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Determination of Optimal Conditions for Extraction of Alcohol-soluble Eugenol Containing Material from Cloves

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ABSTRAK

Oleoresin bunga cengkih (jirim ekstrak larut lengkap) mengandungi satu sebatian anti oksida eugenol. Ekstraksi eugenol daripada bunga cengkih dikaji dengan menggunakan etanol 90%. Ujian yang berbeza dari segi saiz zarah, suhu ekstraksi, masa ekstraksi dan nisbah pelarut/pepejal dilakukan dan keadaan optimum dinilai. Indeks biasan digunakan untuk memonitor parameter-parameter ekstraksi. Corak ekstraksi pecahan bunga cengkih hancur yang mempunyai saiz zarah 500 μm, 355 μm, 250 μm, dan kurang daripada 250 μm dengan nisbah pelarut/pepejal 4:1 pada suhu bilik telah dikaji. Adukan dilakukan dengan penggoncang salingan. Saiz zarah optimum bagi ekstraksi pada suhu bilik ialah 250 μm. Ujian terhadap suhu dilakukan dalam julat 30°C hingga 70°C pada nisbah pelarut/pepejal yang sama untuk julat saiz zarah antara 250 μm hingga 355 μm. Suhu optimum untuk saiz zarah 250 μm ialah 50°C manakala untuk saiz zarah 355 μm pula suhu optimum ialah 60°C.

ABSTRACT

Clove oleoresin (total soluble extract matter) contains an antioxidant compound eugenol. Extraction of eugenol from cloves was studied using 95% ethanol. Trials varying in particle size, extraction temperature, extraction time and solvent to solid ratio were performed and optimal conditions were evaluated. Refractive index was used to monitor the extraction parameters. Extraction patterns of ground clove fractions with size of 500 μm, 355 μm, 250 μm and less than 250 μm with solvent to solid ratio of 4:1 at room temperature were studied. Agitation was ensured by a reciprocating shaker. Optimal size for the extraction at room temperature was 250 μm. Trials on extraction temperature were varied from 30°C to 70°C with the same solvent to solid ratio for particle size of 250 μm and 355 μm. Optimal temperatures for 250 μm and 355 μm were 50°C and 60°C respectively.

Keywords: eugenol, extraction, cloves, process optimization

INTRODUCTION

It is well known that cloves possess a phenolic compound 4-allyl-2-methoxyphenol, commonly called eugenol with a chemical formula of ($C_{10}H_{12}O_2$). This compound acts as an antioxidant on oleogenous foods. Eugenol also acts as an anti-carminative, anti-spasmodic and antiseptic in pharmacy and as an antimicrobial agent (Purseglove *et al.* 1981). Cloves are cultivated in many parts of Malaysia, particularly in Penang. With a view to substituting this natural antioxidant compound for synthetic chemicals to avoid the possible toxicity, cloves were chosen for eugenol extraction.

The objective of this work was to investigate extraction characteristics and to determine optimal conditions for more efficient extraction of eugenol from cloves.

MATERIALS AND METHODS

Fresh green cloves were obtained from Kampong Batu Laut, Banting, Selangor, Malaysia. Analytical reagent grade, Univar, 95% ethanol was used for the extraction experiments.

Clove Drying

The green buds were removed from the stems and spread in a thin layer on the shelf of an air-circulating oven. The buds were dried at 60°C for 16 hours until a constant weight was obtained, and then the oven temperature was raised to 100°C. The dried buds were stored in the whole state. The stems were dried separately and stored for future analysis.

Preparation of Clove Oleoresin

The ground clove material with a particle size of 355 μm was extracted with 95% ethanol for 2 hours at a solvent to solid ratio of 4:1 and a realistic temperature of 70°C (boiling point of ethanol is 78°C). Agitation was performed by a temperature-controlled reciprocating shaker. The mixture was filtered through filter paper (Whatman No. 10), and the residue extracted again under the same conditions until the refractive index of the extract was close to that of the solvent. The extraction was performed for 8 hours and the total solvent to solid ratio used was in the region of 15:1. The combined filtrate was partially concentrated in a rotary vacuum evaporator and the final concentration was adjusted by heating the extract concentrate at 90°C. The oleoresin was able to withstand temperatures of up to 90°C without detectable quality deterioration, which becomes significant at temperatures above 100°C (Sabel and Warren 1972). The process of heating, cooling and weighing was repeated until the total weight loss on evaporation and drying of the extract solution was about 97%. This extract was further used to prepare a calibration curve.

