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## Effect of pectinase enzyme on distillation rate of eugenol, caryophyllene and eugenyl acetate from essential oil of clove bud (*Syzygium aromaticum*)

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### Abstract

Effect of pectinase enzyme treatment on whole clove bud and ground clove bud were studied for eugenol, caryophyllene, and eugenyl acetate. Pectinase enzyme to whole clove bud caused the eugenol increased from 79,93 to 89,98%, caryophyllene and eugenyl acetate decreased from 5,17 to 2,64% and 7,65 to 4,88%, respectively. Pectinase enzyme to the ground clove bud caused eugenol decreased from 75,99 to 74,08%, caryophyllene increased from 12,95 to 13,63 and eugenyl acetate increased from 5,34 to 6,66%. The oil composition for 8 hours distillation of pectinase enzyme on ground clove bud caused eugenol increased from 44,34 to 90,02%, caryophyllene decreased from 46,06 to 3,47% and eugenyl acetate increased from 0,90 to 4,38%. Enzymatic process on whole clove bud caused eugenol decreased from 85,87 to 81,80%, caryophyllene increased from 2,11 to 4,94% and eugenyl acetate increased from 5,34 to 10,21%.

**Keywords:** *Syzygium aromaticum*, essential oil, chemical composition, pectinase enzyme

### 1. Introduction

The clove tree (*Syzygium aromaticum*) called “cengkeh” in Indonesia, was first cultivated on the island of Moluccas, Indonesia, in Southeast Asia [1]. *S. aromaticum* is widely cultivated in Indonesia, Sri Lanka, Madagascar, Tanzania, and Brazil. The clove oil from *S. aromaticum* have been described as having useful antiseptic, analgesic and used in dental medicine [2].

Clove is an aromatic herb has many useful purposes. The part of the clove used as medicine is the flower bud of the clove. The oil of clove has been used in a variety of health condition including indigestion, generalized stress, parasitic infestation, cough, toothaches and blood impurities [3]. Clove is a natural antiviral, anti microbial, anti tumor, anaesthetic activity, insecticidal activity [4], antioxidant [5], antifungal [6], and anti-giardia activity [7].

The clove species have been demonstrated to produced a wide variety of potentially useful chemical compounds such as sesquiterpenes, tannins, and triterpenoids [8]. the flower buds of *S. aromaticum*, contain an essential oil comprised of a mixture of aliphatic and cyclic volatile terpenes [9]. Several constituents of clove has been identified, mainly eugenol, eugenyl acetate, beta-caryophyllene, 2-heptanone, alpha- humulene, methyl salicylate, isoeugenol, methyleugenol, phenylpropanoides, dehydrodieugenol, gallic acid, and oleanolic acid [10]. The major constituent of the clove essential oils are eugenol,  $\beta$ - caryophyllene,  $\alpha$ -humulene and humulene epoxide [11, 12]. The oils were analyzed by high resolution GC and GC-MS [13].

Pectinase is an enzyme that breaks down pectin. This enzyme are responsible for the degradation of the long and complex molecules called pectin that occur as structural polysaccharides in the middle lamella and the primary cell walls of young plant cells. Chemically, pectic substance are complex colloidal acid polysaccharides, with a backbone of galacturonic acid residues linked by linkage. Over the years, pectinase have been used in several conditional industrial processes, such as textile, tea, coffee, oil extraction, ect [14, 15].

Two major sources of the enzyme pectinase are plant and microorganism. But for both technical and economic point of view microbial source of pectinase has become increasingly important. Nowadays, pectinase is one of the most important enzymes in food processing industries mainly for extraction and clarification of fruits juice and wine [16]. The use of pectinase enzyme not only result in higher yield of juice but also preserves the nutrients, original color and flavor [17].

The objective of this research were to study the effect of particle size and pectinase enzyme on chemical composition (eugenol, caryophyllene and eugenyl acetate) and distillation rate of clove bud (*Syzygium aromaticum*) from Indonesia.

