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Comparison of Two Isolation Methods for Essential Oils from Orange Peel (*Citrus auranticum* L) as a Growth Promoter for Fish: Microwave Steam Distillation and Conventional Steam Distillation

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Abstract

Microwave steam distillation (MSD) is an advanced steam distillation (SD) technique utilizing a microwave oven in the extraction process. MSD of essential oils from the peel of orange (*Citrus auranticum* L.) was studied and the results were compared with those of the conventional SD in terms of extraction time, extraction yield/efficiency, chemical composition, and quality of the essential oils. MSD was superior in terms of saving extraction time (140 min, compared to 7 h in SD) and extraction yield. Scanning electron microscopy (SEM) of orange peel undergone SD and MSD provided evidences as to a sudden rupture of essential oil glands with MSD. Gas chromatography–mass spectrometry analysis of the extracted essential oils indicated that the use of microwave irradiation did not adversely influence the composition of the essential oils. The result of this study demonstrate that MSD can be used as an alternative method to extract orange essential oil as growth promoter for fish.

Keywords: Orange essential oil; Microwave steam distillation; Scanning electron microscopy; Extraction; *Citrus auranticum* L

Introduction

Owing to the increasing demand for fish and declining fish stocks worldwide, aquaculture has become increasingly necessary and profitable. To achieve higher productivity and thus meet world demand, it is essential to use complete and balanced diets with additives that help maintain health and improve fish growth [1].

The growth promoters are natural or synthetic substances, or living organisms that act beneficially in improving the animal body weight gain, feed efficiency ratio, reproductive performance and decreasing mortality [2]. Among the growth promoters, essential oils are commonly recognized as safe for animals, consumers and the environment for being natural products that have decreased side effects or toxicity and better biodegradability [3], besides good market availability.

Extraction is one of the key technologies for sustainable growth of the agro-food industry and economy of the process industry, and also often requiring up to 50% of investments in a new plant and more than 70% of total process energy used in food industries [4]. Existing extraction technologies suffer from considerable technological and scientific bottlenecks which are difficult to overcome, such as reduction of energy consumption, and fulfilment of increasingly stringent legal requirements on emissions product/process and materials safety and control.

Therefore, in the last few years there has been an increasing demand for novel green process technologies, starting from the idea that pollution and hazards have to be eliminated at the source, thus reducing environmental impact and costs [5]. As a consequence, an increased interest exists for improvement, design and development of new green processes for the extraction of essential oil from bio-product. Thus, auxiliary techniques as ultrasound and microwave irradiation have been used to enhance extraction performances [6-8].

Recently, much attention has been given to the application of microwave dielectric heating for the extraction of natural products that typically needed hours or days to reach completion with conventional methods. Using microwaves, full reproducible extractions can now be completed in seconds or minutes with high reproducibility, reducing the consumption of solvent, simplifying manipulation and work-up, giving higher purity of the final product, eliminating posttreatment of waste water and consuming only a fraction of the energy normally needed for a conventional extraction method such as steam distillation or solvent extraction. Several classes of compounds such as essential oils, aromas, pigments, anti-oxidants, and other organic compounds have been extracted efficiently from a variety of matrices (mainly animal tissues, food, and plant materials). Microwave extraction is a research area which has an impact in several fields of modern chemistry. All the reported applications have shown that microwave-assisted extraction is an alternative to conventional techniques for such matrices. The main benefits are decrease of extraction time and solvents used. The advantages of using microwave energy, which is a non-contact heat source, for the extraction of essential oils from plant materials, include: more effective heating, faster energy transfer, reduced thermal gradients, selective heating, reduced equipment size, faster response to process heating control, faster start-up, increased production, and elimination of process steps. Extraction processes performed under the action of microwave radiation are believed to be affected in part by polarization, volumetric and selective heating [9].

Citrus is the largest fruit crop in the world (100 million cubic tons per year) and the orange account for 60% [10]. The remaining orange peel account for approximately 45% of the total bulk [11]. Consequently, significant amounts of orange peel are available as a

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by-product. The orange peel, if treated as waste materials, may create environmental problems, particularly water pollution, due to the presence of biomaterials such as essential oil [12-15], pectin [11,16] and sugar. This problem could be turned into an asset, if potentially marketable active principles such as essential oil could be extracted from the peel. After extraction, the peel could be a high protein stock feed in dry form, increasing the potential return for the orange juice industry and reducing the pollution [11]. Orange essential oil is used to add the orange aroma to products such as carbonated drinks, ice creams, cakes, air-fresheners and perfumes [17]. Recently, other applications make use of the d-limonene, major component of orange essential oil, as green solvent for extraction of fats and oils from olive seeds in combination with microwave energy [18].

