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Research article

Vanillin extraction from Thai vanilla pods using ohmic heating followed by ultrasound- or microwave-assisted extraction

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Abstract

Importance of the work: Typically, vanilla extract is used to enhance the flavor and taste of foods and beverages. With the rising demand for natural extracts, several extraction technologies were explored to improve extraction efficiency and preserve the extract quality.
Objectives: Ohmic heating extraction (OHE) was investigated as a pretreatment for vanilla extraction followed by conventional extraction (CE), ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE).
Materials & Methods: Vanilla pods were subjected to various methods (CE, UAE or MAE) without and with OHE as a pretreatment. The extraction conditions were carried out at 55°C using 1:10 (weight per volume) of vanilla pods and 35% ethanol. The vanilla extracts were compared for their vanillin content, antioxidant activities—using 2,2'-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and ferric ion reducing antioxidant power (FRAP) assays)—and electronic nose profiles to identify the best method in terms of extraction efficiency and extract quality.
Results: The highest vanillin content was produced using CE; however, UAE substantially shortened the extraction time from 12 hr to 0.25 hr. The highest vanillin extraction efficiency was from using OHE, which also created pores on the vanilla pods as was evident from the various pore sizes that appeared on the surface. When OHE was applied before further extraction, a higher amount of vanillin was obtained than without pretreatment, specifically 55.68% for OHE+UAE and 34.39% for OHE+MAE. Nonetheless, the antioxidant capacities of the OHE+CE extracts were the highest based on the DPPH, ABTS and FRAP assays. Odor analysis based on E-nose analysis identified vanillin, benzaldehyde and furfural as the chemicals with high relevance index values.
Main finding: OHE showed potential as a rapid pretreatment method to improve vanillin extraction while maintaining antioxidant activity. Combined OHE and UAE substantially improved the extraction efficiency.

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Introduction

Vanilla (*Vanilla planifolia* Andrews) is an economically important cultivated plant that has a high economic value (Longares-Patrón and Cañizares-Macías, 2006; Dong et al., 2014). In Thailand, vanilla cultivation was initially introduced to the northern highlands by the Royal Project Foundation and although production in the early phase was quite small, the average yield of green beans was approximately 4.01 kg/tree; later, vanilla cultivation supported local communities through economic opportunities (Wongsheree et al., 2013). Krasaekoopt et al. (2010) reported that crude extract from Thai vanilla beans contained natural antimicrobial substances against *Listeria monocytogenes* and *Salmonella* Typhimurium, which could be utilized as a natural preservative in fresh-cut fruits and vegetables. According to Sinha et al. (2008), vanilla extract contains approximately 200 compounds, of which the major component is vanillin (4-hydroxy-3-methoxybenzaldehyde). Vanillin is found naturally in vanilla pods as an organic substance and is widely used as a flavouring agent in foods, beverages, desserts and fragrances, as well as in the pharmaceutical industry (Sinha et al., 2008; Jadhav et al., 2009; Arya et al., 2021). In addition, vanillin has been used in multiple therapeutic applications and it has potential bioactive values such as neuroprotection, anticarcinogenic and antioxidant properties (Arya et al., 2021). In cured vanilla pods, vanillin is naturally found at 1–2% concentration (Sinha et al., 2008). Due to limited supply and the extended curing process, natural vanilla extract commands a high price. In addition, increasing consumer preference toward healthy and more natural products has driven the high demand for natural flavors and ingredients (Shyamala et al., 2007; Jadhav et al., 2009). Consequently, several alternative and practical extraction methods have been investigated to produce natural vanilla extract (Jadhav et al., 2009; Dong et al., 2014).

Extraction is an important process, influenced by several factors such as types of solvent, extraction method, temperature, time and pressure (Moreira et al., 2017). The solvent and extraction methods affect the extract's antioxidant activities (Michiels et al., 2012). Conventional extraction (CE) is a fundamental method for the extraction of bioactive compounds as it does not require complicated equipment and is cheap, but has several disadvantages, such as using a large amount of solvent, being time-consuming and requiring considerable energy during the process; nonetheless, it is commonly used as a reference method to compare the efficiency of alternative methods (Garcia-Vaquero et al., 2020). New extraction methods have been developed to increase the extraction efficiency and to reduce the quantities used and the environmental impacts of organic solvents (Azmir et al., 2013). Ultrasound-assisted extraction (UAE) uses sound waves in the frequency range 20–100 kHz, which is higher than the audible frequency range of human hearing, and involves the occurrence of cavitation and bubbles which grow in size until disrupted, with the resulting high energy producing shock waves that degrade the plant cell wall, improve solvent penetration and later the release of extracted compounds (Rutkowska et al., 2017; Kumar et al., 2021).

