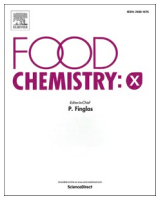
Food Chemistry: X 24 (2024) 102040

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Impact of drying techniques on volatile aroma profiles and formation mechanisms of black soybean thua nao

Pakavit Mathatheeranan [a], Ting-Jang Lu [b,*], Thanakorn Wongprasert [a], Yi-Ci Jhu [b], Hsin-Jo Chang [b], Wei-Yuan Wu [b], Mingchih Fang [c], Kitipong Assatarakul [a], Inthawoot Suppavorasatit [a,*]

[a] Department of Food Technology, Faculty of Science, Chulalongkorn University, Phayatai Road, Wangmai, Pathumwan, Bangkok 10330, Thailand
[b] Institute of Food Science and Technology, National Taiwan University, No. 1, Sec. 4, Roosevelt Road, Taipei 10617, Taiwan
[c] Department of Food Science, College of Live Science, National Taiwan Ocean University, Taiwan

ARTICLE INFO

Keywords:
Fermented black soybean
Drying
Aroma
Lipid oxidation
Maillard reaction

ABSTRACT

Thua nao, a traditional Thai fermented soybean, offers a unique aroma and nutritional value. However, fresh thua nao cannot be stored for long periods due to its high in water activity (a_w). This study examined the effects of various drying methods, including natural sun drying and machine drying methods, namely hot air, microwave vacuum (MIC), and vacuum drying on the qualities of dried black soybean thua nao. Our findings showed that MIC-treated sample showed the lowest a_w and highest crude fat and TVB-N contents. Volatile aroma compounds were categorized according to odor description and clustered using principal component analysis (PCA). Drying methods influenced volatile compounds associated with lipid oxidation (green-fatty attribute) and the Maillard reaction (nutty-roasted, sweet, sulfurous, and smoky attributes). PCA results indicated that volatile profiles of the sun-dried sample differed from the others. This research establishes a correlation between sugar and free fatty acid precursors and aroma attributes.

1. Introduction

Thua nao is a traditional fermented soybean product from Northern Thailand that has an umami taste and strong meaty aroma, which has promoted its consumption as a plant-base food and condiment for vegetarians and vegans. Moreover, thua nao products are salt-free, unlike other condiments such as shrimp paste or other fermented soybean products, which lends credence to their purported health benefits (Chukeatirote, 2015). During fermentation (mainly through indigenous *Bacillus* spp.), the compounds contained within soybeans are hydrolyzed into smaller molecules, such as amino acids, peptides, free fatty acids, and sugars. These small molecules can act as primary precursors for the formation of volatile and nonvolatile compounds in thua nao (Chukeatirote, 2015; Dajanta, Apichartsrangkoon, & Chukeatirote, 2011; Dajanta, Apichartsrangkoon, Chukeatirote, & Frazier, 2011). The volatile aroma compounds in thua nao are mainly formed through microbial activity, lipid oxidation, and the Maillard reaction (Mathatheeranan et al., 2023). Additionally, microbial enzymes can convert bound-form bioactive compounds in the thua nao into free-form compounds, such as phenolic acids and isoflavones, thereby increasing its nutritional value and antioxidant activity (Shin et al., 2014).

After fermentation, fresh thua nao can be consumed directly or used as an ingredient for other food products. However, it cannot be stored at ambient temperature for extended periods due to its high water activity (a_w). Therefore, drying is commonly employed to remove water from thua nao and thus extend the product's quality and safety (Chukeatirote, 2015; Leejeerajumnean, 2003). Studies have shown that drying parameters, namely temperature, pressure, and drying times, significantly impact chemical reactions, such as lipid oxidation and the Maillard reaction (Calín-Sánchez et al., 2020; ElGamal et al., 2023). Drying methods also directly affect physicochemical properties, nutritional value, sensory characteristics, and stability of the dried products (Calín-Sánchez et al., 2020). Based on available studies, the quality of dried thua nao produced from traditional sun drying (SUN) can vary from one batch to another due to its dependency on atmospheric weather conditions, such as temperature, humidity, and airflow velocity (Chukeatirote, 2015; Leejeerajumnean, 2003). In contrast, machine drying techniques, such as hot air drying (HOT), microwave vacuum

* Corresponding authors.
E-mail addresses: tjlu@ntu.edu.tw (T.-J. Lu), inthawoot.s@chula.ac.th (I. Suppavorasatit).

https://doi.org/10.1016/j.fochx.2024.102040
Received 22 October 2024; Received in revised form 20 November 2024; Accepted 21 November 2024
Available online 23 November 2024

drying (MIC), and vacuum drying (VAC), have been found to enhance quality maintenance and production efficiency, control operational cost-effectiveness, reduce drying times, prolong shelf-life, and facilitate commercial product upscaling (Calín-Sánchez et al., 2020; ElGamal et al., 2023).

Nowadays, the demand for dried foods with high nutritional value and health benefits has certainly increased. Dried food products produced using proper drying technologies are considered premium products due to their superior quality and high consumer perception compared to those produced using the traditional SUN method (ElGamal et al., 2023). Despite the availability of studies on volatile aroma compounds and antioxidative activity in fresh thua nao products (Dajanta, Apichartsrangkoon, & Chukeatirote, 2011; Mathatheeranan et al., 2023; Mathatheeranan, Wongprasert, Wang, et al., 2024), to the best of our knowledge, limited information has been available regarding the impact of various drying techniques on volatile aroma compounds and the mechanisms underlying their formation from lipid oxidation and the Maillard reaction in dried thua nao products, particularly those produced from black soybeans.

The current study aimed to investigate the effects of various drying treatments on volatile aroma profiles, free fatty acid profiles, lipid oxidation, and the Maillard reaction in dried black thua nao products. Additionally, volatile aroma profiles were classified based on odor descriptions, Pearson's correlation, and cluster analysis. The current study provides valuable information that can be utilized to enhance the quality and stability of dried thua nao.

2. Materials and methods

2.1. Sample preparation

This study used black soybeans from a Royal Project Foundation in Chiang-Mai province, Thailand for the preparation of thua nao, which followed the methods described by Mathatheeranan et al. (2023). Briefly, black soybeans were cleaned, soaked in refrigerated (4 °C) potable water for 24 h, steamed (100 °C) for 4 h, and then cooled to room temperature. Cooked soybeans (100 g) were inoculated with 0.3 g of lyophilized commercial *Bacillus subtilis* (initial active viable cell count: 6.35×10^6 CFU/g; Beijing Chuanxiu Science and Technology Ltd., Beijing, China). Fermentation was conducted in a chamber under a controlled temperature of 37 °C with 80 % relative humidity for 3 days. Fermented soybeans were then homogenized using a household grinder. Thereafter, 25 g of ground thua nao was formed into flat disks on nonstick Teflon sheets. Samples were then vacuum-sealed and refrigerated at 4 °C until further drying treatments.

2.2. Drying treatments

Drying treatments were conducted according to the methods described by Qin et al. (2022). Thua nao samples (flat disk shape, ~10 cm diameter) were dried using four different techniques: the traditional SUN (uncontrolled drying conditions) and three machine drying methods (controlled conditions), including HOT, MIC, and VAC. For SUN treatment, samples were placed in a basket for air ventilation and covered with a cheesecloth. The samples were then directly exposed to sunlight for 8 h or until a constant weight was achieved. For HOT treatment, samples were dried in an industrial hot air oven (Spring Green Evolution, Thailand) at 50 °C for 8 h or until a constant weight was reached. MIC treatment involved drying samples using a microwave vacuum drying machine (March Cool Industry, Thailand) equipped with two magnetron sources (each with a power output of 800 W) and a vacuum pressure of 800 mbar for 5 min. The temperature of the sample ranged from 45 °C to 46 °C during the drying process. For VAC treatment, samples were placed in a vacuum oven (Memmert, Germany) at 40 °C under a vacuum pressure of 40 mbar for 8 h. A sample with a constant weight was obtained after drying for 8 h. All drying treatments were simultaneously conducted in triplicate to avoid biases.

2.3. Physicochemical analysis

To determine moisture content, samples (2 g) were dried in a hot air oven (Memmert, Germany) at 105 °C until a constant weight was reached, following the AOAC method (AOAC, 2023). The a_w of the sample was then measured using a water activity analyzer (Aqualab, USA). Samples (0.5 g) were ground and blended into 5 mL of distilled water, after which the pH value was determined using a pH meter (Mettler Toledo, Switzerland). Crude fat content was determined by extracting 2 g of sample with petroleum ether (boiling point 40 °C–60 °C, Loba Chemie, India) using a Soxhlet apparatus (Foss, Denmark) for 4 h (Dajanta et al., 2012).

Total volatile basic nitrogen (TVB-N) content was determined following the method described by Maghraby et al. (2013). Samples (2.5 g) were extracted with 25 mL of 0.6 M perchloric acid (Sigma-Aldrich, USA) solution, spiked with antioxidant solutions containing 250 μL of 10,000 mg/kg butylated hydroxytoluene (BHT, KemAus, Australia) and 250 μL of 10,000 mg/kg ethylenediaminetetraacetic acid (EDTA, KemAus, Australia). The mixture was subjected to ultrasonic bath (40 kHz) (Keen, China) for 30 min at a controlled temperature of below 30 °C. Subsequently, the extract was centrifuged (Corning, USA) (4000 rpm for 15 min at ambient temperature) and filtered using Whatman paper No. 1 to obtain the clear extract. The TVB-N content of the sample was determined using steam distillation followed by titration. Thereafter, 10 mL of extract was distilled with 30 % NaOH solution (alkaline conditions) to convert ammonium ions into ammonia gas using a distillation apparatus (Buchi, Switzerland). This ammonia gas was trapped in 30 mL of 4 % (*w/v*) boric acid (KemAus, Australia) solution, after which this solution was titrated with 0.02 N HCl (Sigma-Aldrich, USA). An indicator containing 1 % (*v*/v) each of methyl red (Sigma-Aldrich, USA) and methylene blue (Sigma-Aldrich, USA) was used to determine the endpoint of the titration.

Determination of reducing sugar and total sugar were conducted following the method described by Dajanta et al. (2012). Accordingly, samples (2.5 g) were mixed with 25 mL of 80 % ethanol solution (Loba Chemie, India). The extraction and separation protocols were repeated as described earlier. Reducing sugar content was determined using 3,5-dinitrosalicylic acid (DNS) method. The reagent consisted of 1 g of 3,5-dinitrosalicylic acid (Sigma-Aldrich, USA), 0.2 g of phenol (KemAus, Australia), 0.05 g of sodium sulfite (KemAus, Australia), 1 g of sodium hydroxide (KemAus, Australia), and 20 g of sodium potassium tartrate (KemAus, Australia) dissolved in 100 mL of distilled water. After mixing 1 mL of extract with 1 mL of DNS reagent, the reaction mixture was boiled for 5 min and then cooled in ice water (4 °C). Meanwhile, total sugar content was determined using Anthrone reagent, prepared as a 0.1 % (*w/v*) in sulfuric acid (KemAus, Australia). After mixing 0.5 mL of extract with 1.5 mL of Anthrone reagent (Sigma-Aldrich, USA), the reaction mixture was boiled for 20 min and then cooled in ice water (4 °C). Reducing sugar and total sugar contents were measured using a spectrophotometer (Thermo Scientific, USA) at 540 and 620 nm, respectively. D-glucose (KemAus, Australia), which was used as the reference standard with concentrations ranging from 0.05 to 0.2 mg/mL, was employed for calibration.

2.4. Lipid oxidation indicator

2.4.1. Primary lipid oxidation indicator

Crude lipid extraction was conducted following the methods described by Cho et al. (2017). Briefly, samples (2.5 g) were extracted using 20 mL of 2:1:1 (*v*/v/v) chloroform:methanol:water solution (Loba Chemie, India). Antioxidant solutions containing 200 μL of BHT (10,000 mg/kg) and 200 μL of EDTA (10,000 mg/kg) were spiked into the sample mixtures, which were then shaken using an orbital shaker (Cole Parmer, USA) at a speed of 150 rpm for 30 min at 30 °C. Chloroform

phase was separated via centrifugation (Corning, USA) (4000 rpm, 15 min, ambient temperature). Subsequently, the extract was concentrated using nitrogen gas purging until no residual solvent remained.