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## 2. Experiment Section

### 2.1 Plant collection

Clove buds (*Syzygium aromaticum*) were collected from Central Java, Indonesia. Plant samples were air-dried prior to extraction.

### 2.2 Sample preparation

The clove buds were grinding with size of particle 100 mesh and other sampel without grinding. The samples of grinding and non grinding were incubated by 1% Pectinex Ultra SP- L enzyme solution for 16 hours. Pectinex UltraSP-L was obtained from Novozyme Malaysia.

### 2.3 Extraction of essential oil

50 g of clove buds samples was extracted using clevenger distillation apparatus in ratio of sample and water was 1:6. Hydro-distillation process was running for 8 hours at atmospheric pressure.

### 2.4 Analysis of the oil

Gas chromatography (GC) analysis was performed on an Agilent Technologies HP 6890 Plus Gas Chromatograph

equipped with a FID and fitted with HP-5MS column (30m x 0.25mm, film thickness 0.25  $\mu$ m, agilent technology, Berks, United Kingdom). The analytical condition were: carrier gas  $H_2$  (1mL/min), injector temperature (PTV, programmed temperature vaporization) 250  $^{\circ}C$ , detector temperature 260  $^{\circ}C$ , column temperature programmed from 40  $^{\circ}C$  (2 min hold) to 220  $^{\circ}C$  (10 min hold) at 4  $^{\circ}C$ /min. Samples were injected by splitting and the split ratio was 10:1. The volume injected was 1,0  $\mu$ L. Inlet pressure was 6.1 kPa. An Agilent technologies HP 6890 N plus Chromatograph fitted with a fuse silica capillary HP-5 MS column (30 m x 0.25 mm, film thickness 0.25  $\mu$ m) and interfaced with a mass spectrometer HP 5973 MSD was used for the GC/MS analysis, under the same condition as those used for GC analysis. The condition were the same as describe above with He (1 mL/min) as carrier gas. The MS condition were as follow : ionization voltage 70 eV; emission current rate of 1.0 scan/s.

