monoterpenes (51.52%), sesquiterpenes (21.21%) and phenylpropanoids/aromatic (27.27%) compounds. Comparison was made by pretreating the nutmeg seeds with a pure pectinase (CP) obtained from Sigma-Aldrich. The fraction obtained when pretreated with CP was made up of 32 components with monoterpenes (51.31%), sesquiterpenes (18.75%) and phenylpropanoids/aromatic (28.12%) compounds. The major components of essential oils obtained after pretreatments with PPP and CP were sabinene, α-pinene, β-pinene and myristicin. This research shows that the extraction of *Myristica fragrans* seed kernel essential oil can be improved using PPP when compared with the yield of the control (3.80%) obtained from the seed kernels that were not pretreated with the enzymes prior to hydrodistillation.

Keywords: Pectinase, *Myristica fragrans*, pectinase-assisted extraction, Essential oil, Nutmeg

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INTRODUCTION

The application of enzyme technology in the extraction of natural biomolecules that occur within the plant cell wall is on the increase in recent times (Hosni *et al*., 2013; Chávez-González *et al*., 2015; Mohamad *et al*., 2019; Miljanovic 2020). The critical step for the extraction of these biomolecules is achieved using enzymes such as cellulase and pectinase to breakdown the cell wall polysaccharides and allow access to the storage gland where essential oil is located (Rashed *et al*., 2007; Cheng *et al*., 2015; Sajith *et al*., 2016). This technique assists in the extraction of important biomolecules especially when they occur in low concentrations like the case of essential oils. Essential oils contribute to the flavour and attractiveness of food (Agozzino *et al*., 2007; Burčul *et al*., 2017; Panggabean *et al*., 2019; Fatma *et al*., 2019; Ibrahim *et al*., 2020; Ben-Lagha *et al*., 2020). *Myristica fragrans*, one of the natural sources of essential oils, has gained prominence in many countries (Rahardiyan *et al*., 2020). It is a large, leafy evergreen plant whose origin is traced to the Molucca Isles (Spice Islands) which is now being cultivated in several countries in Africa, America and Asia (Iwu, 1993; Ogunwande *et al*., 2003; Rahardiyan *et al*., 2020). A lot of homes and industries use *Myristica fragrans* due to its sweet taste and pleasant fragrance as a flavour to many kinds of baked goods, confections, puddings, custard, meats, sausages, saucers, vegetables, beverages; and as component of curry powder, teas, and soft drinks; or is added to milk and alcohol (Olaleye *et al*., 2016; Periasamy *et al*., 2016; Naeem *et al*., 2018). *Myristica fragrans* essential oil is also used in the manufacturing of camphor, plasticizers, bases, solvents, perfume, and synthetic pine oil (Naeem *et al*., 2018). In medicine, *Myristica fragrans* has various uses because of its aromatic, stimulant, narcotic, carminative, astringent, aphrodisiac, hypolipidemic, antithrombotic, anti-platelet aggregation, antifungal, antidysenteric, and anti-inflammatory activities, in addition to possessing insecticidal, fungicidal, and bactericidal activities (Muchtaridi *et al*., 2010; Ibrahim *et al*., 2020). These myriads of activities enable *Myristica fragrans* to function as medication for the treatment of abdominal pain, bronchitis, vomiting, flatulence, increasing metabolism, dysentery (infectious diarrhea), constipation, bloating, rheumatism, kidney disorders and liver disease (Al-Jumaily *et al*., 2012; Olaleye *et al*., 2016; Rahardiyan *et al*., 2020). Consumers now prefer natural food ingredients to ones with synthetic chemical additives due to their toxic effects (Sowbhagya *et al*., 2010; Yazdi *et al*., 2019). The increasing demand for natural essential oils for various applications has greatly influenced research in the area of oil extraction and recovery methods (Cheng *et al*., 2015; Bich *et al*., 2017). Pectinases are regarded as one of the most important enzymes used in the degradation of plant cell wall (Doughari *et al*., 2019; Okonji *et al*., 2019) and they account for 25% of the enzymes used in food (Khatri *et al*., 2015; Anand *et al*., 2020) for processes such as the extraction and clarification of fruit juice, vegetable oil extraction, tea and coffee fermentation, in alcoholic beverage and food industries etc (Kuhad *et al*., 2011; Madhu *et al*., 2015; Kubra *et al*., 2018; Adeyefa *et al*., 2020; Satapathy *et al*., 2020). There is a dearth of information in literature on the use of pectinase to assist in the extraction of essential oils from *Myristica fragrans* seed kernels with a view to enhance oil yield. Therefore, this research has focused on the pretreatment of *Myristica fragrans* seed kernels with both partially purified pectinase and pure pectinase obtained from Sigma-Aldrich with a view to enhance the extraction of essential oil.