Peroxide value (PV) was determined following the methods described by Ludwig et al. (2021). Accordingly, crude lipid (50 mg) was dissolved into 10 mL of 7:3 (*v*/v) chloroform:methanol solution. After adding 50 μL of 30 % (*w/v*) ammonium thiocyanate (KemAus, Australia) solution into sample mixture, followed by 50 μL of 0.06 M ferrous chloride (KemAus, Australia) solution, the sample mixture was incubated for 10 min at ambient temperature (without light). A standard curve was prepared using ferric chloride solutions (KemAus, Australia) with concentrations ranging from 1 to 40 μg/mL. The absorbance of the reaction mixture was measured using a spectrophotometer at 500 nm. PV results were reported as milliequivalents of peroxide per kilogram of oil (mEq peroxide/kg oil).

2.4.2. Secondary lipid oxidation indicator

Thiobarbituric acid reactive substances (TBARS) were determined following the methods described by Chang et al. (2009). Briefly, 1 mL of acid extract (TVB-N extraction mentioned in Section 2.3) was mixed with 1 mL of 0.3 % (*w/v*) thiobarbituric acid (TBA, Sigma-Aldrich, USA) reagent. This mixture was boiled for 10 min, and then the reaction was stopped in cooled water (4 °C). The reaction was measured using a spectrophotometer at 532 nm. Malondialdehyde (MDA, Sigma-Aldrich, USA) with concentrations ranging from 0.1 to 1.0 mg/kg was used as the reference standard. TBARS results were reported as milligrams of MDA per kilogram of oil (mg MDA/kg oil).

2.4.3. Free fatty acid analysis

Free fatty acid profiles were determined following the methods described by Namgung et al. (2010). Briefly, crude lipid (50 mg) extracted from chloroform:methanol:water (mentioned in Section 2.4.1) was mixed with 2 mL of 14 % (w/v) boron trifluoride (BF_3, Sigma-Aldrich, USA) in methanol. After adding 200 μL of internal standard of heptadecanoic acid (C17:0, 1 mg/mL in hexane, Sigma-Aldrich, USA), the mixture was incubated at 60 °C for 30 min for methylation, converting fatty acids to fatty acid methyl esters (FAMEs), and then cooled in ice water (4 °C). After incubation, FAMEs were extracted with 4.5 mL of hexane (HPLC grade, Sigma-Aldrich, USA) using a vortex for 30 s. Hexane phase was collected, concentrated using nitrogen gas purging, and then filtered using a nylon 0.22-μm filter.

A gas chromatograph (Agilent Technologies 7890 A, USA) coupled with a mass spectrometer (Agilent Technologies 5975C, USA) was used for analyzing free fatty acid profiles. Accordingly, 1 μL of the FAME extract was injected with a split ratio of 1:50 at 250 °C on a DB-wax column (Agilent, USA, 30 m × 0.25 mm i.d. × 0.25 μm film thickness). Helium gas (ultrahigh purity) at a flow rate of 1.0 mL/min was employed as the mobile phase. The oven temperature program started at 50 °C held for 1 min and then increased at a rate of 25 °C/min to 175 °C and 5 °C/min to 235 °C held for 5 min. Mass spectrometer conditions were set as follows: transfer line temperature at 250 °C, energy of electron impact ionization at 70 eV, and mass range (scan mode) of 40–500 amu. The solvent delay was set at 4 min. The relative concentrations were quantified using the peak area ratio of a target compound against the internal standard. FAME identification was qualified based on the mass spectrum (MS) and retention index (RI).

2.5. The Maillard reaction indicator

Maillard reaction indicators were determined according to the methods described by Mathatheeranan, Wongprasert, Fang, et al. (2024). Briefly, 1 g of sample was mixed with 20 mL of distilled water in a 50-mL centrifuge tube. The extraction using an ultrasonic bath and separation protocols were then repeated as mentioned in Section 2.3 (TVB-N extraction). Prior to spectrophotometric analysis, the extract was diluted 10-fold with distilled water to ensure absorbance values below 1. Maillard intermediate products and Maillard browning were measured using a spectrophotometer (Thermo Scientific, USA) at 294 (UV wavelength) and 420 (visible wavelength) nm, respectively. A single diluted sample was analyzed sequentially at both wavelengths against a distilled water blank.

2.6. Volatile compound analysis

2.6.1. Solid-phase microextraction (SPME)

Highly volatile compounds in the samples were extracted using the SPME technique following the methods published by Mathatheeranan, Wongprasert, Fang, et al. (2024). Briefly, 1 g of sample was mixed with 2 mL of saturated sodium chloride (NaCl, KemAus, Australia) solution and spiked with 10 μL of 2,4,6-trimethylpyridine (104.3 μg/mL in methanol, Sigma-Aldrich, USA) as the internal standard. The headspace vial was incubated in an agitator heat block at 60 °C for 10 min. After equilibration, the SPME fiber (divinylbenzene/carboxen/polydimethylsiloxane, DVB/CAR/PDMS, Sigma-Aldrich, USA) was used to extract volatile compounds at 60 °C for 20 min. Subsequently, the volatile compounds were desorbed from SPME fiber at the injection port (220 °C for 5 min) using the splitless mode with a purge flow to the split vent for 1.2 min.

2.6.2. Direct solvent extraction (DSE)

Solvent extraction coupled with the fractionation technique was conducted following the methods described by Mathatheeranan, Wongprasert, Wang, et al. (2024). Accordingly, 5 g of sample was mixed with 25 mL of dichloromethane (HPLC grade, Sigma-Aldrich, USA) at a ratio of 1:5. Thereafter, 20 μL of 2-ethyl-butanoic acid (1097 μg/mL in methanol, Sigma-Aldrich, USA) and 20 μL of 2,4,6-trimethylpyridine (1043 μg/mL in methanol, Sigma-Aldrich, USA) were added internal standards for acidic and neutral/basic fractions. The sample was shaken using an orbital shaker at a speed of 150 rpm for 30 min. Subsequently, the dichloromethane phase was collected, and residual samples were re-extracted twice under the same protocol. The pooled dichloromethane phase was fractionated into acidic and neutral/basic fractions based on pH solubility. Accordingly, 30 mL of 0.5 M sodium carbonate (KemAus, Australia) solution was mixed into the dichloromethane phase twice in a separatory funnel and then separated into aqueous and solvent layers. This solvent layer was washed twice with 15 mL of saturated NaCl solution to obtain a neutral/basic fraction (NaCl solution was discarded). Meanwhile, the aqueous layer was washed twice with 15 mL of dichloromethane, subsequently discarding the solvent. The aqueous layer was adjusted to create an acidic condition (pH 2) using HCl solution and subsequently extracted three times with 20 mL of dichloromethane. The dichloromethane layer was washed twice with 15 mL of saturated NaCl solution to obtain an acidic fraction. Extracted samples (both acidic and neutral/basic fractions) were concentrated using a Vigreux column in a water bath at 50 °C until the volume of each fraction had reduced to 10 mL. Each fraction was dried using anhydrous sodium sulfate (KemAus, Australia), further concentrated by flushing nitrogen gas to a final volume of 300 μL, and then filtrated using a 0.22-μm nylon filter. Thereafter, 1 μL of the DSE extract was injected at the injection port with split mode (ratio of 1:5) at 220 °C, with a purge flow to the split vent for 1.2 min. The solvent delay was set at 4 min.

2.6.3. Identification and quantitation of volatile aroma compounds

Volatile aroma compounds were analyzed using gas chromatography–mass spectrometry (GC–MS) following the methods published by Mathatheeranan, Wongprasert, Wang, et al. (2024). Volatile compounds were separately analyzed on two different polarity stationary phases, DB-wax (Agilent, USA, 30 m × 0.25 mm i.d. × 0.25 μm film thickness) and DB-5 (Agilent, USA, 30 m × 0.25 mm i.d. × 0.25 μm film thickness) columns, with helium gas (ultrahigh purity) as the mobile phase at a flow rate of 1.0 mL/min. The oven temperature program started at 40 °C held for 5 min and then increased at a rate of 5 °C/min to 220 °C held for

5 min. Mass spectrometer conditions were set as follows: transfer line temperature of 250 °C, energy of electron impact ionization of 70 eV, and mass range (scan mode) of 35–350 amu. Compound identification was conducted by comparing mass spectra (MS) with those reported in the NIST17 library database and matching retention indices (RIs) of both DB-wax and DB-5 columns with references published in the literature (Dajanta, Apichartsrangkoon, & Chukeatirote, 2011; Mathatheeranan, Wongprasert, Wang, et al., 2024). For quantitation, relative concentrations were calculated using the peak area ratio of a target compound to the internal standard present in each fraction (Mathatheeranan, Wongprasert, Wang, et al., 2024). The odor activity value (OAV) was calculated as the ratio between relative concentration and odor threshold of the compound (Mathatheeranan, Wongprasert, Wang, et al., 2024; Shahidi & Hossain, 2022; Van Gemert, 2011).

2.7. Statistical analysis

All results were analyzed in triplicate and expressed as mean ± standard deviation. Analysis of variance (ANOVA) and Duncan's multiple-range test were performed using IBM SPSS Statistics software, version 28 (IBM Corp., Armonk, NY, USA) to determine differences among treatments ($p \leq 0.05$). For data visualization, heatmap cluster analysis was conducted using GraphPad version 9. Principal component analysis (PCA) and Pearson's correlation were conducted using MetaboAnalyst program version 6 from webserver: https://www.metaboanalyst.ca/home.xhtml.

3. Results and discussion

3.1. Physicochemical analysis

The physicochemical properties of dried thua nao samples are summarized in Table 1. Studies have shown that a_w is an important factor for predicting the rate of chemical reactions and shelf-life of dried foods (Calín-Sánchez et al., 2020). All dried thua nao samples had a moisture content and a_w of <10 % and 0.7, respectively. The MIC-treated sample showed the lowest a_w (0.455), followed by those treated using VAC (0.525), HOT (0.585) and SUN (0.670). Evidence shows that a_w levels are affected by the lipid oxidation, the Maillard reaction, and enzymatic reaction, which can degrade nutritional value and decrease sensory properties (Calín-Sánchez et al., 2020). This finding indicates that the quality of the SUN-treated sample may degrade faster than that of samples treated using other methods due to increased lipid oxidation, the Maillard reaction, and enzymatic activity.

The MIC-treated sample was found to have the highest crude fat content at 20.32 g/100 g dry basis (db), whereas other samples yielded crude fat contents ranging from 14.27 to 14.94 g/100 g db (Table 1). MIC treatment may preserve fat in the product due to its short drying time, low temperature, and absence of oxygen interaction. One study found that MIC drying caused internal water molecules to quickly migrate to the outer surface of the food, resulting in enhanced porosity compared to conventional drying methods (Fu et al., 2015). Moreover, porosity occurring during MIC treatment could enhance extraction efficiency compared to the structural shrinkage occurring through conventional drying processes (Oikonomopoulou & Krokida, 2013). Hence, the MIC-treated sample attained the maximum crude fat content through solvent extraction (Table 1).

Table 1
Physicochemical properties of dried thua nao treatments.

parameter	drying treatment			
	SUN	HOT	MIC	VAC
moisture content (g/100 g)	9.59 ± 1.27 ^{d}	8.48 ± 0.29 ^{c}	6.35 ± 0.23 ^{a}	7.63 ± 0.16 ^{b}
water activity (a_w)	0.670 ± 0.002 ^{d}	0.585 ± 0.002 ^{c}	0.455 ± 0.002 ^{a}	0.525 ± 0.005 ^{b}
crude fat content (g/100 g, db)	14.94 ± 0.34 ^{a}	14.27 ± 1.27 ^{a}	20.32 ± 1.45 ^{b}	14.82 ± 0.80 ^{a}
pH	6.92 ± 0.07 ^{a}	6.97 ± 0.04 ^{a}	7.23 ± 0.20 ^{b}	7.20 ± 0.20 ^{b}
TVB-N content (mg/100 g, db)	218.9 ± 4.7 ^{b}	215.3 ± 3.2 ^{b}	322.9 ± 9.0 ^{c}	195.9 ± 6.0 ^{a}

Results expressed as the means ± standard deviation ($n = 3$).
Means within same row with different superscripts were significantly different (p-value ≤0.05).