In order to obtain a better understanding of microwave steam distillation (MSD), comparisons have been made in terms of extraction time, yield, chemical composition, and quality of the orange essential oil with conventional steam distillation (SD). The chemical analysis studies were supplemented by scanning electron micrographs to shed light on the physical action of the two extraction systems.

Materials and Methods

Plant materials

In this study, orange (*Citrus auranticum* L.) peel were collected locally after juice extraction (which separates the external part of the orange (peel), giving a yield approximately of 20% (w/w) of orange peel with respect to the whole fruit). All other chemicals and solvents used were of analytical grade.

Microwave steam distillation apparatus and procedure

A domestic microwave oven (EMM-2007X, Electrolux, 20 L, maximum delivered power of 800 W, 2.45 GHz) was modified for MSD operation shown in Figure 1. The dimensions of the PTFE-coated cavity of the microwave oven were 46.1 cm × 28.0 cm × 37.3 cm. In a typical MSD procedure at atmospheric pressure, batch of four plant material to solvent ratio of orange peel samples (0.20, 0.30, 0.40 and 0.50 g mL^{-1}) were placed in a 1 L flask containing deionized water (400 mL). The microwave oven was operated at 400 W power level. An electrical steam generator and a conventional condenser placed outside a microwave zone are connected to a reactor containing the raw material, via Pyrex connecting tubes. The condenser is connected to a receiving Florentine flask which is preferably a separating funnel to enabling the continuously collected of condensate essential oil and water. This system presents the advantage that the reactor containing aromatic particles could be easily and quickly replaced and cleaned after each cycle of extraction.

The saturated steam was produced by electrical steam generator, passes through the orange bed, whilst the mixture was continuously heated in a microwave cavity. The direct interaction of microwaves with saturated steam favours the release of essential oils trapped inside the cells of plant tissues. A mixture of hot "crude juice" and steam moves thus naturally downwards by earth gravity downwards, and directed towards the condenser to the Florentine flask. The extraction was continued for a period of 140 min. During the extraction process, the collected essential oils were decanted from the condensate in 20 min intervals. To remove water, the extracted essential oils were then dried over anhydrous sodium sulphate and stored in amber vials at 4°C until they were used for analysis. The yield of orange essential oil was found by the following equation

$$Y = \frac{v}{w} \times 100\% \quad (1)$$

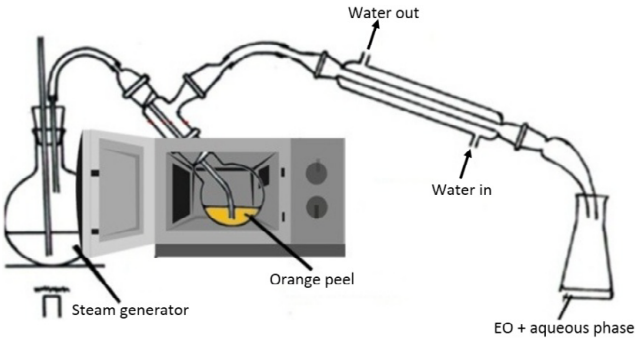

Figure 1: Schematic diagram of the experimental apparatus of microwave steam distillation (MSD).

where Y is the orange essential oil yield (%, w/w), V is the weight or mass of extracted orange essential oil (g) and W is the weight or mass of orange peel (g).

Steam distillation apparatus and procedure

For a rigorous comparison, the same glassware and same operating conditions have been used for conventional SD (the same process but without use of microwave cavity). In this system, the vapour produced by the steam generator crosses the plant, charged with essential oil then passes through the condenser to a receiving Florentine flask (Figure 2). The extraction was continued for a period of 7 h. Also, the sample collection intervals were increased to 60 min.