Determination of Alcohol-Soluble Extract.
The alcohol-soluble extract was determined according to the Malaysian standard for spices and condiments (SIM 1973).

Calibration Curve
The procedure for extracting oleoresins is to use the ground spice with known optimum particle size at selected low boiling point of pure solvent. The extraction is repeatedly performed and the solvent removed via vacuum. Low heat flux must be exercised to the mixture to minimize heat damage to the oleoresin (Langenau 1959). Therefore measurement of the amount of oleoresin for routine monitoring of an extraction process is laborious, time-consuming and difficult, especially when predicting the change in concentration of soluble extract within a short time. Spice oleoresins are not completely soluble in 95% alcohol but are soluble in clove oil (Furia 1972). Clove oleoresin, which is soluble in 95% alcohol, was taken as a basis for preparing the calibration standard for the total soluble materials. A calibration curve was determined by dissolving known weights of oleoresin (1 gm, 2 gm, 3 gm, 4 gm, and 5 gm) dissolved in 50 ml of 95% ethanol. The refractive index of each solution was measured using the Atago 3T refractometer at ambient temperature. The results, plotted in *Fig.1*, were used to monitor the extraction parameters.

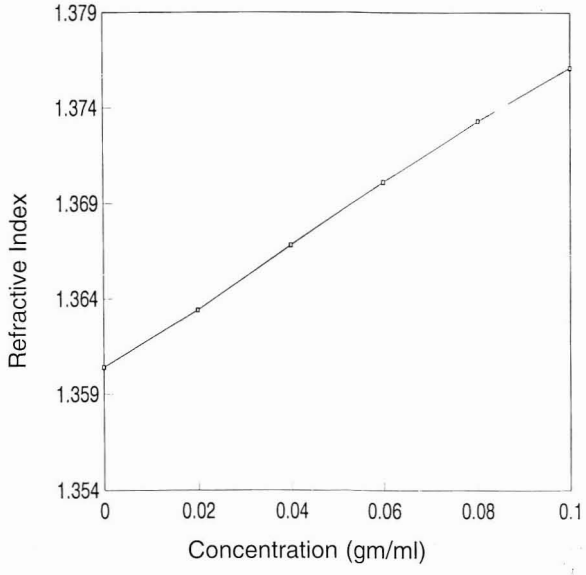

Fig. 1 Relationship between refractive index and concentration of soluble extract in 95% ethanol

Extraction Trials

The cloves used in the extraction trials were finely ground prior to the experiments and used immediately to ensure richness. Heath and Reineccius (1986) recommended that ground cloves be stored for no longer than three months in the manufacturing process.

In this experiment, one kg of dried cloves were ground in a blender (Moulinex, France). Clove particles of different sizes (500 μm, 355 μm, 250 μm and less than 250 μm) were selected by sieving with test sieves (Endecotts Ltd, London, England).

In order to study the effect of particle size on extraction, 5-gm samples of each size were mixed with 25 ml of 95% ethanol in a 50-ml conical flask and shaken for 1 hour at room temperature via a reciprocating shaker. The solid and liquid phases were quickly separated by filtration and the refractive index of each of the extract solutions was measured. The results obtained are plotted in *Fig. 2.*

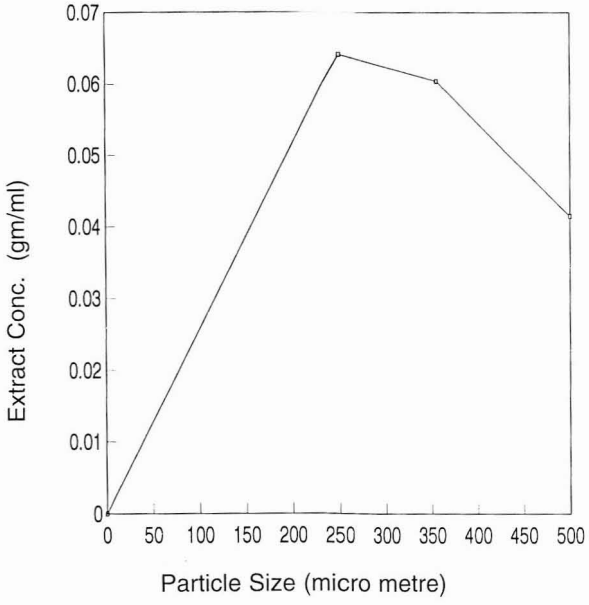

Fig. 2 Extraction curve with different particle sizes

Experimental trials with variation in extraction temperature ranging from 30°C to 70°C were performed using two sample sizes of 250 μm and 355 μm. Five grams of each sample size were mixed with 25 ml 95% ethanol in a 50-ml conical flask and shaken for one hour. The average results of the temperature study are plotted in *Fig 3.*