During fermentation, protease activity from *B. subtilis* hydrolyzes proteins into free amino acids and peptides, which releases ammonia and consequently results in an alkaline pH of around 8 (Leejeerajumnean, 2003; Mathatheeranan et al., 2023) As shown in Table 1, the pH of thua nao ranged from 6.92 to 7.23 after drying, indicating that drying could cause the evaporation of ammonia and other alkaline volatile compounds (Leejeerajumnean, 2003). TVB-N contents were highest in the MIC-treated sample, followed by SUN-, HOT-, and VAC-treated samples. This phenomenon could be related to the efficiency of VAC treatment in removing water molecules and volatile substances, such as ammonia and aroma compounds (Fu et al., 2015; Thamkaew et al., 2021). Additionally, the organic acids, such as acetic acid, 2-methylpropanoic acid, 2-methylbutanoic acid synthesized from microbial fermentation and octanoic acid and nonanoic acid derived from lipid degradation, might contribute to the decrease in pH observed in dried thua nao (Mathatheeranan, Wongprasert, Wang, et al., 2024).

3.2. Indicator of lipid oxidation and the Maillard reaction

PV and TBARS results normally used to assess primary and secondary lipid oxidation products are presented in Fig. 1. PV was detected between 33 and 36 mEq peroxide/kg oil, with no significant difference among drying methods (Fig. 1a). Conversely, TBARS showed a lower value of 104 MDA mg/kg oil in the MIC-treated sample than in other samples (Fig. 1b). This finding could be likely attributed to the shorter drying time of MIC treatment, which could limit lipid oxidation by reducing lipid exposure to heat and oxygen. Consequently, the nutritional quality and bioactive compounds of the MIC-treated sample were preserved quite well (Fu et al., 2015; Thamkaew et al., 2021). Conversely, prolonged exposure to sunlight and heat during SUN drying accelerated lipid oxidation reactions, thereby increasing oxidation parameters, such as acid value, PV, and free fatty acid contents, which may reduce nutritional quality and bioactive compounds in SUN-treated samples.

Fresh thua nao contains high amounts of amino acids, peptides, and sugars, which readily participate in the Maillard reaction (Chukeatirote, 2015; Dajanta, Apichartsrangkoon, Chukeatirote, & Frazier, 2011; Leejeerajumnean, 2003). Reducing sugar and total sugar contents showed similar trends in all treated samples (Fig. 2). The sugar content in the SUN-treated sample was ~2.5 times lower than those in MIC- and VAC-treated samples (Fig. 2a and b). This significant reduction in sugars (Fig. 2) suggested their incorporation as precursors into the Maillard reaction (Fig. 3). Determination of the Maillard reaction indicator at both 294 and 420 nm serves to identify the formation of Amadori products occurring at the initial stages and the degree of browning at the later stages (Liu et al., 2022; Shahidi & Hossain, 2022). Moreover, after evaluating Maillard intermediate products and browning intensity, our results showed that MIC- and VAC-treated samples showed lower levels than did the other samples (Fig. 3). This finding could have been attributed to low temperatures associated with MIC and VAC treatment and a decrease in the rate of Maillard reactions due to low sugar utilization. Conversely, SUN- and HOT-treated samples were dried at high temperatures for longer drying periods, facilitating greater sugar utilization for Maillard reactions.

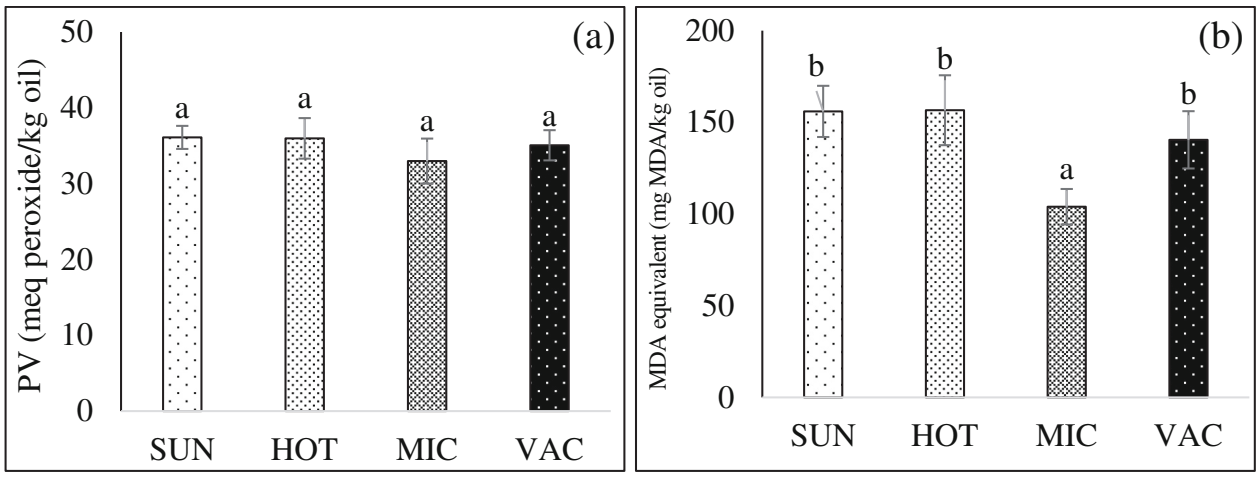


Fig. 1. Primary lipid oxidation indicator by peroxide value (PV) (a), secondary lipid oxidation indicator by TBARS (b) in dried thua nao treatments.
Result expressed as the mean ± standard deviation (n = 3).
Means within different drying treatments with different superscripts were significantly different (p-value ≤0.05).

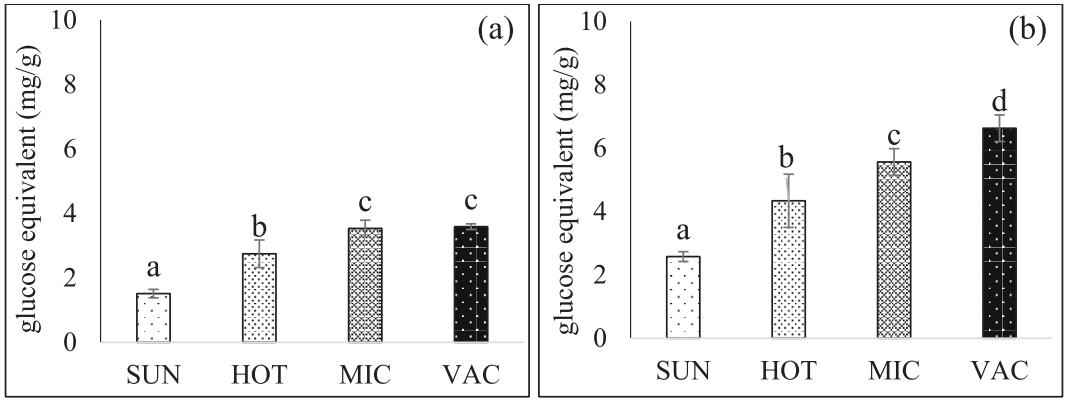


Fig. 2. Reducing sugar content by DNS method (a), total sugar content by Anthrone method (b) in dried thua nao treatments.
Result expressed as the mean ± standard deviation (n = 3).
Means within different drying treatments with different superscripts were significantly different (p-value ≤0.05).

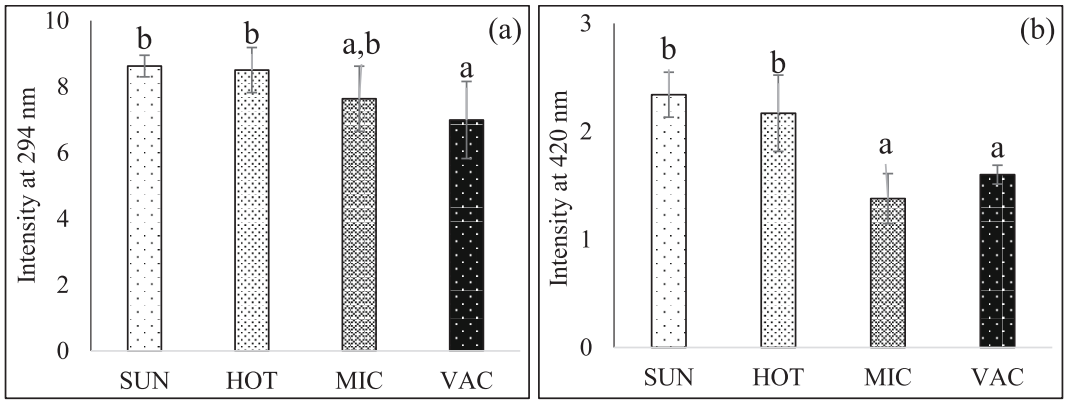


Fig. 3. The Maillard intermediate product (a), Maillard browning (b) in dried thua nao treatments.
Result expressed as the mean ± standard deviation (n = 3).
Means within different drying treatments with different superscripts were significantly different (p-value ≤0.05).

3.3. Free fatty acid composition

Fatty acids are generally stable in triacylglycerol. However, lipid oxidation can trigger triacylglycerol hydrolysis and the release of free fatty acids (Barriuso et al., 2013; Qu et al., 2016). In our study, we determined that free fatty acid profiles reflected the stability of the lipids in dried thua nao samples. The results of free fatty acid profiles are detailed in Table 2.

Table 2
Free fatty acid composition in dried thua nao treatments.

No.	RI wax	compound	formulation	relative concentration (mg/100 g oil)				identification [a]
				SUN	HOT	MIC	VAC	
1	1974	12-methyltridecanoic acid	BCFA iso14:0	3.54 ± 0.36 [a]	3.97 ± 0.35 [a]	3.61 ± 0.51 [a]	4.04 ± 0.47 [a]	MS
2	2023	tetradecanoic acid	C14:0	5.10 ± 0.58 [b]	3.03 ± 0.69 [a]	2.31 ± 0.71 [a]	3.21 ± 0.88 [a]	MS, RI
3	2077	13-methyltetradecanoic acid	BCFA iso15:0	5.11 ± 0.45 [a]	5.21 ± 0.3 [a]	5.14 ± 0.36 [a]	5.01 ± 0.49 [a]	MS
4	2094	12-methyltetradecanoic acid	BCFA anteiso15:0	14.4 ± 1.49 [a]	17.8 ± 1.04 [b]	17.4 ± 1.23 [b]	17.4 ± 1.17 [b]	MS
5	2181	14-methylpentadecanoic acid	BCFA iso16:0	6.42 ± 0.84 [a]	7.58 ± 0.07 [b]	7.65 ± 0.48 [b]	7.79 ± 0.63 [b]	MS, RI
6	2231	hexadecanoic acid	C16:0	312 ± 15.9 [b]	211 ± 36.1 [a]	178 ± 40.0 [a]	224 ± 55.5 [a]	MS, RI
7	2257	(*Z*)-9-hexadecenoic acid	C16:1, 9c (n7)	3.38 ± 0.41 [b]	2.11 ± 0.45 [a]	1.51 ± 0.45 [a]	2.31 ± 0.57 [a]	MS, RI
8	2284	15-methylhexadecanoic acid	BCFA iso17:0	2.99 ± 0.30 [a]	3.30 ± 0.43 [a]	3.26 ± 0.39 [a]	3.18 ± 0.12 [a]	MS
9	2301	14-methylhexadecanoic acid	BCFA anteiso17:0	3.72 ± 0.38 [a]	4.18 ± 0.21 [a, b]	4.53 ± 0.51 [b]	4.14 ± 0.06 [a, b]	MS
10	2357	(*Z*)-10-heptadecenoic acid	C17:1, 10c (n7)	1.72 ± 0.37 [b]	1.24 ± 0.25 [a, b]	0.73 ± 0.42 [a]	1.16 ± 0.48 [a, b]	MS
11	2441	octadecanoic acid	C18:0	77.8 ± 6.62 [b]	52.5 ± 17.5 [a]	39.6 ± 10.4 [a]	50.9 ± 15.3 [a]	MS, RI
12	2461	(*E*)-9-octadecenoic acid	C18:1, 9 t (n9)	338 ± 29.3 [b]	231 ± 65.2 [a, b]	188 ± 54.4 [a]	235 ± 83.5 [a, b]	MS, RI
13	2512	(*Z,Z*)-9,12-octadecadienoic acid	C18:2, 9c, 12c (n6)	634 ± 81.6 [b]	533 ± 98.8 [a, b]	434 ± 52.7 [a]	517 ± 129 [a, b]	MS, RI
14	2573	(*Z,Z,Z*)-9,12,15-octadecatrienoic acid	C18:3, 9c, 12c, 15c (n3)	177 ± 13.7 [b]	124.8 ± 39.9 [a, b]	96.2 ± 28.1 [a]	121 ± 38.2 [a, b]	MS, RI
15	2649	icosanoic acid	C20:0	5.56 ± 0.93 [b]	2.80 ± 1.33 [a]	2.18 ± 0.81 [a]	2.96 ± 1.16 [a]	MS, RI
16	2668	(*Z*)-11-icosenoic acid	C20:1, 11c (n9)	5.00 ± 0.47 [b]	2.16 ± 1.14 [a]	1.79 ± 1.02 [a]	2.16 ± 0.95 [a]	MS
		Total BCFA [b]		36.2 ± 3.82 [a]	42.0 ± 2.40 [a]	41.6 ± 3.48 [a]	41.6 ± 2.94 [a]	
		Total SFA [b]		400 ± 24.1 [b]	270 ± 55.6 [a]	223 ± 52.0 [a]	281 ± 72.8 [a]	
		Total MUFA+PUFA [b]		1160 ± 126 [b]	894 ± 206 [a]	722 ± 137 [a]	878 ± 253 [a]	