The objectives of the current research were to investigate various extraction methods (CE, UAE, MAE and OHE) at their optimum conditions and to evaluate their impact on the crude extract from under-graded Thai vanilla pods in terms of vanillin content and antioxidant activities using 2,2'-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and ferric ion reducing antioxidant power (FRAP) assays. In addition, OHE as a pretreatment for further vanillin extraction was examined to improve the extraction process for the Thai vanilla pods.

Materials and Methods

Sample preparation

Under-graded vanilla pods that had been harvested and cured during 2021 were purchased from the Mae Fah Luang Foundation under Royal Patronage (Thailand). Each pod was 84.89±26.00 mm in length, 3.97±1.38 mm in thickness and weighed 0.87±0.57 g. The vanilla pods (38.34±0.58% moisture content) were vacuumed packed and stored at 3–5°C. Then, the pods were cut using a pair of scissors (Precision Ultra Edge Titanium Scissors Scotch™; 3M Thailand Co., Ltd.; Thailand) into small pieces (about 2.50±0.50 mm long) before being subjected to extraction.

Chemicals and reagents

Vanillin standard, 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid (ABTS), 2,4,6-tris(2-pyridyl)-S-triazine (TPTZ) and Trolox were purchased from Sigma-Aldrich (Germany). Other chemicals used in the experiment were DPPH from Alfa Aesar (the UK), ethanol (AR grade, 95%) from Merck (the USA), ferric chloride hexahydrate from Loba Chemie (India) and potassium peroxodisulfate from PanReac Applichem (Brazil). Hydrochloric acid was obtained from Mallinckrodt Baker (the USA) and the glacial acetic acid was purchased from RCI Labscan (Thailand). Sodium hydroxide (AR grade) and sodium acetate were acquired from Ajax Finechem (Australia).

Comparison of extraction methods

Vanilla pieces were extracted for vanillin using four different extraction methods— CE, UAE, MAE or OHE— under the optimum conditions for each method. From the preliminary experiments, the various extraction conditions for each extraction method were investigated and the optimum conditions were determined specifically based on the highest extraction efficiency (data not shown).

Conventional extraction

Vanilla samples (10 g) were soaked in 100 mL of 35% ethanol (1:10 weight per volume; w/v) at 55°C for 3 hr in a water bath with a shaking speed of 120 revolutions per minute (rpm; model WNB 29; Memmert GmbH & Co. KG; Germany) and passed through Whatman No. 1 filter paper. The extracts were kept in amber bottles until used. The experiment was repeated twice.

Ultrasound-assisted extraction

Vanilla samples (10 g) were placed in a beaker and then submerged in 100 mL of 35% ethanol (1:10 w/v). The samples were immediately extracted using an ultrasound bath (VGT-1860QTD; People's Republic of China) with a frequency of 40 kHz in pulse mode at 55°C for 0.25 hr. Then, they were passed through Whatman No. 1 filter paper and kept in amber bottles until used. The experiment was repeated twice.

Microwave-assisted extraction

Vanilla samples (2 g) were added with 20 mL of 35% ethanol (1:10 w/v) in a vessel. Then, the vessel containing the sample was placed in a microwave digestion system (SK-15 high-pressure segmented rotor, T2 easyTEMP temperature control; ETHOS UP; Italy) at 2,450 MHz frequency and 1,800 W power, capable of operating at the maximum temperature (300°C) and maximum working pressure (100 bar). Extraction was carried out at 55°C for 0.17 hr. Then, the extracts were passed through Whatman No. 1 filter paper and kept in amber bottles until used. The experiment was repeated twice.