Extraction rate of different solvent to solid ratios was studied using optimal size and temperature of 250 μm and 50°C respectively. Five-gram samples were

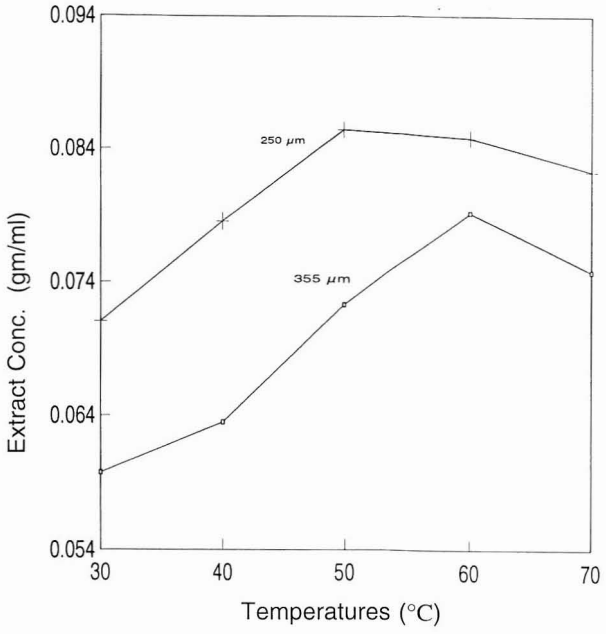

Fig. 3 Extraction curves at different temperatures for two particle sizes.

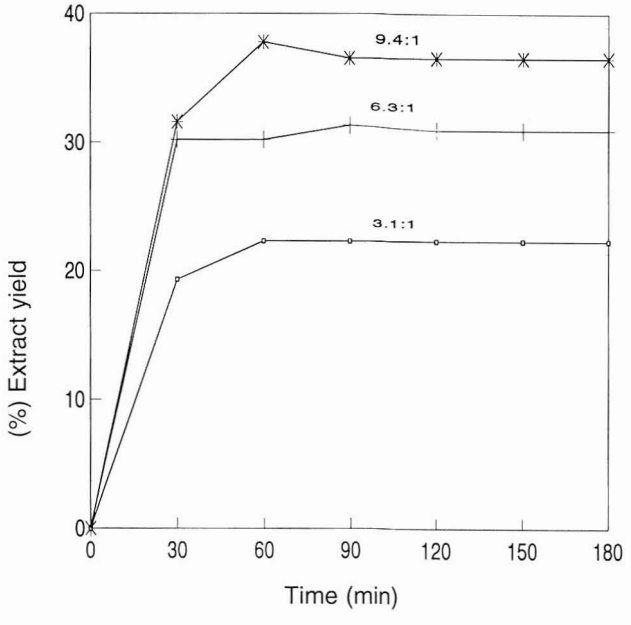

Fig. 4 Extraction curves at different solvent to solid ratios

mixed with 20 ml, 40 ml and 60 ml of 95% ethanol in a100-ml conical flask and shaken for one hour at room temperature. Six samples were prepared for each solvent to solid ratio with an extraction time of 180 minutes. Each sample flask was taken out according to the time interval, filtered, and the total extract solution volume recorded. The refractive index of each of the extract solutions was measured and the results are plotted in *Fig. 4*.

RESULTS AND DISCUSSION

Drying of Cloves

The weight of the bud is about 68 to 70% of total weight of the cloves. During the drying process the cloves lost about 75% of their original fresh green weight. It was found that if the drying is performed as soon as the buds are separated from the clusters, the dried buds exhibit a bright brown colour. If the buds are left for a prolonged period after removal from the clusters, the dried spice will have a whitish shrivelled appearance. The buds and stems cannot be dried together because of their different texture and initial moisture content. The weight lost during drying of the stem is the same as that for the bud, even though the time required to dry the stem is very short compared to the bud. Cloves have naturally warming qualities and become hot after grinding. The temperature rise of the ground sample depends on the quantity and time of grinding.