Results expressed as the means ± standard deviation ($n = 3$).
Means within same row with different superscripts were significantly different (p-value ≤0.05).
[a] Identification method: mass spectra (MS), retention indices (RI).
[b] Branched-chain fatty acid (BCFA), saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA).

The first group of free fatty acids, namely monomethyl branched–chain fatty acids (BCFAs), play a crucial role in human health through its incorporation into complex lipids, such as phospholipids, triacylglycerols, sphingolipids, and cholesterol esters, which are essential components of human skin, vernix caseosa, colostrum, and mature breast milk (Gozdzik et al., 2023). These BCFAs are saturated fatty acids with an additional methyl group at either the n-2 carbon (iso configuration) or n-3 carbon (anteiso configuration) (Diomandé et al., 2015; Gozdzik et al., 2023). In general, soybean contains even-numbered carbon fatty acids since their synthesis pathway involved acetyl-CoA as the building block (two-carbon molecules), which undergoes sequential elongation. The presence of the BCFAs is depended on the microbial activity involved in the fermentation process (Wang et al., 2019). Interestingly, the current study has been the first to report BCFAs in dried thua nao products. Our results found that BCFA content was only slightly affected by drying treatments, with anteiso-15:0 (14.4–17.8 mg/100 g oil) being the most affected, followed by iso-16:0 (6.4–7.7 mg/100 g oil) and iso-15:0 (5 mg/100 g oil). Generally, BCFA formation can be synthesized from amino acids, such as isoleucine for anteiso-15:0 and anteiso-17:0, valine for iso-14:0 and iso-16:0, and leucine for iso-15:0 and iso-17:0, through *B. subtilis* biosynthesis (Diomandé et al., 2015). In addition, studies on fermented soybean products, including natto, miso, and douche, revealed that BCFA content accounted for 1.00 % ± 0.64 %, 0.37 % ± 0.06 %, and 0.08 % ± 0.01 % of total fatty acids, respectively (Wang et al., 2019).

During lipid oxidation, triacylglycerol is hydrolyzed into free fatty acids through autooxidation, photooxidation, and enzymatic reactions (Shahidi & Hossain, 2022). Free fatty acids serve as precursors for hydroperoxide formation, which further decomposes into various secondary oxidation products (Barriuso et al., 2013). Table 2 details the 12 fatty acids detected herein, including saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). The main components of unsaturated fatty acids were linoleic acid (C18:2; 434–634 mg/100 g oil), followed by oleic acid (C18:1; 188–338 mg/100 g oil), linolenic acid (C18:3; 96.2–177 mg/100 g oil), and others (0.8–3.4 mg/100 g oil). Meanwhile, the main SFA components included palmitic acid (C16:0; 178–312 mg/100 g oil), steric acid (C18:0; 39.6–77.8 mg/100 g oil), and arachidic acid (C20:0; 2.18–5.56 mg/100 g oil). As seen in Table 2, the total SFA and unsaturated (MUFA + PUFA) contents in the SUN-treated sample were significantly higher than those in the machine-treated samples, which indicated that lipid degradation by SUN treatment was the most susceptible to free fatty acid formations, followed by HOT, VAC, and MIC treatments. These results could suggest that the SUN-treated sample might have been exposed to oxygen, ultraviolet (UV) radiation, and uncontrolled temperatures, which could promote photooxidation and the generation of various unstable free radicals and reactive oxygen species (ROS), especially singlet oxygen that could attack the double bonds of PUFAs (Lee & Min, 2010).

3.4. Identification and quantification of volatile aroma compounds

Because aroma compounds in thua nao products exhibit a wide range of volatilities, polarities, and pH-dependent solubilities (Mathatheeranan, Wongprasert, Wang, et al., 2024), volatile aroma compounds of dried thua nao samples were analyzed using SPME (for highly volatile compounds) and DSE (for compounds with lower volatility) techniques coupled with GC–MS. From Table 3, acidic fraction (A) predominantly contained compounds containing acidic, furan, and phenolic groups, while the neutral/basic (N/B) fraction was enriched in compounds containing alcohol, ester, ketone, and nitrogen groups. In our previous works, 87 volatile compounds were identified in fresh thua nao products, such as (*E*)-2-hexenal, heptanal, (*E*)-2-nonenal, (*E,E*)-2,4-nonadienal, decanal, 2-decanone, and 2-undecanone (Mathatheeranan et al., 2023; Mathatheeranan, Wongprasert, Wang, et al., 2024). However, those volatile compounds were absent in the dried treated samples (Table 3). This could be attributed to their potential loss or transformation into other compounds during the drying process (Calín-Sánchez et al., 2020; Oikonomopoulou & Krokida, 2013). The present study positively identified 65 volatile compounds, including acids, alcohols, aldehydes, amides, aromatics, esters, furans/furanones, ketones, and pyrazines, using MS and the RI of each compound in both polar and nonpolar columns. Given that the Maillard reaction and lipid oxidation are the primary mechanisms occurring in dried thua nao products, the volatile compounds were grouped based on these possible mechanistic pathways.

3.4.1. Generation of volatile compounds from lipid oxidation

The type of drying method used can significantly influence the formation of volatile compounds in fermented foods by altering the levels

Table 3
Relative concentrations and odor descriptions of volatile aroma compounds in dried thua nao treatments.

No.	RI [a]		compound	fractionation [b]	relative concentration (μg/kg)				odor description [c]	identification [d]
	wax	DB-5			SUN	HOT	MIC	VAC		
1	935	697	3-methyl-butanal	H	308.3 ± 43.7 [b]	417.2 ± 45 [c]	178.6 ± 28.8 [a]	392.5 ± 80.7 [b,c]	fruity, malt	MS, RI
2	951		3-methyl-3-buten-2-one	A	388.5 ± 95.2 [a]	259.4 ± 38.9 [a]	1300.5 ± 273.4 [c]	700.9 ± 125.6 [b]	pungent	MS, RI
3	1006		2-butenal	NB	902.9 ± 84.8 [a]	860 ± 146 [a]	1184.1 ± 317.6 [a,b]	1287.6 ± 87.4 [b]	pungent, plastic	MS, RI
4	1067	802	hexanal	H	1139.2 ± 52.1 [b]	52.5 ± 9.8 [a]	18.3 ± 4.0 [a]	45.1 ± 1.7 [a]	green, fatty	MS, RI
5	1069		2-methyl-1-propanol	A	181.1 ± 55.7 [a]	204.1 ± 86.8 [a]	197.5 ± 69 [a]	129.9 ± 39.4 [a]	fruity, winey, fermented	MS, RI
6	1153		(*E*)-3-penten-2-ol	NB	1293.2 ± 58.4 [a]	1382.6 ± 67.7 [a]	2513.2 ± 284 [b]	2306 ± 179.4 [b]	fruity, sweet, fermented	MS, RI
7	1173	886	2-heptanone	H	313.8 ± 12 [b]	302.4 ± 80 [b]	192.1 ± 26.4 [a]	193.6 ± 23.2 [a]	cheesy, fruity	MS, RI
8	1176	1029	D-limonene	A	ND	217.2 ± 59.6 [b]	83.8 ± 28.7 [a]	ND	citrus, orange	MS, RI
9	1189		1-pentanol	A	262.3 ± 62.1 [b]	ND	166.3 ± 26.8 [a]	140.3 ± 31.7 [a]	pungent, winey, fermented	MS, RI
10	1205		3-methyl-1-butanol	H	637.8 ± 280.4 [c]	91.9 ± 20.4 [b]	25.0 ± 1.6 [a]	120.6 ± 7.8 [b]	fruity, malt, fermented	MS, RI
11	1227		3-methyl-3-buten-1-ol	A	723.9 ± 35.8 [a]	909 ± 330.2 [a]	ND	782.8 ± 140.8 [a]	fruity, sweet	MS, RI
12	1251	989	3-octanone	H	471.9 ± 15.2 [b]	ND	70.9 ± 19.2 [a]	ND	herbal, fruity, mushroom	MS, RI
13	1260	828	methylpyrazine	H	ND	34.9 ± 15.5 [a]	34.6 ± 6.3 [a]	ND	nutty, cocoa, roasted	MS, RI
14	1262	907	2,5-dimethylpyrazine	H	11,096.7 ± 473.7 [d]	8142.9 ± 1001.9 [c]	6604 ± 996.8 [b]	3568.2 ± 563.3 [a]	nutty, cocoa, peanut	MS, RI
15	1298	956	(*E*)-2-heptenal	H	4120 ± 506.1 [b]	210.7 ± 74.5 [a]	114.2 ± 20.2 [a]	ND	green, fatty, pungent	MS, RI
16	1328	924	2,6-dimethylpyrazine	H	117.7 ± 43.8 [b]	85.5 ± 31.1 [a,b]	44.1 ± 6.6 [a]	69.7 ± 13.4 [a,b]	nutty, cocoa, roasted	MS, RI
17	1360	1002	trimethylpyrazine	H	2877.2 ± 222.1 [c]	2357 ± 409 [b,c]	1530.8 ± 285.4 [a]	1932.4 ± 288.3 [a,b]	nutty, cocoa, roasted	MS, RI
18	1372	1104	nonanal	NB	1898.9 ± 206.4 [a]	ND	ND	ND	fatty, waxy, green	MS, RI
19	1383	999	2-ethyl-5-methylpyrazine	H	244.4 ± 21.7 [b]	82.3 ± 16.6 [a]	56.5 ± 10.5 [a]	53.3 ± 5.7 [a]	nutty, cocoa, roasted	MS, RI
20	1387	1094	2-nonanone	H	233.9 ± 26.6 [b]	83.2 ± 33.3 [a]	59.7 ± 13.6 [a]	84.7 ± 11.2 [a]	cheesy, buttery, green	MS, RI
21	1406		ethyl-hydrazine	H	2498.4 ± 315.8 [b]	ND	539.4 ± 121.2 [a]	866.7 ± 126.5 [a]	ammonia-like odor	MS, RI
22	1420		acetic acid	A	1966.1 ± 188 [a]	2020.2 ± 371.8 [a]	2038.3 ± 300.2 [a]	1676.4 ± 213.7 [a]	pungent, sour, vinegar	MS, RI
23	1427	979	1-octen-3-ol	H	5634.9 ± 553.7 [a]	ND	ND	ND	earthy, green, mushroom	MS, RI
24	1445	1080	3-ethyl-2,5-dimethylpyrazine	H	350.5 ± 17.4 [b]	147.6 ± 48.1 [a]	139.2 ± 20.1 [a]	101.2 ± 14.2 [a]	nutty, cocoa, roasted	MS, RI
25	1447	1087	tetramethylpyrazine	H	849 ± 64.3 [b]	751.5 ± 159.2 [b]	234.9 ± 49.7 [a]	769.4 ± 100.7 [b]	nutty, cocoa, coffee	MS, RI
26	1450	903	methional	H	100.3 ± 4.7 [b]	91.8 ± 18.2 [b]	32.9 ± 4.1 [a]	43.5 ± 9.0 [a]	baked potato, tomato	MS, RI
27	1454		2-ethenyl-6-methylpyrazine	H	861.6 ± 85.1 [c]	976.6 ± 101.8 [c]	445.8 ± 81.7 [b]	252.1 ± 49.3 [a]	nutty, coffee, hazelnut	MS, RI
28	1465	995	(*E,E*)-2,4-heptadienal	NB	13,479 ± 1510.6 [a]	18,173.8 ± 1539.9 [b]	18,713.1 ± 3718.4 [b]	16,291.1 ± 326.1 [a,b]	fatty, oily, green	MS, RI
29	1481	963	benzaldehyde	H	368.6 ± 56.4 [a]	595.4 ± 34.1 [b]	586.5 ± 109.3 [b]	379.8 ± 70.3 [a]	nutty, almond, cherry	MS, RI
30	1508		propanoic acid	A	845.3 ± 242.1 [b]	838.6 ± 103.6 [b]	470.3 ± 161.8 [a]	612.7 ± 86 [a,b]	pungent, cheesy, rancid	MS, RI
31	1515	1215	2,3,5-trimethyl-6-ethylpyrazine	H	178.9 ± 8.3 [c]	111.4 ± 50.2 [a,b]	92.5 ± 14.1 [a]	155.9 ± 12.6 [b,c]	cocoa, coffee, roasted	MS, RI
32	1536	791	2-methylpropanoic acid	A	25,184.5 ± 4367.7 [b]	21,222.5 ± 898.7 [a,b]	26,207 ± 2998.2 [b]	19,292.9 ± 1796.6 [a]	cheesy, buttery, rancid	MS, RI
33	1588	1059	(*E*)-2-octenal	H	418 ± 58.8 [a]	ND	ND	ND	green, fatty, waxy	MS, RI
34	1592		(*Z*)-2-octen-1-ol	NB	5208.4 ± 187.9 [b]	2739.5 ± 320.3 [a]	3299.1 ± 977.4 [a]	3433.8 ± 455.8 [a]	fatty, oily, green	MS, RI
35	1597	803	butanoic acid	A	1244.8 ± 182.2 [a]	ND	ND	ND	cheesy, buttery, sweaty	MS, RI
36	1616		4-methyl-benzaldehyde	NB	6019.1 ± 35.8 [a]	5905.7 ± 533.4 [a]	5939.9 ± 951.4 [a]	5532.3 ± 325.3 [a]	fruity, sweet, cherry	MS, RI