MATERIALS AND METHODS

Sample collection

Myristica fragrans seeds were obtained from Ogige Market in Nsukka L.G.A of Enugu State. The seeds were identified at the Department of Pharmacology and Environmental Medicine of the University of Nigeria. The seeds were thereafter deposited at the herbarium of the same department with voucher number PCG/UNN/0334.

Chemicals and Reagents

All the chemicals and reagents used in this study were of analytical

grade and freshly prepared except where otherwise stated.

Methods

Pretreatment of seed kernels of *Myristica fragrans* with pectinase

Myristical fragrans seeds were blended into fine powder and 25 g of the powder was soaked in 25 ml of sodium acetate buffer pH 5.0. A quantity of 2 ml of the partially purified pectinase (PPP) was added to it and incubated at 25˚C for 6 hr (Amudan *et al*., 2011). The above procedure was also used to pre-treat the seed kernels using the pure pectinase obtained from Sigma-Aldrich.

Hydrodistillation of essential oil using the clevenger apparatus.

All the pre-treated *Myristica fragrans* powdered seed kernels were then hydrodistilled for 3 hr in a clevenger apparatus containing 200 ml of distilled water. Powdered *Myristical fragrans* seed kernel (25 g) that was not pretreated with enzymes was also hydrodistilled using a clevenger apparatus to extract the essential oil Briefly, the powdered seed (25 g) material in the clevenger apparatus was heated at 100 ºC. The steam from the boiling material in the round bottom flask of the apparatus (containing oily particles) was evaporated and conveyed through the Clevenger extractor to the condenser. The condenser circulated cold water maintained below 10 ºC provided by an external circulation in a closed circuit. The vapour containing the essential oil was condensed into two phases and directly decanted into a graduated burette to separate the essential oil from the aqueous supernatant and then determine the amount of oil produced. The volatile oils were then collected and analyzed according to the procedure of Al-Jumaily and Al-Amiry (2012). The experiments were performed in duplicates and the average values were used to determine the yield using the formula below:

$$\text{Yield } (\%) = \frac{Amount\ of\ essential\ oil\ recovered\ (g)}{Amount\ of\ plant\ material\ distilled\ (g)} \times 100$$

Preparation of essential oil samples for GC-MS analysis

The essential oil sample was diluted with chloroform to 7%. The inert gas (helium) from the large storage cylinder was introduced through the injection part to the column and the detector. To ensure reproducible retention time and minimize detector dirt, the flow rate of the carrier gas was adjusted. A micro-syringe was used to inject the sample through a heated injection part that vaporized and carried the sample into the column made up of a long tube closely packed with solid particles. The supporting solid was uniformly covered with a thin film of highly boiling liquid as the stationary phase. The mobile and stationary phases separated the sample into individual components which emerged from the column together with the carrier gas and passed through a detector. The components generated a signal registered electrically as detected by the device which was passed to the detector.

Gas chromatography- mass spectrometric (GC-MS) analysis of essential oil

The essential oil was analyzed by electron ionization (EI) method on GC-MS-QP2010SE SHIMADZU, JAPAN. The conditions of the MS employed during the analysis were: ionization voltage 70 eV; ion source temperature 230 ºC; mass scan range: 40–440 mass units. The GC settings were as follows: the column oven temperature was 60 ºC, the injection temperature was 250 ºC, the injection mode was split, flow control mode was linear velocity, while pressure was 144.9 kPa, with the total Flow at 103.1 mL/min, the column flow at 3.22 mL/min, the linear velocity at 46.3 cm/sec, with the purge flow at 3.0 mL/min, and split ratio set at 30.1. The mass range was 45 m/z to 700m/z. The carrier gas used was helium and the samples (1 µL) were injected with a split ratio of 1: 30 according to the procedure of Fan *et al.* (2018). The chemical components were identified through comparison of their retention times and mass spectra with those in the MS data library of the National Institute of Standards and Technology (NIST 11). The relative quantity of each component was determined by calculating the peak area of the TIC chromatogram.