Clove Oleoresin Extract

Extraction of oleoresin using ground clove size of 355 μm, a solvent to solid ratio of 15:1, an extraction time of 8 hr at 70°C and then drying the extract at 90°C yielded 37.4% oleoresin based on dried clove materials. It was observed that the extract colour changed from greenish-brown to brownish green during drying probably due to the oxidation of phenolic compounds as the temperature increases (Dziezak 1989).

Extraction performed according to Malaysian standard for spices and condiments (SIM 1973), that is by using ground clove, a solvent to solid ratio of approximately 40:1, an extraction time of 24 hours at ambient temperature and then drying the extract at 103°C gave 33.97% of oleoresin based on dried material. It was found that the laboratory scale continuous shaking extraction process gave a oleoresin yield close to that obtained by the standard method.

Extraction Characteristics

Fig. 2 shows the variation in the soluble content of the extraction material as a function of particle size. As the particle size increases, the extract concentration increases, and a significant decrease in extraction is observed as the size change from 355 μm to 500 μm. This is because the smaller the parti-

cle size, the greater the interfacial area per unit volume between the solid and the liquid phase, and therefore the greater the rate of transfer of material to the solvent, which is also due to the shorter distance the solute will have to diffuse to the surface of the particle. It was found experimentally that the extraction rate does not always increase when the particle size becomes smaller; it may decrease. This phenomenon is probably due to the fact that the fine particles may wedge themselves in the interstices of the larger particles and thus impede the flow of the solvent. The optimal particle size with maximum extraction concentration was observed to be 250 μm. It was found that in practice this optimal size does not need auxiliary separation devices such as a centrifuge or a rotary vacuum filter.

The variation of the soluble extract concentration as a function of temperature is shown in *Fig. 3*. The extraction procedure is conducted in the temperature range of 30°C to 70°C. It was observed that as the temperature increased the soluble extract solution became increasingly dark. For the 250 μm particle size significant increase in extraction rate or solubilization was noted up to 50°C whereas for particle size of 355 μm the same phenomenon was observed up to 60°C. Further increase in temperature accelerated adverse solubilization reaction. The optimum extraction temperature for 250 μm and 355 μm are 50°C and 60°C respectively. The influence of the particle size on the extraction is apparently more pronounced for particle size of 250 μm and 355 μm (*Fig.3)*, and the larger particle size needs higher extraction temperature. Extraction at low temperature avoids loss of volatile components of the clove and prevents oxidation of phenolic compounds.

Fig.4 shows the extraction pattern using different solvent to solid ratios. The amount of extract solution obtained, was 12 ml for solvent to solid ratio of 3.1:1, 30 ml for 6.3:1 and 50 ml for 9.4:1. Based on the amount of soluble extract solution obtained, it was found that the extract removed at the beginning was approximately 22.31% after 60 min, 31.34% after 90 min and 37.8% after 60 min for the solvent to solid ratios of 3.1:1, 6.3:1 and 9.4:1 respectively. After the initial extraction, the rate of extraction continued at a constant decreasing rate for a further 3 hours. Experiments using higher solvent to solid ratios were found to give different extraction rates for different values of solvent to solid ratios.

The volumes of soluble extract solution holdup by the solid in the filtration process were approximately 40%, 25% and 16.7% for solvent to solid ratios of 3.1:1, 6.3:1 and 9.4:1 respectively. In the laboratory scale experiments, it was found that at low solvent to solid ratios, solid particles suspended in solution formed a packed bed on the filter paper, and the solution holdup amount was therefore quite large. The holdup volume of the extract solution was reduced when the solvent to solid ratio was increased as part of the soluble extract solution was decanted and filtered first, followed by the remaining solid-liquid mixture. Therefore, to minimize the solution absorbed by the solid, the larger particle size is preferred but this would

reduce cell wall rupture as well as the solvent-particle contact area. Thus in solid-liquid extraction (leaching) the extraction plant is always designed to strike the proper balance between the particle size desired for most rapid extraction and that required for good filtration.

CONCLUSION

It can be concluded that the optimal particle size for extraction of eugenol from cloves at room temperature is 250 μm whereas the optimal eugenol extraction temperature for particle size of 250 μm is 50°C and that for particle size of 355 μm is 60°C. Work is currently in progress to identify the antioxidant components and to directly measure the eugenol content in the soluble extract materials without separating it from the other components.

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