(*continued on next page*)

Table 3 (*continued*)

No.	RI [a]		compound	fractionation [b]	relative concentration (μg/kg)				odor description [c]	identification [d]
	wax	DB-5			SUN	HOT	MIC	VAC		
37	1620	1264	(*E*)-2-decenal	NB	54,426.6 ± 7870.2 [a]	50,631.4 ± 8095.5 [a]	58,689.4 ± 9621.3 [a]	47,469.3 ± 1294.6 [a]	fatty, waxy, green	MS, RI
38	1625		phenylacetaldehyde	H	2764.4 ± 291.4 [b]	865.3 ± 116.4 [a]	524.8 ± 115.4 [a]	654.8 ± 94.7 [a]	floral, sweet, honey	MS, RI
39	1639	909	2-methylbutanoic acid	A	45,813.5 ± 6220.1 [a,b]	41,053.7 ± 3463.8 [a]	51,841.9 ± 6834.2 [b]	37,089.5 ± 2859.4 [a]	pungent, cheesy, sweaty	MS, RI
40	1643	1098	acetophenone	H	ND	ND	22.2 ± 3.9 [a]	ND	floral, sweet	MS, RI
41	1681	1133	N-isobutyl-isobutyramide	NB	3576.3 ± 130.2 [a]	3564.4 ± 354.2 [a]	11,408.2 ± 960.4 [b]	2717.2 ± 351.5 [a]	–	MS, RI
42	1738	1295	(*E,Z*)-2,4-decadienal	NB	69,306.3 ± 9222.8 [a]	72,073.5 ± 5876 [a]	72,722.3 ± 13,674.8 [a]	64,515.5 ± 1832.2 [a]	fatty, waxy, green	MS, RI
43	1754	1180	methyl phenylacetate	H	227.4 ± 17.3 [b]	129.8 ± 57.8 [a]	105.1 ± 11.0 [a]	96.5 ± 16.3 [a]	floral, sweet, honey	MS, RI
44	1763	935	3-methyl-2-butenoic acid	A	2383.9 ± 312.4 [a]	2096.6 ± 145 [a]	3229.7 ± 377.8 [b]	1966.7 ± 143.4 [a]	green, phenolic, dairy	MS, RI
45	1771	980	4-methyl-pentanoic acid	A	4048 ± 583.2 [b]	3619.3 ± 219.6 [a,b]	4107.5 ± 390.9 [b]	3289.6 ± 186.6 [a]	pungent, cheesy	MS, RI
46	1783	1319	(*E,E*)-2,4-decadienal	NB	87,547 ± 11,920.1 [a]	74,019.6 ± 7195.1 [a]	90,928.9 ± 14,561.6 [a]	77,398.2 ± 1403.2 [a]	fatty, oily, fried	MS, RI
47	1801	1230	3-methyl-N-isobutyl-butyramide	NB	ND	ND	16,724.6 ± 1130 [a]	ND	–	MS, RI
48	1807	1239	N-(3-methylbutyl)-isobutyramide	NB	ND	ND	13,081.2 ± 641.9 [a]	ND	–	MS, RI
49	1812		(Z)-2-methyl-2-butenoic acid	A	7010.2 ± 905.2 [c]	3176.2 ± 126.3 [a]	4299.7 ± 381.7 [b]	3209.4 ± 340.9 [a]	pungent, cheesy, sour	MS, RI
50	1826	1089	2-methoxy-phenol	H	3897.3 ± 392.1 [b]	3592.8 ± 387.1 [b]	1477.7 ± 262.7 [a]	901.1 ± 170.3 [a]	smoky, woody, phenolic	MS, RI
51	1873	1034	benzyl alcohol	H	144.5 ± 6.1 [c]	78.7 ± 10.6 [b]	30.8 ± 8.2 [a]	45.6 ± 8.3 [a]	floral, sweet	MS, RI
52	1892	1075	4-methylhexanoic acid	A	1199.8 ± 231 [a]	1119.8 ± 97.6 [a]	1176.8 ± 124 [a]	1013.1 ± 66.6 [a]	cheesy, sour	MS, RI
53	1918		heptanoic acid	A	974.8 ± 117.4 [a]	ND	ND	ND	cheesy, sweaty, fermented	MS, RI
54	1925	1337	3-methyl-N-3-methylbutyl-butyramide	NB	ND	ND	10,751.6 ± 935.3 a	ND	–	MS, RI
55	1932	1118	maltol	A	25,333 ± 2838.4 [b]	23,131.2 ± 1265.2 [b]	17,047.4 ± 2128 [a]	13,889.2 ± 962.1 [a]	sweet, caramellic, cotton candy	MS, RI
56	1961	1121	phenylethyl alcohol	NB	7442.3 ± 87.6 [c]	5941.2 ± 501.5 [b]	4868.6 ± 297.8 [a]	6176 ± 218.8 [b]	floral, rose, honey	MS, RI
57	2068	982	phenol	H	1117.4 ± 102.1 [c]	630.4 ± 95.4 [b]	382.9 ± 46.4 [a]	529.8 ± 98 [a,b]	phenolic, plastic, rubbery	MS, RI
58	2124	1181	octanoic acid	A	4729.9 ± 850 [b]	5017.4 ± 835.5 [b]	1443 ± 85.7 [a]	4028.3 ± 66.1 [b]	cheesy, sweaty, rancid	MS, RI
59	2131	1292	nonanoic acid	A	2263.6 ± 504.1 [a]	ND	ND	ND	cheesy, waxy, green	MS, RI
60	2139	1124	2-heptenoic acid	A	1379.8 ± 257.4 [a]	ND	ND	ND	rancid	MS, RI
61	2159		caprolactam	NB	4275.8 ± 119.4 [a]	12,884.9 ± 304.3 [c]	5532.8 ± 1355.5 [a]	8213.7 ± 552 [b]	unpleasant odor	MS, RI
62	2199	1927	methyl hexadecanoate	NB	31,501 ± 1261.2 [a]	51,922.2 ± 5924.4 [b]	24,182.3 ± 13,972.2 [a]	39,319.2 ± 12,006.8 [a,b]	fatty, waxy, oily	MS, RI
63	2441		dodecanoic acid	A	ND	1460.7 ± 517.6 [a]	ND	ND	fatty, waxy	MS, RI
64	2476	1295	4-methyl-benzoic acid	A	ND	3822.4 ± 551.4 [a]	ND	ND	–	MS, RI
65	2498	1275	phenylacetic acid	A	63,753.8 ± 7770.4 [b]	41,581.3 ± 2897.8 [a]	66,188.1 ± 7840.4 [b]	45,909.6 ± 6266.1 [a]	floral, sweet, honey	MS, RI

Results expressed as the means ± standard deviation (n = 3), not detected (ND).
Means within same row with different superscripts were significantly different (p-value ≤0.05).
[a] Retention indices (RI) were determined from on DB-wax and DB-5 columns.
[b] Fractionation: acidic fraction (A), neutral/basic fraction (NB), headspace technique (H).
[c] Odor descriptions were obtained from online database of Good Scents Company (https://www.thegoodscentscompany.com/), Pub Chem (https://pubchem.ncbi.nlm.nih.gov/), and Flavornet (https://www.flavornet.org/).
[d] Identification method: mass spectra (MS), retention indices (RI).

of free fatty acids and their subsequent oxidation. Free fatty acids with double bonds trigger the formation of hydroperoxides, which further degrade into oxidized volatile compounds (Qu et al., 2016; Shahidi & Hossain, 2022). This result suggested that increased free fatty acid content observed in the SUN-treated sample (Table 2) could significantly accelerate the formation of oxidized volatile compounds through photooxidation compared to autoxidation. SUN treatment, in particular, induced the formation of various volatile compounds, including nonanal, 1-octen-3-ol, (*E*)-2-octenal, butanoic acid, heptanoic acid, nonanoic acid, and 2-heptenoic acid. The high content of free fatty acids (Table 2)

observed in the SUN-treated sample compared to other treatments contributed to the increase in the formation of oxidized volatile compounds (Table 3).

Hexanal content was significantly higher in the SUN-treated sample than in HOT- (20 times), VAC- (20 times), and MIC-treated (60 times) samples. Additionally, hexanal and 1-octen-3-ol have been commonly used as indicators of linoleic acid (C18:2) autoxidation (Mathatheeranan, Wongprasert, Fang, et al., 2024; Shahidi & Hossain, 2022). Moreover, (*E*)-2-heptenal content was substantially higher in the SUN-treated sample than in the HOT- (20 times) and MIC-treated (35 times) samples (not detected in VAC-treated sample). This finding could imply that the SUN-treated sample was directly exposed to singlet oxygen, which is the major ROS causing photooxidative oxidation, and oxygen during the extended drying process. These reactions triggered the formation of specific oxidized volatile compounds, such as 1-octen-3-ol, (*E*)-2-octenal, and (*E*)-2-heptenal (Ludwig et al., 2021; Shahidi & Hossain, 2022). Notably, (*E*)-2-heptenal has been identified as a marker specifically indicating linoleic acid oxidation through this pathway (Lee & Min, 2010).