RESULTS

Enzyme assisted extraction

Treatment of *Myristica fragrans* seed kernels with PPP before hydrodistillation was able to release 4.70% of the essential oil from the seed kernel while pretreatment with CP before hydro distillation released 7.60% of the essential oil. The conventional extraction of the essential oil through hydro-distillation gave the percentage yield of 3.80% as shown in Table 1.

Table 1: Pectinase assisted extraction of essential from *Myristica fragrans* seeds

Treatment	Percentage
Distillation ^{NP}	3.80
Distillation + Pectinase ^{PPP}	5.80
Distillation + Pectinase^{CP}	8.20

NP= No enzyme pretreatment; CPP = partially purified pectinase enzyme; CC = Pure enzyme from Sigma

GC-MS analysis

The separation and identification of the components were carried out using the GC-MS as shown by the chromatograms in Figures 1 and 2.GC-MS analysis of the essential oils from the seed kernels of *Myristica fragrans* earlier pretreated with PPP showed 33 constituents with 51.52% monoterpenes, 21.21% sesquiterpenes and 27.27% phenylpropanoids/aromatic compounds while the fraction obtained when pretreated with CP is made up of 32 components with 51.31% monoterpenes, 18.75% sesquiterpenes and 28.12% phenylpropanoids/aromatic compounds (Table 2 and 3). The chemical compositions of fractions obtained after pretreatment with PPP and CP respectively were characterized by the high contents of sabinene, α-pinene, and β-pinene in the monoterpene class, safrole and α-copaene as the major sesquiterpenes and myristicin and methyl eugenol occurring as the main components in phenylpropanoid class as shown in tables 2 and 3.

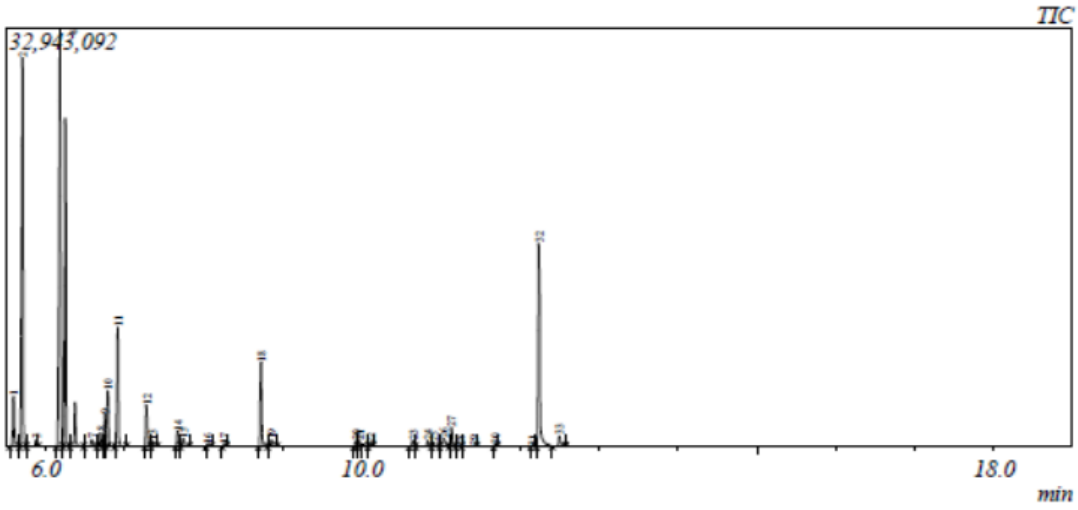

Figure 1: Chromatogram of the GC-MS analysis, the peak numbers and retention times of the components of the essential oil obtained after pretreatment pretreatment with partially purified pectinase