Gas chromatography-mass spectrometry

Essential oils composition was determined by gas chromatography coupled to mass spectrometry (GC–MS) analysis on a Hewlett-Packard 6890 gas chromatograph coupled to a 5973A mass spectrometer, using two fused-silica-capillary columns with different stationary phases. The non-polar column was HP5MS™ (30 m length, 0.25 diameter and 0.25 µm film thickness) and the polar one was a Stabilwax™ consisting of Carbowax™-PEG (60 m length, 0.25 mm diameter and 0.25 µm film thickness). GC–MS spectra were obtained using the following conditions: carrier gas He; flow rate 1.0 mL min^{-1}; split 1:50; injection volume 1.0 µL; injection temperature 300°C; oven temperature progress from 100 to 250°C at 10°C min^{-1}; the ionisation mode used was electronic impact at 70 eV. Most constituents were tentatively identified by comparison of their GC Kovats retention indices (RI), determined with reference to an homologous series of C_5–C_{28} *n*-alkanes and with those of authentic standards available in the authors' laboratory. Identification was confirmed by comparison of their mass spectral fragmentation patterns with those stored in the MS database (National Institute of Standards and Technology and Wiley libraries) and with mass spectra literature data [19,20]. For each compound on the chromatogram, the percentage of peak area relative to the total peak areas from all compounds was determined and reported as relative amount of that compound.

Scanning electron microscopy (SEM)

Microhistological analysis was done by using a scanning electron microscope Merk FEI, Type Inspect-S50. Single orange peels were individually packed in pieces of perforated aluminium foil to permit gas exchange, rapidly frozen in liquid nitrogen before being transferred to a pre-cooled (-60°C) plate of a Pearse tissue dryer and dried overnight under vacuum. Samples were then coated with a thin gold film.

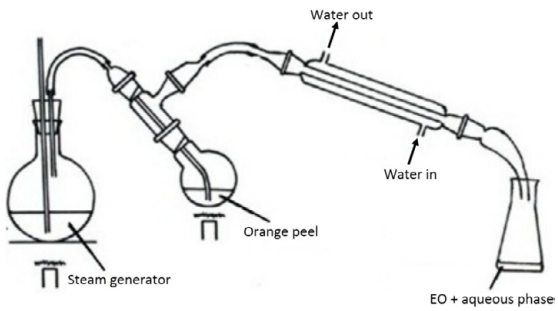

Figure 2: Schematic diagram of the experimental apparatus of conventional steam distillation (SD).

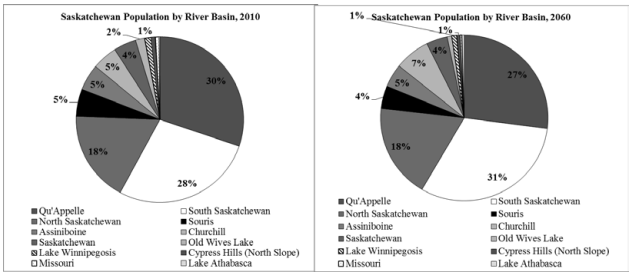

Figure 3: Yield (%) profile of the orange essential oil obtained by conventional steam distillation (SD) method as a function of extraction time.

Physical constants

Orange essential oil have been analysed and compared according to the EOA standard. The usual physical constants defining the essential oil have been determined at 20°C: specific gravity and refractive index.

Results and Discussion

Comparison of extraction time and extraction yield of orange essential oil

Based on the effect of the length of extraction time to orange essential oil yield for a various variables of plant material to solvent ratio, it can be seen that the extraction process has reached a diffusion phase. It can be seen from the decrease in the rate of extraction and fewer orange essential oil obtained during the extraction of entering a time of 4 hour for SD and 80 minutes for MSD.

In Figure 3 can be seen the relationship between the extraction time to the yield obtained from a various variables of plant material to solvent ratio using SD method. A significant increase in yields seen within the period four hours early. After five hours, the increase in yield is not very significant. This is because the essential oil content in the raw materials have started to decrease. Between the sixth and seventh hour yield profile shows horizontal lines indicate that essential oils contained in the plant material is up. Based on these figures seen total yield obtained after the process runs for 7 hours ranged from 0.59-0.70%. From Figure 3 also seen the final yield with the extraction time for 7 hours, where there is greatest yield on the plant material to solvent ratio 0.40 g mL^{-1} whereas the lowest yield on the plant material to solvent ratio 0.50 g mL^{-1}.

