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Effect of Pre-Treatment on Bitterness and Quality of Osmodehydrated Lemons

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Abstract

Lemons are rich in beneficial phytochemicals and antioxidants, including phenolic compounds and flavonoids. However, some of these compounds impart a bitterness that can limit its application in food products and affect consumer acceptance. This study aimed to reduce the bitterness of osmodehydrated lemon using a combination of pre-treatment, osmotic dehydration, and drying processes. Fresh lemons, lemons immersed in 2 % NaCl for 3 nights, and lemons stored at room temperature for 3 days were analyzed for bitter compounds. These samples underwent 4 cycles of blanching at either 35 or 80 °C, followed by cooling, osmotic dehydration in a 60 °Brix sugar solution with heating at 80 °C for 45 min and soaking in syrup for an additional 30 min, and finally drying at 70 °C until reaching a water activity below 0.6. Results indicated that the optimal condition for reducing bitterness involved storing fresh lemons at room temperature for 3 days, soaking them in 2 % NaCl solution for 3 nights, and blanching at 80 °C, followed by osmotic dehydration and drying. The naringin, limonin, and hesperidin contents were reduced by 34-fold, 24-fold, and 10-fold, respectively, compared to those in fresh lemons. Additionally, its sensory properties were well accepted by panelists. Although the production cost at the laboratory scale was high, it is expected to be lower at pilot or commercial scales. The production method is deemed suitable for entrepreneurial ventures in the lemons processing industry.

Keywords: Lemon fruit, Osmodehydrated lemon, Bitterness, Naringin, Limonin, Hesperidin, Blanching

Introduction

Lemon (*Citrus limon*), a fruit of the evergreen tree in the Rutaceae family, is the 3rd most cultivated citrus fruit after mandarin and orange [1]. It consists of several parts: flavedo, albedo, juice vesicles, segment walls, and seeds. Globally, lemons are utilized for a wide range of culinary and non-culinary purposes. Its juice, containing 5-6 % citric acid and with a pH of around 2.2, is particularly valued for its sour flavor and applications in cooking, baking, and cleaning [2].

Citrus fruits are rich in a variety of phytochemicals, including vitamin C, carotenoids, flavonoids, and limonoids [3]. Citrus flavonoids are particularly noteworthy due to their chemoprotective effects. They exhibit a range of biological activities, including antioxidant, antimicrobial, anticarcinogenic, antiviral, anti-allergic, and anti-inflammatory effects [4,5]. Additionally, citrus flavonoids can inhibit human platelet aggregation [4,6]. Citrus fruits are also a significant source of dietary fiber, providing a well-balanced mix of soluble and insoluble fibers [7]. Among the many classes of flavonoids, flavanones are the most abundant in citrus fruits, with naringin and hesperidin being the most prevalent flavanones found in the tissues and peels [5,8-10].

A significant challenge in citrus fruit processing is managing its bitterness .Various chemical metabolites in citrus fruits contribute to this bitterness, including naringin, tangeretin, nobiletin, sinensetin, quercetin, limonin, nomilin, and neohesperidin [11,12]. Among these, limonin and naringin are particularly significant in causing the pronounced bitter taste [11]. The type and concentration of compounds responsible for bitterness can vary depending on the specific part of the fruit, its stage of maturity, growing conditions, and the fruit variety [12]. In fruits, the non-bitter compound limonoate A-ring lactone can be converted into the bitter compound limonin under acidic conditions. This conversion occurs more rapidly at lower pH levels [13]. The highest concentrations of bitter compounds are typically present in immature fruits, but these levels diminish as the fruits mature.

Bitterness can lead to diminished product quality, decreased consumer acceptability, and reduced economic value of citrus-based products [14]. Globally, various methods such as physical, chemical, and microbiological methods are being explored to reduce bitterness in citrus fruits. Additionally, artificial sweeteners, resins, and enzymes are being employed to mitigate bitterness and enhance the overall taste. The primary mechanisms for reducing bitterness in citrus fruits include 1) removal of bitter compounds; 2) removal of physical barrier; 3) flavor enhancers and bitter compound scavengers; 4) enzymatic treatment; and 5) genetic engineering.