Ohmic-heating extraction

A laboratory-scale ohmic heater operating at 50 Hz and with the electrode distance fixed at 4.0 cm was designed by Boonchai Haruthaithanasan (as illustrated in Piyapanrungrueang et al., 2016). Vanilla samples (20 g) were soaked in 200 mL of 35% ethanol (1:10 w/v) at 100 V (resulting in an electric field strength of 25 V/cm) with the final temperature at 55°C, resulting in an extraction time of 0.30 hr. The extracts were passed through Whatman No. 1 filter paper and kept in amber bottles until used. The experiment was repeated twice.

The extracts obtained from each method were analyzed for vanillin content and antioxidant activities (based on DPPH, ABTS and FRAP assays).

Determination of vanillin content

Vanilla extract (10 mL) was added to a 100 mL flask and the volume was adjusted to 100 mL using reverse osmosis (RO) water. Then, 2 mL of diluted sample was removed and mixed with 2 mL of 0.1N NaOH in a 100 mL flask. Then, it was diluted with RO water to 100 mL and analyzed using an ultraviolet-visible light spectrophotometer (UV-1900; Shimadzu Corp.; Japan) at a wavelength of 348 nm. The measured absorbance was compared with the vanillin calibration curve (coefficient of determination = 0.9978) to obtain the vanillin concentration in the crude extract, as shown in Equation 1:

$$y = 1.2646x - 0.0264 \quad (1)$$

where y is the vanillin concentration in the crude extract and x is the measured absorbance of the crude extract.

Then, the vanillin content was calculated based on the initial weight of the vanilla pods used in each extraction.

Determination of antioxidant activities

2,2-Diphenyl-1-picrylhydrazyl antioxidant scavenging assay

The analysis was modified from the method of Maduwanthi and Marapana (2021). Samples of DPPH solution (0.05 mM) were prepared (the absorbance at 517 nm was adjusted to be in the range 0.700±0.02). The extract (150 µL) was mixed with DPPH solution (2,850 µL) and stored for 0.50 hr in the dark. Later, the absorbance was measured at 517 nm using the UV-visible spectrophotometer; the % DPPH radical inhibition was calculated using Equation 2 and compared with a standard curve of Trolox solution, expressed as milligrams of Trolox equivalents per gram of sample:

$$\% \text{ DPPH radical inhibition} = (Ac - As) / Ac \times 100 \quad (2)$$

where Ac is the absorbance of the control and As is the absorbance of the sample.

2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulphonic acid radical cation scavenging activity

The method for investigating the ABTS radical cation scavenging activity was modified from Re et al. (1999). The ABTS radical cation was generated by mixing 7 mM ABTS and 2.45 mM potassium persulfate solution at a ratio of 2:1; the mixture was kept in the dark and refrigerated for 12 hr. The ABTS solution was diluted with ethanol until its absorbance at 734 nm was in the range 0.700±0.02. Later, 10 mL of ABTS solution and 100 µL of vanilla extract were reacted in the dark for 6 min. The absorbance was measured at 734 nm using the UV-visible spectrophotometer. The results were calculated as the percentage of free radical scavenging using Equation 3 and compared to a standard curve of Trolox concentrations, which were expressed in milligrams of Trolox equivalents per gram of sample:

$$\% \text{ ABTS radical inhibition} = (Ac - As) / Ac \times 100 \quad (3)$$

where Ac is the absorbance of the control and As is the absorbance of the sample.

Ferric reducing antioxidant power

The FRAP assay was analyzed according to the method of Kubola and Siriamornpun (2008). FRAP reagent was prepared by mixing 100 mL of 300 mM acetate buffer at pH 3.6, 10 mL of 10 mM TPTZ (in 40 mM of hydrochloric acid), 10 mL of 20 mM ferric chloride hexahydrate and 12 mL of distilled water. Then, 60 µL of extract, 180 µL of distilled water and 1,800 µL of FRAP reagent were added and kept at 37°C for 4 min. The absorbance was measured at 593 nm using the UV-visible spectrophotometer. The absorbance was compared with a standard curve of Trolox solution and reported as milligrams of Trolox equivalents per gram of sample.