In addition to SD method, this study also used the MSD method. Orange essential oil extraction with MSD method performed for 140 minutes. In the Figure 4 can be seen the relationship between the extraction time to the yield obtained from a various variables of plant material to solvent ratio. A significant increase in yields seen within the period 80 minutes early. After 80 minutes the yield increase is not very significant. Between 100 minutes to 140 minutes a yield profile shows horizontal lines indicate that essential oils contained in the plant material is up. Based on these figure seen total yield obtained after extraction process runs for 140 minutes ranged between 0.65-0.75%.

From Figure 4 also seen the greatest yield on the plant material to solvent ratio 0.50 g mL^{-1} whereas the lowest yield on the the plant material to solvent ratio 0.20 g mL^{-1}. This data is different when compared with the Figure 3 which shows the yield of the extraction time profiles for the same material but different methods used. In Figure 3 shows that the extraction of orange essential oil with SD method on the plant material to solvent ratio 0.50 g mL^{-1} indicates the lowest yield whereas in Figure 4 shows that the extraction of orange essential oil with MSD method on the plant material to solvent ratio 0.50 g mL^{-1} has the highest yield. It is very closely associated with microwave heating is evenly distributed during the extraction of essential oils. So it can be said that the use of microwave can recognized as efficient extraction technique that dramatically reduces time and increases the yield.

Composition of essential oil

A total of 7 compounds were identified in orange essential oil using SD method and 17 compounds were identified in orange essential oil using MSD method (Table 1). The essential oils extracted by MSD or SD are rather similar in their composition. The same number of volatile secondary metabolites is found in the essential oils isolated by MSD or SD, with similar yields. Limonene is the most abundant component in the essential oil extracted from oranges peel with equivalent relative amounts for both extraction methods: 92.42% and 93.39%, respectively, for MSD and SD. α-Terpineol, an oxygenated monoterpene, is present at 0.86% and 0.84%, respectively for MSD and SD. Therefore, in this application, microwave irradiation highly accelerated the extraction process, without major modifications in the volatile oil composition, phenomenon which was already described [21].

Structural changes after extraction

The images from the surfaces of orange peels obtained by SEM before and after the extraction are shown on Figure 5. Figure 5a is a micrograph of the untreated gland (i.e., before the extraction). Images from the orange peels undergone a 7 h SD (Figure 5b) and a 140 min MSD (Figure 5c) are also shown for comparison. Both extraction methods resulted in apparent physical changes in the orange glands. While MSD destroyed the glands in 140 min, the extraction with SD did not start by that time. This indicates that microwaves (i.e., the irradiation) cause the glandular walls to crumble or rupture more rapidly and more efficiently. The gland undergone SD (Figure 5b) is wrinkled while that undergone MSD (Figure 5c) is not. Such differences can be attributed to a difference in the rate of heat transfer between the two extraction methods. MSD utilizes three ways of heat transfer within the sample: irradiation, conduction and convection. As a result, with MSD, heat is produced more quickly from within the glands as well as from the outside [13]. With SD, heat transfer can occur through conduction and convection only.

Evaluation of physical properties

Physical properties (refractive index, specific gravity and acid value) of orange essential oils extracted by both SD and MSD are shown in

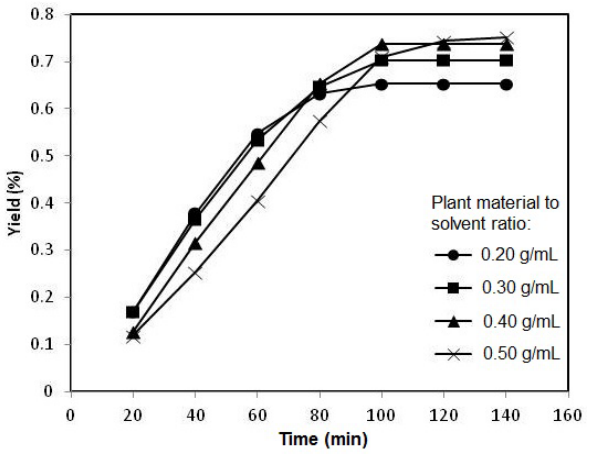

Figure 4: Yield (%) profile of the orange essential oil obtained by microwave steam distillation (MSD) method as a function of extraction time.