This research aimed to reduce the bitterness of osmodehydrated lemon made from whole lemons (seeds excluded). The methods employed for bitterness reduction are tailored to the context and capabilities of the local entreprenuers, avoiding chemicals or food additives. The pre-treatment strategies included 1) using lemons harvested at different post-harvest dates; 2) pickling the lemons in brine; and 3) applying heat treatment (blanching). Additionally, using sugar solution at 60 °Brix was consequently conducted after blanching. The study evaluated the effectiveness of each method individually, as well as in combination, to identify the most effective approach for producing osmodehydrated lemon that are minimally bitter and acceptable to consumers without the use of chemicals, enzymes, or food additives.

Materials and methods

Raw materials

Lemon fruits, (*Citrus limon L.*). Burm f., ranging from maturity stages 2 to 4 (fruit color: green - yellow to yellow), were meticulously hand-picked from lemon trees in the early morning at a local farm and transported to laboratory at Naresuan University for subsequent analysis.

Investigation of color profile and phytochemical composition of lemon fruits

Upon reaching the laboratory, the fresh lemon fruits were randomly divided into 3 groups. The 1st group was designated as the fresh lemon fruit, while the 2nd group were immersed in a 2 % sodium chloride (NaCl) solution for 3 nights, and the 3rd group was left at room temperature for 3 days. Subsequently, all sample groups underwent the following analyses.

Color profile of lemon fruits

The color of the lemon fruits was assessed using the Color Reader CR-20 from Konica Minolta, Japan. The color was expressed in terms of L^*, a^* and b^*, where L^* represents the lightness of the samples a^*, indicates redness, and b^* indicates yellowness.

Total phenolic content of lemon fruits

Extracts of fresh lemon fruit and osmodehydrated lemon

The fresh lemon and osmodehydrated lemon samples were sliced crosswise into approximately 5 mm thick sheets. Subsequently, the lemon slices were cut into triangular shapes to utilize all parts of the lemon, ensuring removal of seeds. The extraction was performed using the method previously described by Thaweesang [15] with slight modifications. The samples, finely chopped to 5 g, were soaked in a 100 mL ethanol solution (95 %) for 24 h at room temperature. Following soaking, the mixture was filtered through Whatman® No. 1 filter paper to separate the lemon pieces from the solution, yielding the extract. The extract was then stored in a 100 mL amber glass bottle, wrapped with aluminum foil, and refrigerated at 4 ± 2 °C for further analysis.

Total phenolic content

The total phenolic content of the extracts from fresh lemon fruit and osmodehydrated lemon was determined using the Folin-Ciocalteu colorimetric method [16] with slight modifications. Specifically, 0.4 mL of the extracts were mixed with 2 mL of 10 % v/v Folin-Ciocalteau reagent and 1.6 mL of 7.5 % w/v sodium bicarbonate aqueous solution. After allowing the mixture to stand for 30 min, the absorbance was measured at 760 nm. The absorbance values were then subsequently converted to antioxidant activity using a gallic acid standard curve, with concentrations ranging from 0 to 100 ppm. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of sample, or mg GAE/g.

Total flavonoid content of lemon fruits

The same extract used to determine the total phenolic content was also utilized to assess the total flavonoid content. The total flavonoid content was determined using a modified colorimetric assay based on the method outlined by Wuttisin and Boonsook [17] with quercetin as the standard across concentrations ranging from 0 to 250 ppm. To determine the flavonoid content, 0.5 mL of the extract was combined with 3.0 mL of distilled water, followed by thorough mixing with 0.3 mL of 5 % (w/v) sodium nitrite, left to stand for 5 min at room temperature. Subsequently, 0.6 mL of 10 % (w/v) aluminum chloride was added, succeeded by 2 mL of 1 M sodium hydroxide, and the mixture was incubated for 6 min. Absorbance readings were taken at 510 nm using a UV/Vis spectrophotometer (Thermo, Genesys 20, USA). A standard curve was constructed by correlating absorbance with quercetin concentration, covering a range from 0 to 250 ppm. Results were quantified as milligrams of quercetin equivalents per gram of sample (mg QE/g), with the entire procedure conducted in triplicate for accuracy.