Energy and extraction efficiency

The energy used and the extraction efficiency of the optimum conditions for each extraction method (CE, UAE, MAE and OHE) were calculated according to Equations 4 and 5, which were adapted from Piyapanrungrueang et al. (2016).

$$\text{Energy} = \text{Power} \times \text{Time} \quad (4)$$

where the energy is measured in kilowatt-hours per gram.

$$\text{Extraction efficiency} = \text{Vanillin content} / \text{Energy} \quad (5)$$

where extraction efficiency is measured in milligrams per kilowatt-hour, vanillin is measured in milligrams per gram and energy is measured kilowatt-hours per gram.

The energy used for extraction was based on 1 g of sample. The extraction efficiency indicated the amount of vanillin being extracted by using one energy unit. Therefore, each extraction method could be readily compared.

Study of effect of ohmic heating extraction as pretreatment prior to extracting vanillin using conventional extraction, ultrasound-assisted extraction or microwave-assisted extraction with selected conditions

To further improve vanillin extraction, OHE was used as a pretreatment to likely initiate pore formation and facilitate solvent extraction. Vanilla pieces were pretreated with OHE prior to extraction using one of the three methods (CE, UAE or MAE) under the same optimum conditions for each method as explained in the previous section. These combined treatments were defined as OHE+CE, OHE+UAE and OHE+MAE. The resultant extracts were analyzed for the vanillin content, antioxidant capacities and electronic nose (e-nose) profile. In addition, pieces of extracted vanilla from each extraction method and unextracted ones were subjected to stereomicroscopy.

Stereomicroscopy

Samples of vanilla pods following extraction using OHE+CE, OHE+UAE or OHE+MAE were photographed under a stereomicroscope (S8 APO; Leica; the USA) with 80x magnification. Cross-section images were compared with unextracted ones.

E-nose analysis

Each sample (1 mL) of the vanilla extract from OHE+CE, OHE+UAE and OHE+MAE was transferred into a 20 mL vial that was then cramp-sealed using an aluminum cap with a Teflon septum. A Heracles NEO 300 electronic nose (Alpha MOS SA; France) and the Alphasoft version 2021 software incorporated with the AroChemBase database were used for the volatile compound analysis. Each sample vial was incubated at 80°C for 10 min at 500 rpm agitation speed. Hydrogen gas was used as a carrier gas at 30 mL/min constant flow rate. A headspace autosampler was used to inject the headspace gas under the following conditions: injection volume 5 mL, injection speed 125 µL/s, injection with split mode at a rate of 10 mL/min and trapping temperature 60°C. Separation of volatile compounds was achieved using two metal capillary columns (MXT-5 and MXT-1701; 10 m length × 0.18 mm internal diameter × 0.4 µm film thickness of absorbing polymer) under an oven temperature program with an initial temperature 50°C that was increased to 250°C at a heating rate of 2°C/s. The flame ionization detection was operated at 260°C.

Statistical analyses

The vanillin content, vanillin extraction efficiency and antioxidant capacities of each method were compared. All data were reported as mean±SD values. Analysis of variance was carried out using the IBM SPSS Statistics software (version 28; Thaisoftup Co. Ltd.; Thailand). Duncan's new multiple range test was used to determine the differences among means at the 95% confidence level.

Results and Discussion

The global market for natural extracts is rising due to consumer demand for authenticity and superior quality. Approximately 100–200 flavor components are present in natural vanilla extracts (Longares-Patrón and Cañizares-Macías, 2006). Since the method of the vanilla extraction is imperative to flavor profiles, there have been numerous alternative techniques investigated to preserve volatile compounds, especially minimizing vanillin degradation, as well as to reduce the usage of solvent (and possible even solvent-free) for environment sustainability. However, each method of extraction has its own advantages and limitations. Extraction efficiency could be contributed to several factors such as extraction method, type of solvent, solid to solvent ratio, pH, temperature, time and energy consumption (Jadhav et al., 2009). Therefore, the current study examined advanced methods (UAE, MAE and OHE) for Thai vanilla extraction and compared the results to those of the conventional method (CE). The combined process using OHE as a pretreatment followed by the investigated alternatives was carried out to improve vanillin extraction from under-graded Thai vanilla pods.