In addition, autoxidation can spontaneously occur in dried thua nao, providing another pathway for the generation of oxidized volatile compounds. Our findings showed that the content of oxidized volatile compounds, such as (*Z*)-2-octen-1-ol, (*E*)-2-decenal, (*E,E*)-2,4-heptadienal, (*E,Z*)-2,4-decadienal, and (*E,E*)-2,4-decadienal, were similar across all treated samples. This result aligns with the finding of Shahidi and Hossain (2022), who reported that autoxidation is associated with the presence of conjugated diene compounds, such as (*E,E*)-2,4-heptadienal, (*E,Z*)-2,4-decadienal, and (*E,E*)-2,4-decadienal. Lipid oxidation could spontaneously occur during drying treatments, as supported by the PV and TBARS results (Fig. 1). Additionally, fatty acids (e.g., octanoic acid) and ketones (e.g., 2-heptanone and 2-nonanone) were detected in all treated samples, whereas heptanoic acid, 2-heptenoic acid, nonanoic acid, and 3-octanone were only detected in the SUN-treated sample. In addition, it had been reported that aliphatic ketones (e.g., 2-heptanone, 3-octanone and, 2-nonanone) can be formed through autoxidation and enzymatic reactions of microbial lipases and lipoxygenases (Mathatheeranan, Wongprasert, Fang, et al., 2024). These results suggested that the formation of oxidized volatile compounds in the SUN-treated samples was influenced by both photooxidation and autoxidation, whereas controlled drying methods primarily impacted autooxidation.

The possible mechanisms for the formation of these oxidation products have been well documented. For instance, (*E*)-2-decenal, 3-octanone, and 2-nonanone are obtained from oleic acid (C18:1); hexanal, (*E*)-2-heptenal, (*E*)-2-octenal, (*E*)-2-decenal, (*E,E*)-2,4-decadienal, and 2-heptanone are derived from linoleic acid (C18:2); and 2-butenal, (*E*)-2-heptenal, and (*E,E*)-2,4-heptadienal are derived from linolenic acid (C18:3) (Shahidi & Hossain, 2022; Liu et al., 2023; Mathatheeranan et al., 2023; 2024a; 2024b).

Lipid oxidation can induce aromatic compound formation via amino acid degradation. Consequently, aromatic compounds, such as phenylacetaldehyde, benzaldehyde, and phenylacetic acid were detected in all treated samples (Table 3). Hidalgo and Zamora (2019) reported that phenylalanine degradation is induced by 13-hydroperoxide of linoleic acid, which triggered the formation of phenylpyruvic acid, β-phenylethylamine, and phenylacetaldehyde. Phenylacetaldehyde, as an intermediate, can further react with lipid hydroperoxides to produce benzaldehyde and phenylacetic acid.

3.4.2. Generation of volatile compounds from the Maillard reaction

Fresh thua nao contains abundant smaller sugars, amino acids, and dicarbonyl compounds, which serve as precursors for the Maillard reaction (Liu et al., 2022; Shahidi & Hossain, 2022;Dajanta, Apichartsrangkoon, & Chukeatirote, 2011; Mathatheeranan, Wongprasert, Wang, et al., 2024). Drying treatment can induce the Maillard reaction, further enhancing the development of volatile compounds, such as pyrazines, maltol, methional, and 2-methoxy-phenol.

As seen in Table 3, the most prominent pyrazine in dried thua nao samples was 2,5-dimethylpyrazine, followed by trimethylpyrazine, tetramethylpyrazine, and 2-ethenyl-6-methylpyrazine. The contents of 2,5-dimethylpyrazine, 2-ethyl-5-methylpyrazine, 2-ethenyl-6-methylpyrazine in SUN- and HOT-treated samples were two to three times higher than those in MIC- and VAC-treated samples. This result aligned with the results of the Maillard browning indicators (Fig. 3), suggesting lower temperatures during MIC and VAC treatments could decrease pyrazine formation. Moreover, 3-ethyl-2,5-dimethylpyrazine in the SUN-treated sample was found to be two to three times higher than that in samples treated using others drying methods, which could possibly be attributed to the maximal utilization of reducing sugars in the Maillard reaction. In addition, pyrazine formation in fresh thua nao products can be synthesized through *B. subtilis* activity, specifically via an enzymatic reaction involving L-threonine and glycolytic intermediates, such as aminoacetone and 2-amino-3-butanone (Mathatheeranan, Wongprasert, Wang, et al., 2024).

Beside pyrazines, maltol was found to have the highest concentration in the SUN-treated sample (25,333 μg/kg), followed by HOT- (23,131 μg/kg), MIC- (17,047 μg/kg), and VAC-treated (13,889 μg/kg) samples. In general, maltol is spontaneously formed during the fermentation of soybeans, such as thua nao, soy sauce, and doenjang (Diez-Simon et al., 2020;Jo et al., 2011; Mathatheeranan, Wongprasert, Wang, et al., 2024). One study found that fresh thua nao samples had a maltol concentration of 71,900 μg/kg (wet basis) (Mathatheeranan, Wongprasert, Wang, et al., 2024). Maltol can also be generated through the dehydration/fragmentation of sugar products during the Maillard reaction (Diez-Simon et al., 2020; Liu et al., 2022; Mathatheeranan, Wongprasert, Wang, et al., 2024).

We found that methional and 2-methoxy-phenol contents were two times higher in SUN- and HOT-treated samples than in MIC- and VAC-treated samples. Methional could be synthesized via Streacker degradation from methionine, with higher temperatures generating more content (Diez-Simon et al., 2020). One study reported a methional content of 16.4–20.1 μg/kg (wet basis) in fresh thua nao (Mathatheeranan, Wongprasert, Wang, et al., 2024). Meanwhile, 2-methoxy-phenol is found in fermented soybean products, including thua nao, natto, soy sauce, and doenjang (Dajanta, Apichartsrangkoon, & Chukeatirote, 2011; Jo et al., 2011; Liu et al., 2018; Mathatheeranan, Wongprasert, Wang, et al., 2024). It has been reported that ferulic acid, a compound presented in soybean seed coat, was converted by *B. subtilis* activity into various aromatic compounds, including 2-methoxy-phenol, 4-vinyl-2-methoxy-phenol, 4-ethyl-2-methoxyphenol, and vanillin, which were identified in fresh thua nao products (Mathatheeranan, Wongprasert, Wang, et al., 2024).

Additionally, the high temperatures and extended drying time of the SUN-treated sample have been found to accelerate the decomposition of plant cell materials and glycoside structure, which yield sugars and phenolic acids (ElGamal et al., 2023). Oxidation–reduction reactions can interconvert benzyl alcohol to benzaldehyde and phenylacetaldehyde to phenylethyl alcohol, as observed in Table 3. In addition, benzaldehyde formation could be synthesized by *B. subtilis* activity through the enzymatic conversion of phenylalanine to phenylpyruvic acid, followed by decarboxylation to benzaldehyde during thua nao fermentation process (Mathatheeranan et al., 2023). The concentrations of phenylacetaldehyde, methyl phenylacetate, and benzyl alcohol were two times higher in SUN-treated sample than in those treated using other methods.

3.4.3. Volatile compound formation unrelated to lipid oxidation and the Maillard reaction

The Maillard reaction and lipid oxidation during drying treatments did not produce volatile acids and phenylethyl alcohol. Volatile acids, including 2-methylpropanoic acid, 3-methylbutanoic acid, 2-methylbutanoic acid, could be derived from the catabolism of branched-chain

amino acids (L-valine, L-leucine, and L-isoleucine) by *B. subtilis* activity. Additionally, phenylethyl alcohol can be synthesized from phenylalanine metabolism (Kai, 2020; Mathatheeranan, Wongprasert, Wang, et al., 2024). In contrast, short-chain organic acids, such as acetic acid (via glycolysis pathway), propanoic acid (via propanoate metabolism), and butanoic acid (via butanoate metabolism) were reported as byproducts of glucose utilization by *B. subtilis* (Jo et al., 2011; Mathatheeranan, Wongprasert, Wang, et al., 2024).

Various drying treatments promote different levels of volatile compound loss. Under the neutral pH (6.92–7.20), volatile acid compounds in dried thua nao products exhibit lower volatilities, resulting in most of them being retained in the food samples. However, these same volatile acids readily volatilize under acidic conditions, explaining their detection in the acidic fraction.

Interestingly, the branch-chained volatile acid with the highest concentration detected in all treated samples was 2-methylbutanoic acid (37,089–51,841 μg/kg), followed by 2-methylpropanoic acid (19,292–26,207 μg/kg), 4-methylpentanoic acid (3289–4107 μg/kg), 3-methyl-2-butenoic acid (1966–3229 μg/kg), and 4-methylhexanoic acid (1013–1199 μg/kg). These volatile acids were most prevalent in the MIC-treated sample due to short drying time and low heat generation. Moreover, butanoic acid was only detected in the SUN-treated sample, suggesting its loss during HOT, MIC, and VAC, whereas acetic acid and propanoic acid were detected in all treated samples. Furthermore, lower concentrations of volatile acids were observed in the VAC-treated sample, possibly due to volatile compounds loss under high vacuum conditions and prolonged operation time.

Although phenylethyl alcohol was found in all treated samples, its content was lowest in the MIC-treated sample. This finding can be explained by the fact that microwave energy is readily absorbed by polar functional groups in molecules and porosity at the surface structure, causing their rapid evaporation from the food matrix (Calín-Sánchez et al., 2020; Mathatheeranan, Wongprasert, Fang, et al., 2024). Furthermore, MIC treatment also promoted the formation of specific amide volatile compounds, which are formed through the reaction between carboxylic acids and amines, with microwave energy acting as a catalyst (Zarecki et al., 2020). The content of N-isobutyl-isobutyramide was three times higher in the MIC-treated sample than in the other samples, whereas 3-methyl-N-isobutyl-butyramide, N-(3-methylbutyl)-isobutyramide, and 3-methyl-N-3-methylbutyl-butyramide were not detected in any other sample.

3.5. Aroma-active compounds through OAV analysis

Evidence has shown that to identify aroma-active compounds in food, volatile compounds with an OAV ≥ 1 can be potentially considered as aroma-active compounds in foods (Liu et al., 2018; Mathatheeranan et al., 2023). Hence, the OAVs of all volatile compounds were calculated, with our results and summarized in Table 1S. The OAV results were normalized and displayed as heatmap analysis (Fig. 4). The heatmap showed differing OAV levels, ranging from high (red) to low (blue). Active-aroma compounds in dried thua nao products were grouped based on similarities in their aroma attributes, following classifications outlined by Mathatheeranan, Wongprasert, Wang, et al. (2024). As seen in Fig. 4, 30 aroma compounds with OAVs ≥1 were grouped into seven attributes, including green-fatty, nutty-roasted, sweet, sulfurous, woody-smoky, fermented-sour, and floral.