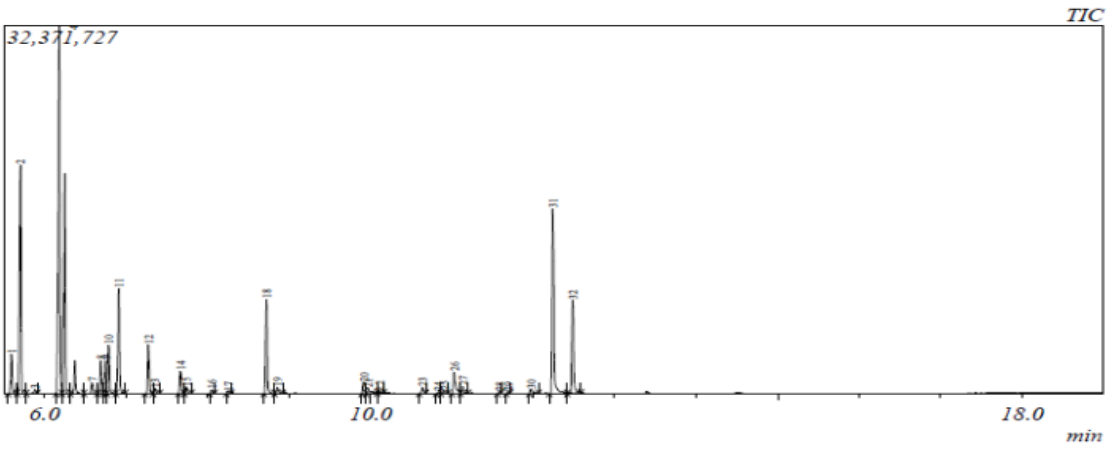

Figure 2: Chromatogram of the GC-MS analysis, the peak numbers and retention times of the components of the essential oil obtained after pretreatment pretreatment with pure pectinase

DISCUSSION

For many bioactive compounds stored inside the cell, the breakdown of the cell wall has remained a critical step of extraction. In the present studies, biocatalysts used for pretreatments assisted in degrading the cell wall material to release the intracellular constituents with 52.60% and 115.79% differences in essential oil yield compared to the control. The research carried out by Fan *et al*. (2018) on the effect of pectinase and other enzymes on the extraction of essential oil from clove (*Syzygium aromaticum)* resulted in about 50.00% yield. Yazdi *et al*. (2019) demonstrated the effect of pretreatment of pistachio green hull with pectinase on the extraction of phenolic compounds which resulted in more than 11.00% yield in the quantity extracted compared to the control sample of about 6.50%. In investigating the effect of enzymes on the extraction efficiency of the hypotensive drug, genoposidic acid, pretreatment with pectinase gave a value of 1.13% while pretreatment with glucanase gave a yield 0.74% (Cao, 2010). The use of pectinase was also investigated in the pretreatment and the recovery of total phenolics with a percentage yield of 346.58 mg gallic acid equivalents (GAE)/100 ml while the control (without pretreatment) was 238.35 mg GAE/100 ml (Koley *et al*., 2011). In another study on the effect of pretreatment of the flesh of 'Sunlady' cantaloupe with pectinase before treatment to extract beta-carotene resulted in a yield of 76.71 µg/100 g while the sample without pretreatment (control) had 68.15 µg/100 g Nattaporn *et al*., 2011). These studies agree with the present study which showed that enzyme pretreatment of samples to breakdown the cell wall pectin facilitates the extraction of constituents stored within the cell.

The components of the essential oils obtained from the seed kernels of Nutmeg earlier pretreated with PPP and CP respectively before carrying out hydrodistillation are monoterpenes which include sabinene (23.4% and 23.38%), α-pinene (19.03% and 13.14%) and β-pinene (15.24% and 10.87%). These components constitute the major fractions of essential oils from the seed kernels of *Myristica fragrans.* Others found are the sesquiterpenes which include safrole (0.44% and 1.01%) and alpha-copaene (1.02% and 0.45%), the phenylpropanoid/aromatic compound which are made up of; myristicin (11.94% and 11.95%) and methyl eugenol (0.81% and 1.96%) for both the PPP and CP respectively.

The result obtained here is similar to that of Muchtaridi *et al*. (2010) who reported 32 components with sabinene, 4-terpineol, myristicin and α-pinene as the major components. Al-Jumaily and Al-Amiry (2012) reported 47 components in *Myristica fragrans* seed kernels with α- pinene, β-pinene, sabinene, Myrecene as the major components of the

essential oil. Jukic *et al*. (2016) also reported 17 components of essential oils in *Myristica fragrans* seed kernels with β-pinene, α- pinene, myristicin and terpine 4-ol as the main components. Ogunwande *et al*. (2003) reported the presence of 37 components made up of sabinene, α-pinene, α-phellandrene and terpinen-4-o1 as the major constituents.

Table 2: Components of the essential oils obtained by pretreatment of the seed kernels of *Myristica frangrans* with partially purified pectinase.