Naringin, limonin and hesperidin contents of lemon fruits

Sample extraction for naringin and hesperidin

The fresh lemon and osmodehydrated lemon samples were initially sliced crosswise into approximately 5 mm thick sheets. Following this, the lemon slices were cut into triangular shapes to ensure the utilization of all parts of the lemon, with careful removal of seeds. Subsequently, the samples underwent extraction, following the method outlined by Nipornram *et al.* [18] with some modifications. The samples, finely chopped to 5 g, were soaked in 10 mL of HPLC grade methanol in a 120 mL amber glass bottle, which was then immersed in an ultrasonic bath (model S30/H, Elma, Germany), serving as the ultrasound source for the experiment. To maximize ultrasonic energy, the liquid level in the immersed bottle was set lower than that of the liquid in the bath. The ultrasonic bath, a rectangular container measuring 240×137×100 mm^3, housed transducers operating at a frequency of 37 kHz, annealed to the bottom. Extraction was carried out under the conditions of 280 watts power, 50 °C temperature, and 1 h extraction time. Following extraction, the mixture was filtered through Whatman® No. 1 filter paper to separate the lemon pieces from the solution, yielding the extract. This extract was subsequently employed for the analysis of Naringin and Hesperidin contents using HPLC.

Sample extraction for limonin

The fresh lemon and osmodehydrated lemon samples were initially sliced crosswise into approximately 5 mm thick sheets. Subsequently, the lemon slices were cut into triangular shapes to ensure the utilization of all parts of the lemon, with careful removal of seeds. Following this preparation, the samples underwent extraction, following a method outlined by Chinapongtitiwat *et al.* [19] with some adjustments. Specifically, the samples, finely chopped to 5 g, were soaked in 60 mL of dichloromethane within a 120 mL amber glass bottle. This bottle was then shaken in an incubator water bath at room temperature (30 ± 2 °C) at a speed of 120 rpm for 4 h. After extraction, the mixture was filtered through Whatman® No. 1 filter paper to separate the lemon pieces from the solution. The solvent was evaporated using a vacuum evaporator at 30 °C for 2 min or until 2 mL of the sample remained. The concentrated sample was then adjusted to a volume of 10 mL with HPLC grade acetonitrile, transferred to a 15 mL amber glass bottle, wrapped with aluminum foil, and stored frozen at –80 ± 2 °C for further analysis.

HPLC analysis for naringin and hesperidin

The extracts from fresh lemon fruit, osmodehydrated lemon, and the standard solutions of naringin and hesperidin underwent filtration through Millipore filter membranes with a pore size of 0.45 µm. Subsequently, the extracts and standards were quantitatively analyzed for naringin and hesperidin content using an HPLC Shimadzu LC-2030C Plus chromatograph equipped with a UV-Visible detector set at 280 nm and a C18 reversed-phase column (Agilent TC-C18, 250×4.6 mm, 5 µm) maintained at 37 °C. The mobile phase comprised 2 solvents: 0.5 % acetic acid (A) and 100 % acetonitrile (B). A linear solvent gradient in volume ratios was established as follows: 10 - 30 % B over 20 min, with the gradient then increased to 35 % B at 25 min and maintained at 35 % B for 5 min. The flow rate was set at 1 mL/min, and the injection volume was 20 µL [18]. Identification of naringin and hesperidin was based on their retention times compared with the standards (Sigma-Aldrich, Germany). The analysis was conducted at least 3 times, with only the mean values reported. The naringin and hesperidin content were determined using the external standard method, with concentrations ranging from 10 to 200 ppm. The results were expressed as milligrams per 100 g of dried weight or mg/100 g DW.

HPLC analysis for limonin

The extracts from fresh lemon fruit, osmodehydrated lemon, and the standard solution of limonin underwent filtration through Millipore filter membranes with a pore size of 0.45 µm. Following this preparation, the samples were subjected to HPLC analysis, following a method outlined by Bilal *et al.* [20] with some modifications. Subsequently, the extracts and standards were quantitatively analyzed for limonin content using an HPLC Shimadzu LC-2030C Plus chromatograph equipped with a UV-Visible detector set at 210 nm and a C18 reversed-phase column (Agilent TC-C18, 250×4.6 mm, 5 µm) maintained at 37 °C. The mobile phase comprised acetonitrile, methanol, and water (HPLC grade) in a ratio of 10:41:49, eluted isocratically. The flow rate was set at 1 mL/min, and the injection volume was 20 µL. Identification of limonin was based on its retention time compared with the standards (Sigma-Aldrich, Germany). The analysis was conducted at least 3 times, with only the mean values reported. The limonin content was quantified by employing the external standard method, covering concentrations from 1 to 20 ppm. The results were reported as milligrams per 100 g of dried weight or mg/100 g DW.