The heatmap revealed that the SUN-treated sample dominated the aroma profile when compared to the other treatment methods, as indicated by its red color on the heatmap. This dominance was associated with lipid oxidation (green-fatty attribute) and the Maillard reaction (nutty-roasted, sweet, sulfurous, and wood-smoky attributes). Green-fatty attribute could be linked to aroma volatile compounds derived from lipid oxidation, particularly in the SUN-treated sample. Notably, hexanal and (*E*)-2-heptenal showed an OAV value 20-fold higher than that in the other treated samples. Moreover, 1-octen-3-ol, nonanal,

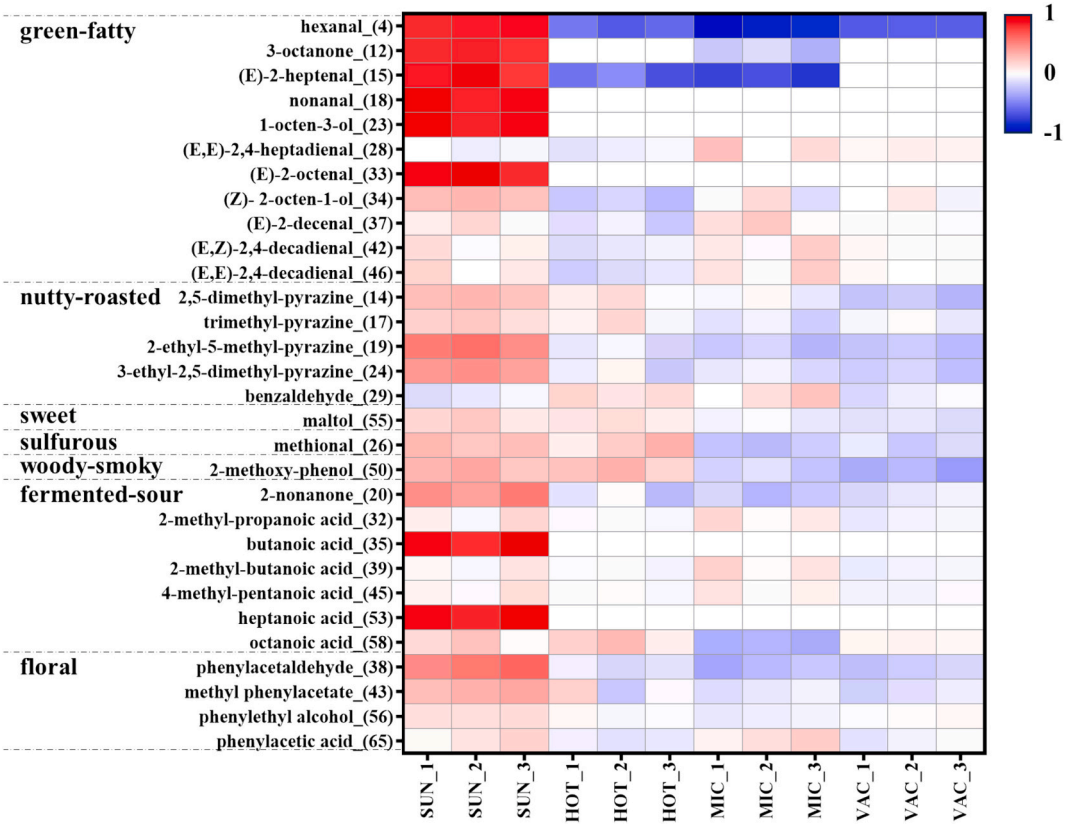


Fig. 4. Heatmap analysis of volatile profiles of dried thua nao treatments. Three replicated measurements were expressed on heatmap.

heptanoic acid, (*E*)-2-octenal, and butanoic acid were only found in the SUN-treated sample, whereas (*E,E*)-2,4-decadienal, (E,Z)-2,4-decadienal, and (*E,E*)-2,4-heptadienal were observed similarly across all treated samples. This result indicates that the intensity of the green-fatty attribute was highest in the SUN-treated sample given that it contained oxidized volatile compounds with high OAVs >100 (Table 1S).

Moreover, aroma-active compounds from the Maillard reaction, such as pyrazine, maltol, methional, and 2-methoxy-phenol, have been associated with nutty-roasted, sweet, sulfurous, and woody-smoky attributes, respectively. Compounds with a nutty-roasted attribute, including 2,5-dimethylpyrazine, trimethylpyrazine, 2-ethyl-5-methylpyrazine, 3-ethyl-2,5-dimethylpyrazine, and benzaldehyde, had been detected at different OAVs levels across all treated samples. Notably, the OAVs of these aroma attributes (nutty-roasted, sweet, sulfurous, and woody-smoky) were two times higher in the SUN- and HOT-treated samples than in the other samples (Table 1S). This result indicated

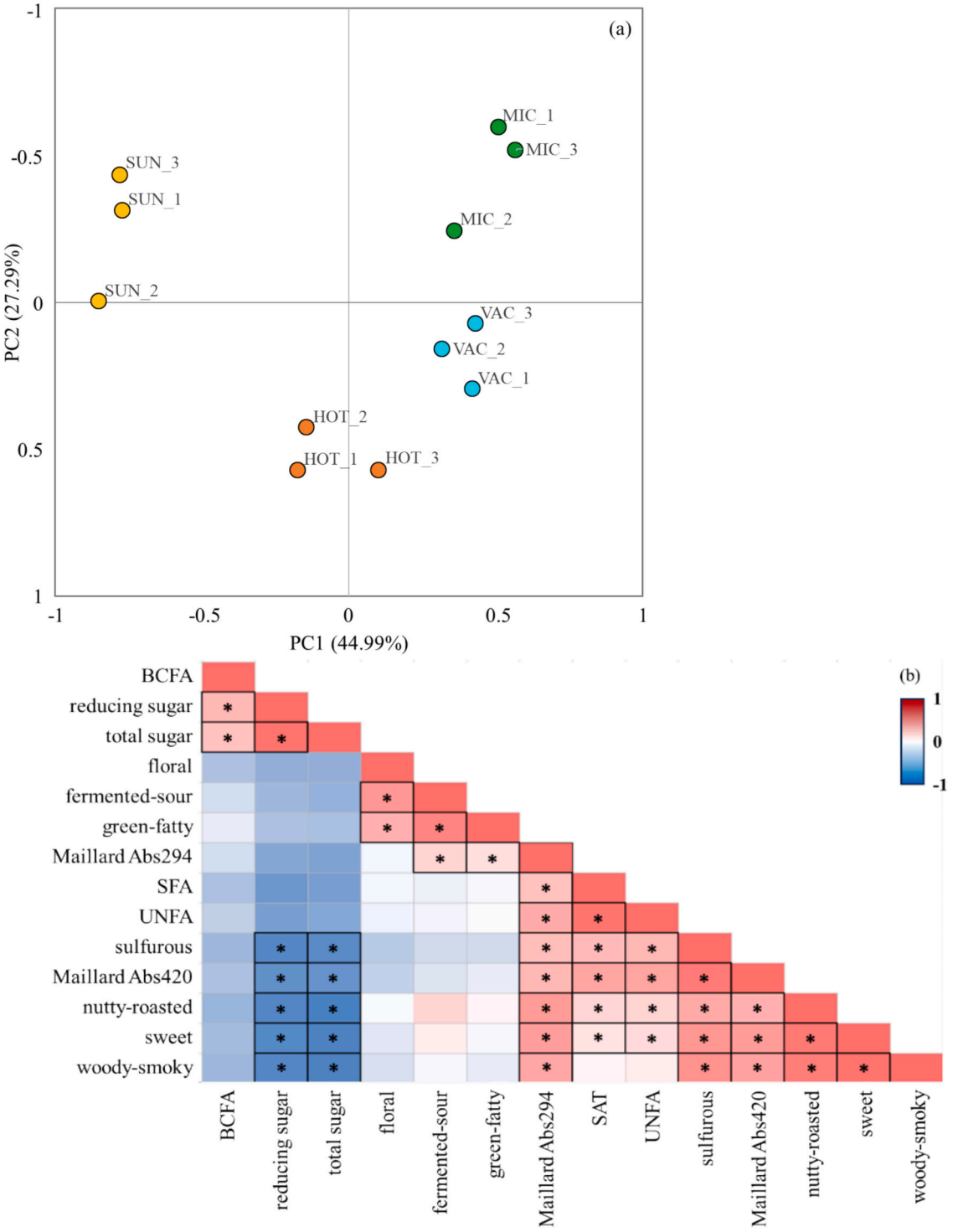


Fig. 5. PCA plot of volatile compounds with OAV ≥1 of dried thua nao treatments (a), Pearson's correlation on sugar contents, fatty acid composition, the Maillard indicators and aroma attributes of dried thua nao treatments, branched-chain fatty acid (BCFA), saturated fatty acid (SFA), unsaturated fatty acid (UNFA) (b).
* Significantly different at *p*-value ≤0.05.

that SUN and HOT treatment promoted greater aroma intensity than did MIC and VAC treatment.

For the fermented-sour attribute, all treated samples exhibited similar OAVs for branch-chained volatile acids (e.g., 2-methylbutanoic acid, 4-methylpentanoic acid, and 2-methylpropanoic acid). However, the SUN-treated sample showed higher OAVs for compounds associated with lipid oxidation (e.g., nonanone, heptanoic acid, and octanoic acid) than did the other samples, indicating a more complex fermented-sour attribute. Regarding the floral attribute, compounds including phenylacetaldehyde, phenylethyl alcohol, methyl phenylacetate were detected in all treated samples.

These results indicate that volatile compounds generated through lipid oxidation and the Maillard reaction significantly affected the aroma profiles of dried thua nao products. We concluded that all dried treated samples retained active-aroma compounds characteristic of fresh thua nao, although their relative intensities varied significantly across treatment methods.

3.6. PCA and correlations among aroma-active compounds

PCA was used to investigate the relationship between four dried thua nao samples and the formation of respective volatile compounds. Only volatile compounds with OAVs $\geq$1 were considered given that they would have been potentially perceived by human during consumption. The total variance explained by PC1 and PC2 was 72.28 % as shown in the PCA plot (Fig. 5a), indicating clear separation among samples.

PCA analysis revealed differences in aroma attributes between SUN and machine drying treatments (HOT, MIC, and VAC) considering that the SUN-treated sample presented with various oxidized volatile compounds. The HOT-treated sample positioned between the SUN- (left side) and MIC- and VAC-treated samples (right side), suggesting a profile of volatile compounds associated with the Maillard reaction similar to that in the SUN-treated sample but with different oxidized volatile compounds. Conversely, both MIC- and VAC-treated samples clustered on the opposite side of the PCA plot from the SUN-treated sample due to their lower levels of volatile compounds derived from both the Maillard reaction and lipid oxidation. This result suggests that drying conditions, such as temperature, process time, UV radiation, and microwave energy, significantly affected aroma-active compound formation in dried thua nao products.

Pearson's correlation demonstrated an association between sugar content, free fatty acid, Maillard indicators, and aroma attributes (Fig. 5b). Notably, our results showed that reducing sugar and total sugar content were significantly negatively correlated with the Maillard browning indicator (Abs 420 nm) and aroma attributes derived from the Maillard reaction (e.g., sulfurous, nutty-roasted, sweet, and woody-smoky). In contrast, the Maillard reaction products (e.g., Maillard indicators of Abs 294 nm and Abs 420 nm, and aroma attributes) exhibited a positive correlation with lipid degradation (free SAT and UNFA contents). These findings align with the observations presented by Han et al. (2023), who reported that reduced sugar levels could enhance the sensory perception of nutty-toasted and burnt-like aromas, which are associated with pyrazine and furan formation from the Maillard reaction. Furthermore, these results support those reported by Liu et al. (2022), who showed that free fatty acid degradation into reactive carbonyl compounds contributes to Maillard intermediate formation, which influences aroma compound development.

4. Conclusion

The type of drying treatment significantly influenced the physicochemical properties and aroma compound formation in dried thua nao products. Among the drying treatments employed herein, MIC treatment was the most effective in reducing a_w while retaining crude fat content and TVB-N levels given its short drying process. Conversely, SUN treatment was less successful in reducing a_w compared to machine drying (HOT, MIC, and VAC). Moreover, the type of drying treatment influenced not only lipid stability but also the Maillard reaction. Notably, we found that SUN treatment promoted lipid oxidation, which increased the levels of free fatty acids and oxidized volatile compounds. Secondary oxidation products, such as hexanal, (*E*)-2-heptenal, nonanal, 1-octen-3-ol, and (*E*)-2-octenal, had a noticeable impact on the higher green-fatty attribute in the SUN-treated sample than in the other samples. Conversely, the Maillard reaction in the SUN- and HOT-treated samples enhanced the nutty-roasted, sweet, sulfurous, and woody-smoky attributes more than it did in the MIC- and VAC-treated samples. Aroma-active compounds associated with fermented-sour and floral attributes were less influenced by the type of drying treatment. Furthermore, PCA techniques revealed distinct aroma profiles in the SUN-treated sample unlike samples treated using machine drying. This study provides insights into the formation of aroma volatile compounds in dried thua nao products, with our findings suggesting that refining the drying process could enhance the formation of pleasant aroma compounds, maintaining nutritional quality and extending food stability.