## 3. Result and Discussion

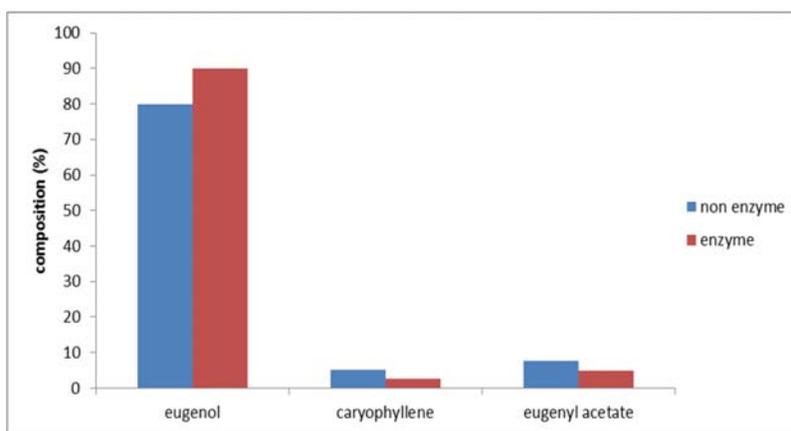


Fig 1: Effect of pectinase enzyme on oil composition of whole clove bud

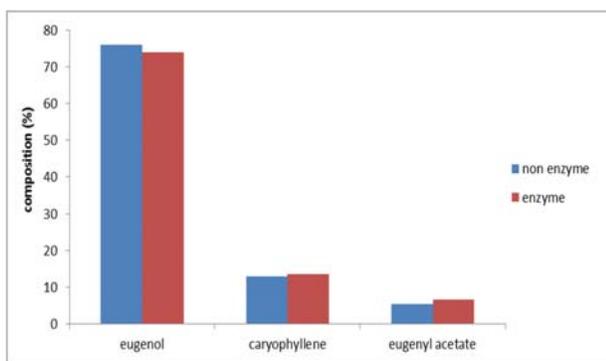


Fig 2: Effect of pectinase enzyme on oil composition of ground clove bud

Effect of pectinase enzyme on treatment of clove bud was performed on Fig 1 and Fig 2. Samples were used whole (without grinding) and ground (with grinding) clove bud. Particle size of clove bud contributed to rate of distillation and oil compositions [18]. Enzymatic process of whole sample caused increase of eugenol from 79,93 to 89,98%, and decrease of caryophyllene and eugenyl acetate from 5,17 to 2,64 and 7,65 to 4,88%, respectively. Enzymatic treatment of ground clove bud caused decrease of eugenol from 75,99 to

74,08%, and increase of caryophyllene and eugenyl acetate from 12,95 to 13,63% and 5,34 to 6,66%, respectively

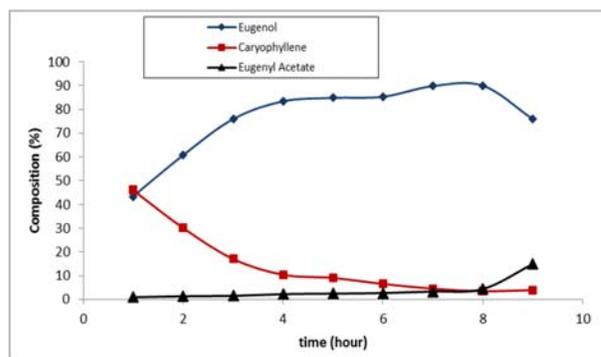
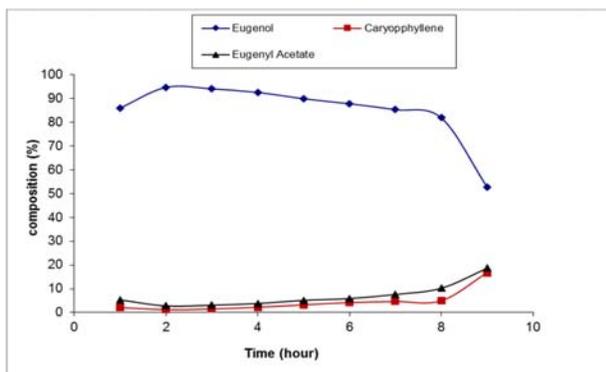


Fig 3: Chemical composition of ground clove bud at various time of distillation by enzymatic treatment

Distillation rate at variation of time can influence of composition of the oil. The ground clove bud with pectinase enzyme treatment showed the different composition for distillation from 1 to 8 hours. The composition of 1 hour distillation were eugenol of 44,34%, caryophyllene 46,06%

and eugenyl acetate 0,90%, The change of main composition after 8 hour distillation distillation were eugenol from 44,34 to 90,02%, caryophyllene from 46,06 to 3,47% and eugenyl acetate from 0,90 to 4,38%.



**Fig 4:** Chemical composition of whole clove bud at various time on distillation by enzymatic treatment

The Enzymatic treatment of whole clove bud for 8 hour distillation showed decrease of eugenol from 85,85% to 81,80%, increase of caryophyllene from 2,11 to 4,94 and increase of eugenyl acetate from 5,34 to 10,21%. Pectinase enzyme treatment of sample with different of particle size give the different chemical compositions.

#### 4. Conclusion

Treatment of pectinase enzyme at whole clove bud and ground clove bud contributed to composition of eugenol, caryophyllene and eugenyl acetate. Effect of enzymatic treatment in whole clove bud increased eugenol and decreased caryophyllene and eugenyl acetate. Effect of enzymatic treatment in ground clove bud decreased eugenol and increased caryophyllene and eugenyl acetate. Distillation rate of the oil with pectinase treatment showed different composition againts of distillation time for 8 hours.

#### 5. Acknowledgement

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