Effect of debittering process prior production on physico-chemical properties and bitterness of osmodehydrated lemon

The fresh lemon fruits were subjected to pre-treatment conditions as part of the osmodehydrated lemon production process. The experimental design employed was a Factorial in Complete Randomized Design involving 3 independent factors: The day after harvest (0 and 3 days), soaking conditions (soaking in 2 % NaCl and non-soaking), and the blanching temperature (35 and 80 °C).

For treatments days 0 and 3, followed by non-soaking in 2 % NaCl, lemon fruits with different day after harvest were meticulously washed with tap water and sliced cross-sectionally into rings with a thickness of 0.5 mm. For treatments days 0 and 3, followed by soaking in 2 % NaCl, lemon fruits with different day after harvest underwent a process involving washing with tap water, scrubbing with NaCl, and immersing in the 2 % NaCl solution for 3 nights. Subsequently, they were washed with tap water and sliced cross-sectionally into rings with a thickness of 0.5 mm. All 8 treatments were thereafter subjected to blanching at 2 different temperatures for a duration of 3 min, followed by soaking in cold water for 2 min. This process of blanching and cooling was repeated 3 times before blanching was continued for an additional 10 min, followed by soaking in cold water for 2 min. After draining the water, the lemon slices were blanched at approximately 35 or 80 °C in a 60 °Brix syrup for 45 min, and then left to soak in the syrup without heating for another 30 min. The obtained osmodehydrated lemon products were drained on a perforated sheet for 10 min before being dried in a hot air oven at 70 °C for 4 h or until reaching a water activity (a_w) value of 0.6, as measured by the LabSwift-a_w from Novasina, Switzerland.

After cooling to room temperature, the osmodehydrated lemon product was individually packed in polyethylene bag. Five of these bags were then packed into Ziplock Grip Standable Seal Bags made of waterproof kraft paper.

All samples of osmodehydrated lemon underwent analysis for yield, color (measured with the Color Reader CR-20 from Konica Minolta, Japan), and hardness (measured using the 4411 Instron from England). Hardness was determined as the peak compression force (in Newtons) at target deformation using the HDP/BS probe, positioned 5.5 cm from the sample, with a speed of 100 mm/min.

Chemical analyses of the osmodehydrated lemon samples included determination of moisture content (following AOAC [21] guidelines), water activity (measured using the LabSwift-a_w device from Novasina, Switzerland), total phenolic content, total flavonoid content, and concentrations of naringin, limonin and hesperidin.

Additionally, sensory evaluation was conducted using the Randomized Complete Block Design (RCBD) with 30 untrained panelists employing a 9-point hedonic scale method to assess the liking scores for various attributes. Panelists tasted and evaluated attributes including palatability, color, size, odor, texture, bitterness, flavor, and overall liking. The osmodehydrated lemon sample with the highest overall liking score was selected.

Evaluation of production cost of osmodehydrated lemon products

After identifying the appropriate debittering conditions, the production costs at the laboratory scale were calculated. These costs would assist the entrepreneur in conducting further analyses for scaling up to pilot or commercial levels.

Statistical analysis

The data were presented as mean values ± standard deviation from triplicate experiments. Statistical analyzes were performed using IBM SPSS Statistics 22 software. Significant differences were assessed using analysis of variance (ANOVA), followed by Duncan's New Multiple Range Test at a 95 % confidence level.

Results and discussion

Investigation of color profile and phytochemical composition of lemon fruits

Color profile

Table 1 presents the color measurements of 3 lemon fruit samples: fresh lemon, fresh lemon immersed in a 2 % NaCl solution for 3 nights, and fresh lemon left at room temperature for 3 days. Fresh lemon immersed in a 2 % NaCl solution for 3 nights exhibited the highest L^* value, followed by fresh lemon, and fresh lemon left at room temperature for 3 days, respectively ($p < 0.05$). The highest a^* value was observed in the fresh lemon, followed by fresh lemon left at room temperature for 3 days, and fresh lemon immersed in a 2 % NaCl solution for 3 nights, respectively ($p < 0.05$). The b^* values of fresh lemon and fresh lemon immersed in a 2 % NaCl solution for 3 nights were the highest and showed no significant difference ($p > 0.05$). However, they were both higher than those of fresh lemon left at room temperature for 3 days ($p < 0.05$).