CRediT authorship contribution statement

Pakavit Mathatheeranan: Writing – original draft, Methodology, Investigation, Formal analysis. **Ting-Jang Lu:** Writing – review & editing, Supervision, Resources, Conceptualization. **Thanakorn Wongprasert:** Investigation, Formal analysis. **Yi-Ci Jhu:** Investigation, Formal analysis. **Hsin-Jo Chang:** Investigation, Formal analysis. **Wei-Yuan Wu:** Investigation, Formal analysis. **Mingchih Fang:** Writing – review & editing, Supervision, Conceptualization. **Kitipong Assatarakul:** Writing – review & editing, Resources. **Inthawoot Suppavorasatit:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The author is an Editorial Board Member/Editor-in-Chief/Associate Editor/Guest Editor for *[FOCHX]* and was not involved in the editorial review or the decision to publish this article.

Data availability

Data will be made available on request.

Acknowledgements

This research project was supported by the Second Century Fund (C2F), Chulalongkorn University, Thailand, and funded by Thailand Science Research and Innovation Fund Chulalongkorn University.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.102040.

References

AOAC. (2023). Official methods of analysis of AOAC international. In *Association of Official Analytical Collaboration International* (22nd ed.) Gaithersburg, MD, USA.

Barriuso, B., Astiasarán, I., & Ansorena, D. (2013). A review of analytical methods measuring lipid oxidation status in foods: A challenging task. *European Food Research and Technology, 236*, 1–15. https://doi.org/10.1007/s00217-012-1866-9

Calín-Sánchez, Á., Lipan, L., Cano-Lamadrid, M., Kharaghani, A., Masztalerz, K., Carbonell-Barrachina, Á. A., & Figiel, A. (2020). Comparison of traditional and novel drying techniques and its effect on quality of fruits, vegetables and aromatic herbs. *Foods, 9*(9), 1261. https://doi.org/10.3390/foods9091261

Chang, C. T., Hsu, C. K., Chou, S. T., Chen, Y. C., Huang, F. S., & Chung, Y. C. (2009). Effect of fermentation time on the antioxidant activities of tempeh prepared from fermented soybean using *Rhizopus oligosporus*. *International Journal of Food Science and Technology, 44*(4), 799–806. https://doi.org/10.1111/j.1365-2621.2009.01907.x

Cho, K. M., Lim, H. J., Kim, M. S., Hwang, C. E., Nam, S. H., Joo, O. S., … Shin, E. C. (2017). Time course effects of fermentation on fatty acid and volatile compound profiles of cheonggukjang using new soybean cultivars. *Journal of Food and Drug Analysis, 25*(3), 637–653. https://doi.org/10.1016/j.jfda.2016.07.006

Chukeatirote, E. (2015). Thua nao: Thai fermented soybean. *Journal of Ethnic Foods, 2*(3), 115–118. https://doi.org/10.1016/j.jef.2015.08.004

Dajanta, K., Apichartsrangkoon, A., & Chukeatirote, E. (2011). Volatile profiles of thua nao, a Thai fermented soy product. *Food Chemistry, 125*(2), 464–470. https://doi.org/10.1016/j.foodchem.2010.09.030

Dajanta, K., Apichartsrangkoon, A., Chukeatirote, E., & Frazier, R. A. (2011). Free-amino acid profiles of thua nao, a Thai fermented soybean. *Food Chemistry, 125*(2), 342–347. https://doi.org/10.1016/j.foodchem.2010.09.002

Dajanta, K., Chukeatirote, E., & Apichartsrangkoon, A. (2012). Improvement of thua nao production using protein-rich soybean and *Bacillus subtilis* TN51 starter culture. *Annals of Microbiology, 62*, 785–795. https://doi.org/10.1007/s13213-011-0319-1

Diez-Simon, C., Eichelsheim, C., Mumm, R., & Hall, R. D. (2020). Chemical and sensory characteristics of soy sauce: A review. *Journal of Agricultural and Food Chemistry, 68*(42), 11612–11630. https://doi.org/10.1021/acs.jafc.0c04274

Diomandé, S. E., Nguyen-The, C., Guinebretière, M. H., Broussolle, V., & Brillard, J. (2015). Role of fatty acids in *Bacillus* environmental adaptation. *Frontiers in Microbiology, 6*, 813. https://doi.org/10.3389/fmicb.2015.00813

ElGamal, R., Song, C., Rayan, A. M., Liu, C., Al-Rejaie, S., & ElMasry, G. (2023). Thermal degradation of bioactive compounds during drying process of horticultural and agronomic products: A comprehensive overview. *Agronomy, 13*(6), 1580. https://doi.org/10.3390/agronomy13061580

Fu, X., Lin, Q., Xu, S., & Wang, Z. (2015). Effect of drying methods and antioxidants on the flavor and lipid oxidation of silver carp slices. *LWT- Food Science and Technology, 61*(1), 251–257. https://doi.org/10.1016/j.lwt.2014.10.035

Gozdzik, P., Magkos, F., Sledzinski, T., & Mika, A. (2023). Monomethyl branched-chain fatty acids: Health effects and biological mechanisms. *Progress in Lipid Research, 101226*. https://doi.org/10.1016/j.plipres.2023.101226

Han, Y., You, M., Wang, S., Yuan, Q., Gao, P., Zhong, W., … Wang, X. (2023). Enzymatic methods for the preparation of fragrant rapeseed oil: Effect of reducing sugars on flavor production using the Maillard reaction. *LWT- Food Science and Technology, 189*, Article 115497. https://doi.org/10.1016/j.lwt.2023.115497

Hidalgo, F. J., & Zamora, R. (2019). Formation of phenylacetic acid and benzaldehyde by degradation of phenylalanine in the presence of lipid hydroperoxides: New routes in the amino acid degradation pathways initiated by lipid oxidation products. *Food Chemistry: X, 2*, Article 100037. https://doi.org/10.1016/j.fochx.2019.100037

Jo, Y. J., Cho, I. H., Song, C. K., Shin, H. W., & Kim, Y. S. (2011). Comparison of fermented soybean paste (doenjang) prepared by different methods based on profiling of volatile compounds. *Journal of Food Science, 76*(3), C368–C379. https://doi.org/10.1111/j.1750-3841.2011.02068.x

Kai, M. (2020). Diversity and distribution of volatile secondary metabolites throughout *Bacillus subtilis* isolates. *Frontiers in Microbiology, 11*, 559. https://doi.org/10.3389/fmicb.2020.00559

Lee, J., & Min, D. B. (2010). Analysis of volatile compounds from chlorophyll photosensitized linoleic acid by headspace solid-phase microextraction (HS-SPME). *Food Science and Biotechnology, 19*, 611–616. https://doi.org/10.1007/s10068-010-0086-y

Leejeerajumnean, A. (2003). Thua nao: Alkali fermented soybean from *Bacillus subtilis*. *Silpakorn University International Journal, 3*, 277–292.

Liu, Q., Lei, M., Zhao, W., Li, X., Zeng, X., & Bai, W. (2023). Formation of lipid-derived flavors in dry-cured mackerel (*Scomberomorus niphonius*) via simulation of autoxidation and lipoxygenase-induced fatty acid oxidation. *Foods, 12*(13), 2504. https://doi.org/10.3390/foods12132504

Liu, S., Sun, H., Ma, G., Zhang, T., Wang, L., Pei, H., … Gao, L. (2022). Insights into flavor and key influencing factors of Maillard reaction products: A recent update. *Frontiers in Nutrition, 9*, Article 973677. https://doi.org/10.3389/fnut.2022.973677

Liu, Y., Su, H., & Song, H. L. (2018). Comparison of four extraction methods, SPME, DHS, SAFE, versus SDE, for the analysis of flavor compounds in natto. *Food Analytical Methods, 11*, 343–354. https://doi.org/10.1007/s12161-017-1005-0

Ludwig, V., Berghetti, M. R. P., Ribeiro, S. R., Rossato, F. P., Wendt, L. M., Thewes, F. R., … Wagner, R. (2021). The effects of soybean storage under controlled atmosphere at different temperatures on lipid oxidation and volatile compounds profile. *Food Research International, 147*, Article 110483. https://doi.org/10.1016/j.foodres.2021.110483

Maghraby, O., Hassouba, M. M., & El-Mossalami, E. (2013). Effect of methodology on the determination of total volatile basic nitrogen as an index of quality of meat and fish. *Egyptian Journal of Food Safety, 1*(1), 23–34. https://doi.org/10.21608/ejfsj.2013.135900

Mathatheeranan, P., Wongprasert, T., Fang, M., Lu, T. J., & Suppavorasatit, I. (2024). Effect of drying techniques on the stability of volatile compounds and antioxidative activities of dried Thai fermented black soybean (thua nao). *Journal of Agriculture and Food Research, 101305*. https://doi.org/10.1016/j.jafr.2024.101305

Mathatheeranan, P., Wongprasert, T., Nan, T. N., Suwannakul, E., Wang, Y., Lu, T. J., … Suppavorasatit, I. (2023). Volatile profiles and aroma-active components of northern Thai fermented soybean (thua nao) produced from controlled and uncontrolled fermentations. *International Journal of Gastronomy and Food Science, 100763*. https://doi.org/10.1016/j.ijgfs.2023.100763

Mathatheeranan, P., Wongprasert, T., Wang, Y., Fang, M., Lu, T. J., & Suppavorasatit, I. (2024). Characterization of potent odorants in northern Thai fermented black and yellow soybeans (thua nao) using solvent-assisted flavor evaporation, aroma extract dilution analysis techniques and omission studies of aroma reconstitution models. *Journal of Food Composition and Analysis, 128*, Article 106031. https://doi.org/10.1016/j.jfca.2024.106031

Namgung, H. J., Park, H. J., Cho, I. H., Choi, H. K., Kwon, D. Y., Shim, S. M., & Kim, Y. S. (2010). Metabolite profiling of doenjang, fermented soybean paste, during fermentation. *Journal of the Science of Food and Agriculture, 90*(11), 1926–1935. https://doi.org/10.1002/jsfa.4036

Oikonomopoulou, V. P., & Krokida, M. K. (2013). Novel aspects of formation of food structure during drying. *Drying Technology, 31*(9), 990–1007. https://doi.org/10.1080/07373937.2013.771186

Qin, H. W., Yang, T. M., Yang, S. B., Yang, M. Q., Wang, Y. Z., & Zhang, J. Y. (2022). Effects of different pre-drying and drying methods on volatile compounds in the pericarp and kernel of amomum tsao-ko. *Frontiers in Plant Science, 13*, Article 803776. https://doi.org/10.3389/fpls.2022.803776

Qu, Q., Yang, X., Fu, M., Chen, Q., Zhang, X., He, Z., & Qiao, X. (2016). Effects of three conventional drying methods on the lipid oxidation, fatty acids composition, and antioxidant activities of walnut (*Juglans regia* L.). *Drying Technology, 34*(7), 822–829. https://doi.org/10.1080/07373937.2015.1081931

Shahidi, F., & Hossain, A. (2022). Role of lipids in food flavor generation. *Molecules, 27*(15), 5014. https://doi.org/10.3390/molecules27155014

Shin, E. C., Lee, J. H., Hwang, C. E., Lee, B. W., Kim, H. T., Ko, J. M., … Cho, K. M. (2014). Enhancement of total phenolic and isoflavone-aglycone contents and antioxidant activities during cheonggukjang fermentation of brown soybeans by the potential probiotic *Bacillus subtilis* CSY191. *Food Science and Biotechnology, 23*, 531–538. https://doi.org/10.1007/s10068-014-0073-9

Thamkaew, G., Sjöholm, I., & Galindo, F. G. (2021). A review of drying methods for improving the quality of dried herbs. *Critical Reviews in Food Science and Nutrition, 61*(11), 1763–1786. https://doi.org/10.1080/10408398.2020.1765309

Van Gemert, L. J. (2011). *Odour thresholds. Compilations of odour threshold values in air, water and other media*. Utrecht (The Netherlands): Oliemans Punter & Partners BV.

Wang, D. H., Yang, Y., Wang, Z., Lawrence, P., Worobo, R. W., & Brenna, J. T. (2019). High levels of branched chain fatty acids in natto and other Asian fermented foods. *Food Chemistry, 286*, 428–433. https://doi.org/10.1016/j.foodchem.2019.02.018

Zarecki, A. P., Kolanowski, J. L., & Markiewicz, W. T. (2020). Microwave-assisted catalytic method for a green synthesis of amides directly from amines and carboxylic acids. *Molecules, 25*(8), 1761. https://doi.org/10.3390/molecules25081761