Influence of different extraction techniques on vanillin content, extraction efficiency and antioxidant activities

The number of vanilla orchards has been steadily growing in Northern Thailand, especially in Chiang Rai and Chiang Mai (Wongsheree et al., 2013). This study investigated the influence of several extraction technologies on the extraction of vanillin from vanilla grown in Thailand. Table 1 shows

Table 1 Vanillin content, energy used and extraction efficiencies of vanillin extract using different extraction methods

Extraction method	Vanillin content (mg/g)	Energy (kW-hr/g)	Extraction efficiencies (mg/kW-hr)
CE	15.14±0.09^{a}	5.40	2.80±0.02^{d}
UAE	7.56±0.56^{b}	0.12	62.99±4.63^{b}
MAE	6.58±0.93^{b}	0.32	20.38±2.92^{c}
OHE	4.28±0.01^{c}	0.007	611.43±0.01^{a}

CE = conventional extraction at 55°C for 3 hr; UAE = ultrasound-assisted extraction at 55°C for 0.25 hr; MAE = microwave-assisted extraction at 55°C for 0.17 hr; OHE = ohmic heating extraction at 25 V/cm and 55°C. Mean ± SD within each column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

that CE provided the highest vanillin content (15.14±0.09 mg/g) with the highest energy consumed, leading to the lowest extraction efficiency (2.80±0.02 mg/kW-hr). On the other hand, OHE could reduce the extraction time to 0.30 hr for obtaining vanillin content of 4.28±0.01 mg/g. When the vanilla pods that had been submerged with 35% ethanol were placed in the ohmic heater under an electric field strength of 25 V/cm, it took only 0.30 h to reach the final temperature of 55°C. These extraction conditions used only 0.007 kW-hr/g energy content and provided a relatively high extraction efficiency (611.43±0.01 mg/kW-hr). Ohmic heating can promote rapid heating and about 90% of the electrical energy may be converted into heat energy in the ohmic heating process (Ribeiro et al., 2022). Therefore, a very short time (within 0.30 hr) could be possible to reach the extraction temperature of 55°C. The vanillin contents extracted using by UAE and MAE were not significantly different. However, UAE had significantly greater (approximately three times more) extraction efficiency (62.99±4.63 mg/kW-hr) than MAE (20.38±2.92 mg/kW-hr). Dong et al. (2014) reported that MAE had the highest extraction power with the shortest extraction time among their four methods being studied (maceration, MAE, UAE and pressure-assisted extraction). The study by Longares-Patrón and Cañizares-Macías (2006) focused on microwave-assisted extraction (150 W power, 20 cycles of 1 min exposure time with 3 min delay time) that was applied to Mexican vanilla beans, producing a vanillin content of 21.36±0.42–25.96±0.60 mg/g in the crude extract. In another study, a vanillin content of 180 µg/mL was achieved using CE with intense extraction conditions of 95°C for 8 hr, while UAE provided rapid release of vanillin (140 µg/mL) after 1 hr (Jadhav et al., 2009). Similar results were found in the current study, where CE delivered the highest vanillin content at the cost of a lengthy extraction time and high energy consumption. In contrast, OHE was a viable extraction method with high efficiency that rapidly released vanillin but at a minimal level (Table 1).

The antioxidant capacity of food matrices potentially arises from the synergistic action of numerous bioactive compounds (Michiels et al., 2012). In addition to vanillin, which affects antioxidant capacity, there are also other phenolic substances such as p-hydroxybenzaldehyde, vanillic acid and p-hydroxybenzoic acid (McCormick, 2018). Radical scavenging activity assay indicated that the antioxidant activity of vanilla extracts from CE was the highest, compared among the four extraction techniques (Table 2). The crude vanilla extracts from CE had antioxidant capacities based on DPPH, ABTS and FRAP assays of 8.32±0.43 mg Trolox equivalents/g sample, 3.22±0.30 mg Trolox equivalents/g sample and 20.37±2.19 mg Trolox equivalents/g sample, respectively. Vanilla extracts with high vanillin concentrations have increased antioxidant capacity (Rojas-López and Cañizares-Macías, 2013). Vanillin (1,000 µg/mL) inhibited colon cancer cells during cell division (Arya et al., 2021). Apart from vanillin, other substances can contribute to antioxidation, such as vanillic acid, 4-hydroxy-3-methoxybenzyl alcohol, and 4-hydroxybenzyl alcohol (Shyamala et al., 2007). Based on the DPPH assay, the antioxidant activities of the vanilla extracts from MAE were 2.80 times and 6.28 times more prominent than those from UAE and OHE, respectively. Nonetheless, there were no significant differences for both the ABTS and FRAP assays of crude extracts between UAE and MAE. Dong et al. (2014) reported that their highest antioxidant activity of crude vanilla extract was from MAE and the antioxidant activities determined based on DPPH assay increased with a longer extraction time. Different extraction methods could affect the amounts of bioactive compounds, which are influenced by various extraction factors such as time, temperature, frequency and the type of solvent. For example, Jadhav et al. (2009) reported an increase in the vanillin content during the extraction of vanilla using UAE since as the extraction time increased (10–70 min), ultrasound disrupted the cell walls. Consequently, the ultrasound waves helped to accelerate the solvent's ability to penetrate the cell walls and release bioactive compounds.