The pre-treatment process of washing with tap water, scrubbing, and immersing lemon fruits in a 2 % NaCl solution for 3 nights contributes to the higher lightness values observed in the fresh lemon immersed in a 2 % NaCl solution for 3 nights sample compared to the other samples. This might be due to the loss of pigment during pre-treatment process causing the fruit sample paler [22]. Additionally, the fruit skins appeared smooth and shiny in comparison to the other samples.

Table 1 Physico-chemical properties of lemon fruit.

Lemon	Color			Total phenolic (mg GAE/g)	Total flavonoids (mg QE/g)	Naringin content (mg/g)	Limonin content (mg/g)	Hesperidin content (mg/g)
	L^*	a^*	b^*					
Fresh lemon	63.72 ± 1.23^{b}	5.51 ± 0.74^{a}	48.21 ± 2.56^{a}	0.53 ± 0.03^{ns}	2.36 ± 0.07^{b}	0.034 ± 0.03^{ns}	0.22 ± 0.04^{b}	0.177 ± 0.00^{c}
Fresh lemon immersed in 2 % NaCl solution for 3 nights	67.65 ± 0.77^{a}	0.94 ± 0.29^{c}	48.28 ± 2.40^{a}	0.56 ± 0.07	3.85 ± 0.05^{a}	0.019 ± 0.00	0.06 ± 0.01^{c}	0.219 ± 0.00^{a}
Fresh lemon left at room temperature for 3 days	60.15 ± 0.43^{c}	3.60 ± 0.12^{b}	30.19 ± 1.92^{b}	0.60 ± 0.03	2.08 ± 0.10^{c}	0.025 ± 0.00	0.29 ± 0.02^{a}	0.211 ± 0.00^{b}

Data is mean ± SD of triplicates, values with different letters within a same column present significant difference ($p < 0.05$), ns = not significantly different ($p > 0.05$).

Phytochemical composition of lemon fruits

Total phenolic content

The total phenolic content (TPC) of 3 samples of lemon fruits showed no significant difference ($p > 0.05$) and ranged from 0.53 ± 0.03 to 0.60 ± 0.03 mg GAE/g, as shown in **Table 1**. The results indicate that the pre-treatment process did not impact the TPC of the lemon fruits. Vichaibun and Kanchanaphu [23] reported that lemon juice contained 319.3 ± 6.0 μg/g TPC. Additionally, Xi *et al.* [24] reported significant variations in total phenolic content (TPC) among different cultivars and fruit parts, ranging from 3.17 to 4.71 mg/g GAE FW in peels, 2.43 to 3.46 mg/g FW in pulp, 0.29 to 0.52 mg/g FW in juice, 1.63 to 3.04 mg/g FW in whole fruit, and 2.12 to 3.36 mg/g FW in seeds. Peels exhibited the highest TPC, followed by whole fruit and seeds, with juice having the lowest TPC. The TPC observed in the present study was lower than those reported in the literature. These discrepancies could be attributed to factors such as varieties, growing conditions, and differences in extraction methods.

Total flavonoid content

The pre-treatment process significantly affected the total flavonoid content of the lemon fruits ($p < 0.05$), as detailed in **Table 1**. Fresh lemon immersed in a 2 % NaCl solution for 3 nights exhibited the highest total flavonoid content (3.85 ± 0.05 mg QE/g), followed by fresh lemon (2.36 ± 0.07 mg QE/g), and fresh lemon left at room temperature for 3 days (2.08 ± 0.10 mg QE/g), respectively. Xi *et al.* [24] reported significant variations in total flavonoid content among different fruit parts and cultivars. The total flavonoid content ranged from 5.12 to 8.30 mg/g QE FW in peels, 3.86 to 5.38 mg/g FW in pulp, 0.26 to 0.44 mg/g FW in juice, 3.16 to 9.27 mg/g FW in whole fruit, and 18.61 to 25.33 mg/g FW in seeds. The total flavonoid content observed in the present study was lower than those reported in the literature. Literature indicates that genetic diversity, along with biological, environmental, seasonal and annual variations, significantly affected the flavonoids content in agricultural products [25].