Peak number	Chemical compound	Molecular weight (g/mol)	Retention time (min)	Compound (%)
1	Alpha-thujene	136	5.595	2.48
2	Alpha-pinene	136	5.707	19.03
3	Camphene	136	5.884	0.34
4	Sabinen	136	6.182	23.40
5	Beta-pinene	136	6.251	15.24
6	Beta-myrcene	136	6.374	2.15
7	Alpha-phellandrene	136	6.586	0.40
8	3-Carene	136	6.694	0.66
9	4-Carene	136	6.750	1.48
10	m-Cymene	134	6.787	2.92
11	m-Mentha-6,8-diene	136	6.913	6.61
12	Gamma-terpinene	136	7.278	2.09
13	Bicyclo(3.1.0)hexanol	154	7.358	0.28
14	Cyclohexene	136	7.672	0.84
15	Linalool	154	7.741	0.54
16	5-Caranol	154	8.052	0.18
17	Dihydrocarveol	154	8.258	0.12
18	Terpinen-4-ol	154	8.725	4.05
19	Alpha-Terpineol	154	8.860	0.41
20	Safrole	162	9.927	0.44
21	1-vinyladamatane	162	9.968	0.54
22	m-allylpyrocatechin	162	10.115	0.14
23	2Azidomethylcyclohexene	196	10.644	0.40
24	Alpha-cubebene	204	10.834	0.33
25	Nerol acetate	196	10.909	0.22
26	Methyl eugenol	178	11.037	0.81
27	Alpha-copaene	204	11.136	1.02
28	Beta-Copaene	204	11.244	0.05
29	Octyne	110	11.416	0.06
30	Alpha-baergamotene	136	11.691	0.14
31	Germacrene D	204	12.160	0.05
32	Myristicin	192	12.243	11.94
33	Elemicin	208	12.501	0.66

Table 3: Components of essential oils obtained after pretreatment of the seed kernel of Nutmeg with pure pectinase obtained from Sigma Aldrich

Peak no.	Chemical compound	Molecular weight (g/mol)	Retention time (min)	Compound (%)
1	Alpha-thujene	136	5.594	2.19
2	Alpha-pinene	136	5.705	13.14
3	Camphene	136	5.884	0.24
4	Sabinen	136	6.182	23.38
5	Beta-pinene	136	6.249	10.87
6	Beta-myrcene	136	6.374	1.87
7	Alpha-phellandrene	136	6.585	0.65
8	3-Carene	136	6.693	1.71
9	4-Carene	136	6.749	1.71
10	m-Cymene	134	6.787	2.87
11	m-Mentha-6,8-diene	136	6.913	6.87
12	Gamma-terpinene	136	7.277	2.79
13	Bicyclo(3.1.0)hexanol	154	7.357	0.32
14	Cyclohexene	136	7.670	1.32
15	Linalool	154	7.743	0.42
16	5-Caranol	154	8.051	0.21
17	Dihydrocarveol	154	8.256	0.13
18	Terpinen-4-ol	154	8.726	5.24
19	Alpha-Terpineol	154	8.860	0.45
20	Safrole	162	9.918	1.01
21	1-vinyladamatane	162	9.967	0.69
22	m-allylpyrocatechin	162	10.116	0.13
23	2Azidomethylcyclohexen	196	10.643	0.45
24	Alpha-cubebene	204	10.833	0.08
25	Nerol acetate	196	10.908	0.19
26	Methyl eugenol	178	11.029	1.96
27	Alpha-copaene	204	11.135	0.45
28	Caryophylene	204	11.585	0.10
29	Alpha-baergamotene	136	11.692	0.10
30	Isoeugenol methyl ester	178	11.973	0.46
31	Myristicin	192	12.242	11.95
32	Elemicin	208	12.485	6.05

CONCLUSION

The demand for natural sources of food ingredients has spurred the search for essential oils from various sources with increasing attention paid to methods that can enhance recovery. The result from this research shows that both the PPP and CP enzymes can be employed for the improvement in the extraction of essential oils when compared with the value of 3.8% from the extraction without pretreatment. The results of the *Myristica fragrans* essential oil components from these studies and those of other studies as reported in literature shows that *Myristica fragrans* have similar components occurring in different quantities but mostly belonging to the monoterpene, sesquiterpene and phenylpropanoid/ aromatic benzene classes. As seen from the results, enhanced extractions were achieved using PPP and CP with the CP

giving more quantitative yield while the quality of the components were similar in both oils.

Author contributions

ESOO designed the work and provided the samples, chemicals, and reagents. IW performed the experiment and wrote the article together with OKO, OVE. ESOO edited the manuscript while CFC supervised the study.

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