No	Compounds	SD (%)	MSD (%)
1	α-Pinene	0.67	0.55
2	β-Pinene	0.94	0.69
3	Myrcene	1.87	1.95
4	Limonene	93.39	92.42
5	δ-Carene	0.74	0.00
6	Cyclohexene	1.55	0.12
7	α-Terpineol	0.84	0.86
8	Cyclotrisiloxane	0.00	0.79
9	Sabinene	0.00	0.20
10	Octyl aldehyde	0.00	0.19
11	n-Octanol	0.00	0.10
12	n-Nonadecanoic	0.00	0.50
13	Hexasiloxane	0.00	0.18
14	Thiocynic acid	0.00	0.25
15	Dimethoxybenzylidene	0.00	0.20
16	γ-Gurjunene	0.00	0.32
17	δ-Guaiene	0.00	0.12
18	n-Decanal	0.00	0.16
19	β-Fenchyl alcohol	0.00	0.30

Table 1: Chemical compositions of essential oils obtained by steam distillation (SD) and microwave steam distillation (MSD) of orange peel.

Table 2. For comparison purposes, standard properties obtained from EOA are also shown in the same Table. The refractive indices and specific gravities of essential oils obtained from orange peel for MSD fall within the ranges specified by EOA. While the refractive indices and specific gravities of essential oils obtained from orange peel for SD doesn't fall within the ranges specified by EOA. However, the acid values of both essential oils are in the range indicated by EOA. Therefore, considering physical properties of the extracted essential oils, MSD as an extraction technique offer important advantages over traditional alternatives and does not introduce any problems to the essential oils extracted from the orange peel.

Conclusions

MSD offered substantial advantages over conventional SD. A similar extraction yield was achieved at significantly shorter extraction time when using MSD instead of SD. SEM images of orange peel undergone MSD and SD indicated that microwaves cause a quick rupture of the glandular walls resulting in a higher extraction efficiency at a shorter time. GC–MS results indicated that there were no significant differences between the essential oils obtained by MSD and those obtained by SD proposing MSD as an excellent alternative for SD with no adverse effects on the composition of the extracted essential oils. Compared to many solvent extraction techniques such as Soxhlet and accelerated solvent extraction, MSD is modern, green and fast. Thus, it can be concluded that MSD can be used as an efficient method to extract orange essential oil as growth promoter for fish.

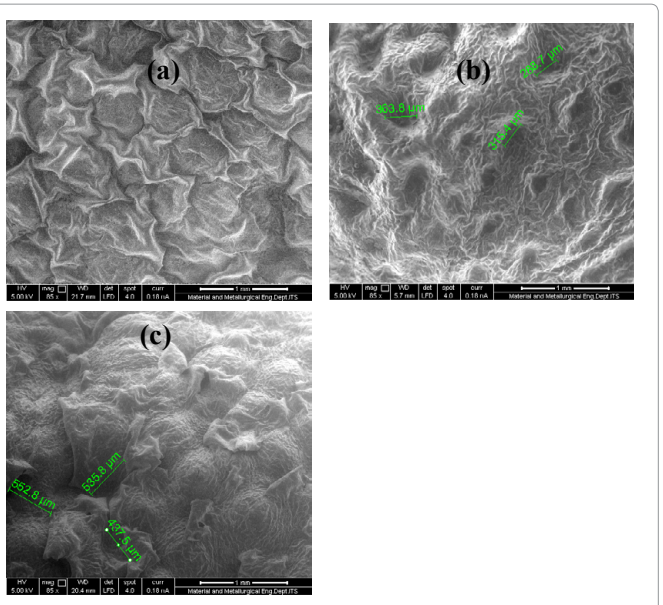

Figure 5: Structure of orange flavedo prior (a), after steam distillation (SD) extraction (b) and after microwave steam distillation (MSD) extraction (c).

Physical properties	EOA	SD	MSD
Specific gravity (25°C)	0.8420-0.8460	0.8500	0.8430
Refractive index (20°C)	1.4723-1.4737	1.4710	1.4728
Acid value	<5	2.8500	2.8500

Table 2: Physical properties of essential oils from orange peel obtained by steam distillation (SD) and microwave steam distillation (MSD).

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