Naringin content

The naringin content of 3 samples of lemon fruits showed no significant difference ($p > 0.05$) and ranged from 0.019 ± 0.00 to 0.034 ± 0.03 mg/g, as indicated in **Table 1**. Similar to the total phenolic content, the pre-treatment process did not influence the naringin content in the lemon fruits. Peterson *et al.* [8] reviewed the flavanones, the dominant flavonoid class in grapefruit, lemons, and limes. For lemons, total flavanones were found to be 26 mg/100 g, with hesperidin (15.78 mg/100 g) and eriocitrin (9.46 mg/100 g) being the major components, while naringin content was reported as 0.18 mg/100 g. The naringin content observed in this study (1.9 - 3.4 mg/100 g) is approximately ten times higher than the value previously reported.

Limonin content

Fresh lemon left at room temperature for 3 days showed the highest limonin content (0.29 ± 0.02 mg/g), followed by fresh lemon (0.22 ± 0.04 mg/g) and fresh lemon immersed in a 2 % NaCl solution for 3 nights (0.06 ± 0.01 mg/g), respectively ($p < 0.05$) (**Table 1**). Huang *et al.* [26] reported limonin content in lemons at various stages: 48.91 mg/100 g dry weight during the fruit falling period, 103.39 mg/100 g during the young fruit period, 23.41 mg/100 g during the fruit expanding period, and 1.02 mg/100 g at maturity. The limonin content was notably lower at the mature stage. However, Bilal *et al.* [20] reported that rough lemon (*Citrus jambhiri*) contained no limonin. Variations in results can be attributed to factors such as lemon variety, seasonal conditions, geographical location, and management practices. Guadagni *et al.* [27] found that the bitterness threshold for limonin in orange juice was 6.5 ppm, or 0.0065 mg/g. Thus, the limonin content of 0.06 ± 0.01 mg/g in fresh lemons immersed in a 2 % NaCl solution for 3 nights exceeded the threshold for detectable bitterness.

Hesperidin content

Fresh lemon immersed in a 2 % NaCl solution for 3 nights exhibited the highest hesperidin content (0.219 ± 0.00 mg/g), followed by fresh lemon left at room temperature for 3 days (0.211 ± 0.00 mg/g), and fresh lemon (0.177 ± 0.00 mg/g), respectively ($p < 0.05$) (**Table 1**). Hesperidin concentration varies among different parts of citrus fruits, with higher amounts found in the citrus flavedo, albedo, membrane, and pith compared to other parts such as juice vesicles and seeds

[28]. The reported concentration of hesperidin in lemon juice is 20.5 mg/100 mL [9,29,30], which is consistent with the levels found in lemons immersed in a 2 % NaCl solution for 3 nights (21.9 mg/100 g) and those left at room temperature for 3 days (21.1 mg/100 g), but slightly higher than the 17.7 mg/100 g observed in fresh lemons in this study. This aligns with the hesperidin content of 15.78 mg/100 g reported by Peterson *et al.* [8].

Effect of pre-treatments prior production on physico-chemical properties and bitterness of osmodehydrated lemon products

Color profile of osmodehydrated lemon products

Pre-treatment showed significant effect on the color of the osmodehydrated lemon ($p < 0.05$), as shown in **Table 2**. While there is no obvious trend for the redness (a^*) and yellowness (b^*) values, samples that blanched at 85 °C showed significantly lower lightness score (L^*) than the correspondent samples that blanched at 35 °C. It showed that higher blanching temperature darkened the color of the osmodehydrated lemon compared to samples blanched with lower blanching temperature. The color changes may be due to non-enzymatic browning and formation of brown pigments [31]. According to Kresic *et al.* [32], non-enzymic browning is responsible for tissue darkening during drying due to both condensation of reducing sugars with amino acids and pigment conversion.

Color is a critical attribute of food appearance, as undesirable color changes can reduce consumer acceptance and market value [33]. The color of both raw materials and final products is closely linked to other quality attributes such as freshness, sensory characteristics, nutritional value, and potential defects. Additionally, color is strongly correlated with antioxidant activity, oxidation, and Maillard reactions, and it can indirectly influence these processes [34]. It is often used as an indicator to assess the severity of heat treatment and to predict the quality degradation resulting from blanching processes [35].

Table 2 Physico-chemical properties of osmodehydrated lemon product from different pre-treatment process.