Table 2 Antioxidant activities of vanillin extract using different extraction methods

Extraction method	Extraction time (min/hr)	DPPH	ABTS	FRAP
		mg Trolox/g sample		
CE	180/3	8.32±0.43^{a}	3.22±0.30^{a}	20.37±2.19^{a}
UAE	15/0.25	1.12±0.40^{c}	1.32±0.88^{b}	5.99±1.58^{b}
MAE	10/0.17	3.14±0.18^{b}	1.72±0.29^{b}	7.87±0.96^{b}
OHE	18/0.30	0.50±0.02^{d}	0.21±0.16^{c}	3.09±0.72^{c}

CE = conventional extraction at 55°C for 3 hr; UAE = ultrasound-assisted extraction at 55°C for 0.25 hr; MAE = microwave-assisted extraction at 55°C for 0.17 hr; OHE = ohmic heating extraction at 25 V/cm and 55°C; DPPH = 2,2'-diphenyl-1-picrylhydrazyl assay; ABTS = 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assay; FRAP = ferric ion reducing antioxidant power assay.

Mean ± SD within each column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Use of ohmic heating extraction as pretreatment to improve extraction

Typically, ohmic heating converts electrical energy into thermal energy within a product at a rapid heating rate (Loypimai et al., 2015; Piyapanrungrueang et al., 2016). Therefore, moisture adjustment would be an important step before using OHE. For example, black rice bran was added with water to increase the moisture content to 30–40% (wet basis) before ohmic heating-assisted solvent extraction to acquire anthocyanin (Loypimai et al., 2015). In the current study, as the moisture content of the Thai vanilla pods was 38.34±0.58%, the pods in ethanol could be directly treated using ohmic heating. Although OHE had the highest extraction efficiency (611.43±0.01 mg/kW-hr), the vanillin yield was the least of all extraction methods investigated (Table 1). To obtain a greater amount of vanillin, OHE was used as a pretreatment to improve the extraction process. Table 3 shows the results of vanillin extraction when the Thai vanilla pods were treated with OHE prior to other extraction techniques. The highest vanillin content (19.25±3.04 mg/g) was obtained from OHE+CE, whereas the contents from OHE+UAE (12.19±2.09 mg/g) and OHE+MAE (13.17±1.65 mg/g) were not significantly different. Based on analysis using a t test, the vanillin contents in the crude extracts were not significantly different between CE and OHE+CE (data not shown). However, significantly increased vanillin contents were obtained by using OHE as a pretreatment followed by either UAE or MAE. A substantial increase in extraction efficiency was noticed with OHE+UAE from 62.99±4.63 mg/kW-hr to 95.34±7.92 mg/kW-hr). The ability of OHE to enhance vanillin extraction and increase extraction efficiency is displayed in Fig. 1, showing a micrograph of untreated vanilla pieces before extraction, treated pieces with OHE and treated pieces with OHE followed by other extraction techniques. Small pores were observed on the vanilla pods treated with OHE at 25 V/cm for 0.30 h (Fig. 1B), indicating that ohmic heating could initiate pores on plant cells.