Treatment	Color			Harness (N)	Moisture content (%)	a_w	Total phenolic (mg GAE/g)	Total flavonoids (mg QE/g)	Naringin content (mg/g)	Limonin content (mg/g)	Hesperidin content (mg/g)
	*L**	*a**	*b**								
Day 0, 0 %, 35 °C	37.44 ± 0.26^{a}	0.81 ± 0.01^{d}	8.92 ± 0.53^{d}	105.54 ± 4.57^{a}	37.2 ± 18.0^{c}	0.49 ± 0.00^{g}	0.93 ± 0.09^{a}	0.96 ± 0.02^{d}	0.001 ± 0.00^{b}	0.007 ± 0.00^{c}	0.021 ± 0.00^{d}
Day 0, 0 %, 80 °C	27.11 ± 0.19^{f}	1.14 ± 0.01^{c}	9.19 ± 0.06^{cd}	54.24 ± 3.50^{d}	56.2 ± 04.0^{b}	0.56 ± 0.01^{d}	0.80 ± 0.02^{abc}	0.59 ± 0.04^{f}	ND	0.008 ± 0.00^{c}	0.016 ± 0.00^{f}
Day 0, 2 %, 35 °C	30.43 ± 0.20^{e}	1.50 ± 0.09^{b}	9.48 ± 0.34^{c}	63.67 ± 4.10^{c}	80.2 ± 15.0^{a}	0.57 ± 0.00^{e}	0.90 ± 0.07^{ab}	1.38 ± 0.04^{c}	ND	0.000 ± 0.00^{d}	0.023 ± 0.00^{e}
Day 0, 2 %, 80 °C	30.68 ± 0.30^{e}	0.75 ± 0.18^{de}	8.83 ± 0.08^{de}	55.47 ± 1.26^{d}	68.2 ± 06.0^{ab}	0.59 ± 0.00^{a}	0.73 ± 0.01^{cd}	0.76 ± 0.08^{e}	ND	0.011 ± 0.00^{a}	0.044 ± 0.00^{a}
Day 3, 0 %, 35 °C	34.22 ± 0.02^{d}	1.15 ± 0.05^{c}	10.44 ± 0.06^{b}	108.33 ± 1.03^{a}	81.2 ± 07.0^{a}	0.58 ± 0.00^{b}	0.77 ± 0.15^{bcd}	2.45 ± 0.16^{b}	0.002 ± 0.00^{a}	0.011 ± 0.00^{a}	0.033 ± 0.00^{b}
Day 3, 0%, 80 °C	30.52±0.24^{e}	1.81±0.04^{a}	12.49±0.28^{a}	57.18±3.54^{d}	55.2±03.0^{b}	0.50±0.00^{f}	0.75±0.05^{bcd}	1.03±0.06^{d}	0.001±0.00^{b}	0.011±0.00^{a}	0.014±0.00^{g}
Day 3, 2%, 35 °C	35.10 ± 0.16^{b}	1.65 ± 0.16^{ab}	10.43 ± 0.07^{b}	70.42 ± 1.14^{b}	79.2 ± 04.0^{a}	0.56 ± 0.00^{d}	0.62 ± 0.11^{de}	2.88 ± 0.07^{a}	0.001 ± 0.00^{b}	0.011 ± 0.00^{a}	0.014 ± 0.00^{g}
Day 3, 2%, 80 °C	34.63 ± 0.15^{c}	0.58 ± 0.12^{e}	8.44 ± 0.03^{e}	43.83 ± 3.61^{e}	57.2 ± 06.0^{b}	0.52 ± 0.00^{e}	0.48 ± 0.11^{e}	1.30 ± 0.08^{c}	0.000 ± 0.00^{c}	0.009 ± 0.00^{b}	0.018 ± 0.00^{e}

Data is mean ± SD of triplicates, values with different letters within a same column present significant difference ($p < 0.05$). ND = not detected.

Hardness of osmodehydrated lemon products

Table 2 illustrates the hardness of osmodehydrated lemon products. Samples Day 0 and Day 3 non-soaking in NaCl and heating at 35 °C exhibited the highest hardness values, while the sample Day 3 soaking in 2 % NaCl concentration and heating at 80 °C revealed the lowest hardness value ($p < 0.05$), indicating the influence of both heating and NaCl concentration on the hardness of osmodehydrated lemon products. Specifically, all samples subjected to pre-treatment at 80 °C showed lower hardness values compared to their counterparts treated at 35 °C, highlighting the significant impact of heating on reducing the hardness of osmodehydrated lemon

products. Higher temperatures (80 °C) can weaken the tissue structure of both peels and albedo, causing these tissues to lose their integrity during subsequent blanching, osmotic dehydration and drying processes [36], as observed in the present study.