Table 3 Vanillin content, energy used and extraction efficiencies of ohmic heating extraction as pretreatment for vanillin extract followed by different extraction methods

Extraction method	Vanillin content (mg/g)	Energy (kW-hr/g)	Extraction efficiencies (mg/kW-hr)
OHE+CE	19.25±3.04^{a}	5.41	3.56±0.56^{c}
OHE+UAE	12.19±2.09^{b}	0.13	95.34±7.92^{a}
OHE+MAE	13.17±1.65^{b}	0.33	39.20±3.90^{b}

CE = conventional extraction at 55°C for 3 hr; UAE = ultrasound-assisted extraction at 55°C for 0.25 hr; MAE = microwave-assisted extraction at 55°C for 0.17 hr; OHE = ohmic heating extraction at 25 V/cm and 55°C.
Mean ± SD within each column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

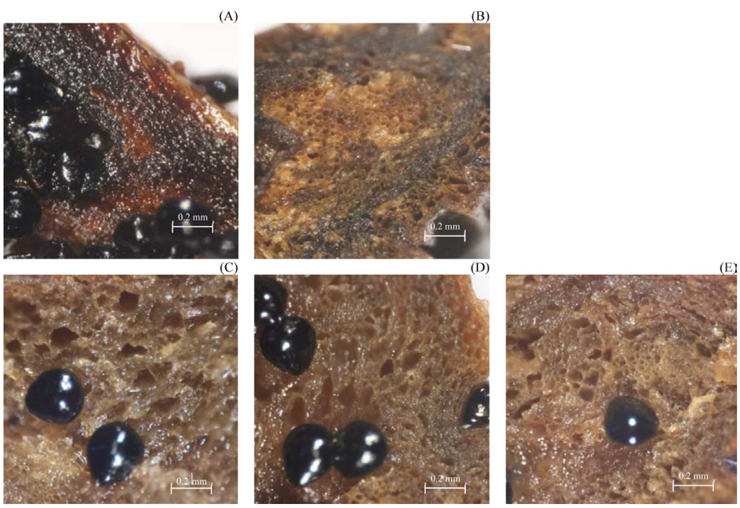

Fig. 1 Micrograph (80x) showing vanilla pieces under various treatment conditions: (A) untreated vanilla ; (B) pretreated vanilla with OHE; (C) treated vanilla with OHE+CE; (D) treated vanilla with OHE+UAE; and (E) treated vanilla with OHE+MAE, where CE = conventional extraction at 55°C for 3 hr; UAE = ultrasound-assisted extraction at 55°C for 0.25 hr; MAE = microwave-assisted extraction at 55°C for 0.17 hr; OHE = ohmic heating extraction at 25 V/cm and 55°C

Since most agricultural products contain ionic components (salts and acids), they are able to conduct an electric current. When sufficient moisture content is provided, an electric current can pass through the plant cell membrane, leading to cell membrane breakdown (Nair et al., 2014). Figs. 1C, 1D and 1E show larger pore sizes occurring on the vanilla pods, through which the solvent could easily diffuse and pass through the cell membrane (Donsì et al., 2010).

Vanillin is known for its antioxidant activities since it is the main phenolic compound found in cured vanilla beans (Shyamala et al., 2007; Dong et al., 2014; Arya et al., 2021). Similar results were observed for the vanilla extracts from CE with or without OHE as a pretreatment which contained the largest antioxidant activities from all three assays. Increased antioxidant activities were determined in the vanilla extracts from OHE+UAE and OHE+MAE (Table 4) since OHE may assist with the release of vanillin before further extraction (Fig. 1B). Antioxidant activities performed based on the three assays yielded comparable results for OHE+UAE and OHE+MAE ($p > 0.05$).

Volatile compounds in vanilla extracts based on flash gas chromatography electronic nose

The overall odor intensities were in the order OHE+MAE > OHE+UAE > OHE+CE (Table 5). This tendency was observed in both columns (MXT-5 and MXT-1701) of the electronic nose. From the relevance indices of chemicals in the vanilla extracts coming from different extraction methods, it was found that overall odor profiles of each vanilla extract were different. The major volatile compounds determined based on the electronic nose were vanillin, benzaldehyde and furfural. For OHE+CE, the relevance indices were 90.21% for vanillin, 89.23% for benzaldehyde and 89.19% for furfural. Although the highest extraction efficiency was obtained from OHE+UAE, OHE followed by UAE impacted the crude extract odor profile, with the major volatile compound (vanillin) in the extract being diminished. This could have been due to the decrease in the relevance indices compared to those of OHE+CE (90.21%) and OHE+UAE (39.09%). The odor profile of OHE+CE was dissimilar to those obtained from OHE+UAE and OHE+MAE, which might have been due to the greater extent of vanillin being extracted. The sensory descriptors for each chemical are displayed in Table 5.

Table 4 Antioxidant activities of ohmic heating extraction as a pretreatment for vanillin extract followed by different extraction methods

Extraction method	DPPH	ABTS	FRAP
	mg Trolox/g sample		
OHE+CE	10.38±0.76^{a}	4.66±0.46^{a}	19.89±2.08^{a}
OHE+UAE	7.06±1.12^{b}	3.75±0.39^{b}	12.19±1.25^{b}
OHE+MAE	8.01±0.79^{b}	3.83±0.42^{b}	13.37±0.71^{b}

CE = conventional extraction at 55°C for 3 hr; UAE = ultrasound-assisted extraction at 55°C for 0.25 hr; MAE = microwave-assisted extraction at 55°C for 0.17 hr; OHE = ohmic heating extraction at 25 V/cm and 55°C; DPPH = 2,2'-diphenyl-1-picrylhydrazyl assay; ABTS = 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assay; FRAP = ferric ion reducing antioxidant power assay.
Mean ± SD within each column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Table 5 Volatile compounds, retention time, intensity, relevance index and their sensory descriptors detected in vanilla extracts that were OHE pretreated then extracted with different extraction methods

Extraction method	Retention time (s)		Intensity		Chemical name	Relevance index (%)	Sensory descriptors
	MXT-5 column	MXT-1701 column	MXT-5 column	MXT-1701 column			
OHE+CE	17.42	19.37	19,801,121^{c}	12,024,641^{c}	1. Vanillin	90.21	Vanilla, Sweet
					2. Benzaldehyde	89.23	Almond, Bitter
					3. Furfural	89.19	Woody, Bread
OHE+UAE	17.44	19.41	20,413,732^{b}	12,927,414^{b}	1. Furfural	91.80	Woody, Bread
					2. Benzaldehyde	71.08	Almond, Bitter
					3. Vanillin	39.09	Vanilla, Sweet
OHE+MAE	17.37	19.29	21,102,407^{a}	13,440,784^{a}	1. Furfural	86.01	Woody, Bread
					2. Benzaldehyde	57.58	Almond, Bitter
					3. Vanillin	46.28	Vanilla, Sweet

CE = conventional extraction at 55°C for 3 hr; UAE = ultrasound-assisted extraction at 55°C for 0.25 hr; MAE = microwave-assisted extraction at 55°C for 0.17 hr; OHE = ohmic heating extraction at 25 V/cm and 55°C.
Values within each column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

When compared specifically on the relevance index of vanillin from the extracts using OHE as a treatment and those without OHE, increases in the relevance indices were detected of 39.09% (OHE+UAE) from 22.19% and of 46.28% (OHE+MAE) from 16.21% (data not shown). A slight change was observed with the extracts from OHE+CE, increasing from 82.74% (CE) to 90.21% (data not shown).

The proposed extraction techniques using OHE as a pretreatment before CE, UAE or MAE were capable of extracting vanillin from under-graded Thai vanilla pods at a selected ethanol concentration at 35%. OHE+CE provided the maximum amount of vanillin content (19.25±3.04 mg/g), whereas OHE+UAE had the highest extraction efficiency (95.34±7.92 mg/kW-hr). Both OHE+UAE and OHE+MAE were feasible for rapid extraction of vanillin (12.19±2.09–13.17±1.65 mg/g) within 0.47–0.55 hr. In terms of extraction efficiency, OHE+UAE was the desirable extraction method for Thai vanilla pods. However, OHE+MAE was also suitable, since this method reasonably preserved the antioxidant activities and overall odor profiles of the vanilla extracts. Further study should be carried out on the application of these vanilla extracts in food matrices, specifically focusing on odor and flavor profiles.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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