Chemical composition of osmodehydrated lemon products

Moisture content

Significant differences ($p < 0.05$) in chemical composition and properties of osmodehydrated lemon products were observed across various pre-treatment methods, as depicted in **Table 2**. Although there were notable variations in the moisture content of osmodehydrated lemon products, no discernible trend was apparent. The moisture content ranged from 2.37 ± 0.18 to 2.81 ± 0.07 %. According to Zainun [37], the standard moisture content for dehydrated fruit candy should be between 12 and 21 % to ensure prolonged storage without deterioration from microorganisms. Excessive moisture content can make the products more susceptible to damage, particularly mold growth, which poses a risk of food poisoning [38]. Since the osmodehydrated lemon had a moisture content of only 2.37 ± 0.18 to 2.81 ± 0.07 %, it is expected to have a long shelf life.

Water activity

Similar to the moisture content, significant differences were observed in the water activity of the osmodehydrated lemon products, with values ranging from 0.49 ± 0.00 to 0.59 ± 0.00. Water activity is a critical parameter and is commonly used to optimize food quality. By monitoring and controlling water activity, the safety and shelf life of food products can be effectively predicted [39]. The water activity of the osmodehydrated lemon indicates that it is safe from microbial spoilage, as pathogenic bacteria cannot grow at water activity levels below 0.86, and yeast and mold cannot grow below 0.62 [40]. According to Jangam *et al.* [41], food with water activity between 0.4 and 0.6 is classified as a dry product, while food with water activity between 0.65 and 0.75 is considered to have intermediate moisture content. Therefore, with a water activity of 0.49 ± 0.00 to 0.59 ± 0.00, the osmodehydrated lemon is classified as a dry product.

Total phenolic content

On Day 0, all samples of osmodehydrated lemon products exhibited significantly higher total phenolic content compared to their counterparts on Day 3, with values ranging from 0.48 ± 0.11 mg GAE/g to 0.93 ± 0.09 mg GAE/g. With the exception of the sample on Day 3 that was treated with 2 % NaCl at 80 °C, all other samples exhibited higher total phenolic content compared to the lemon fruits. Although there were no significant differences in total phenolic content between samples blanched at 80 °C and those blanched at 30 °C, the samples blanched at 80 °C showed lower values overall. This reduction is likely due to higher temperatures (80 °C) weakening the tissue structure of both peels and albedo, which can compromise tissue integrity during subsequent blanching, osmotic dehydration, and drying processes [36].

Total flavonoid content

The total flavonoid content was lower in samples blanched at 80 °C compared to those blanched at 35 °C, highlighting the effect of blanching temperature on flavonoid levels. The total flavonoid content of most osmodehydrated lemon samples were lower than those of the lemon fruits. Higher temperatures (80 °C) can weaken the tissue structure of both peels and albedo, compromising tissue integrity during subsequent blanching, osmotic dehydration, and drying processes [36].

Naringin content

Notable variations in naringin content were observed, with all samples displaying very low concentrations, ranging from 0.000 to 0.002 mg/g. Specifically, only the sample from Day 3, which was not soaked in NaCl, showed a significant decrease ($p < 0.05$) in naringin content when the blanching temperature was increased from 35 to 80 °C. In contrast, naringin levels in the other samples did not differ significantly ($p > 0.05$). Most osmodehydrated lemon samples had lower naringin content compared to fresh lemon fruits. However, Ioannou *et al.* [42] reported that naringin is relatively heat-stable compared to other flavonoids, with only 20 % degradation occurring at 130 °C over 2 h. The observed decrease in naringin content in the osmodehydrated lemon could be attributed to the 4

blanching and cooling cycles, followed by osmotic dehydration and drying process.

Limonin content

As shown in **Table 2**, the limonin content in osmodehydrated lemon products varied significantly ($p < 0.05$), ranging from 0.000 to 0.011 mg/g. Notably, samples from Day 3 generally had higher limonin content compared to those from Day 0, except for the sample from Day 0 that was treated with 2 % NaCl and heated at 80 °C. All osmodehydrated lemon samples had lower limonin content compared to fresh lemon fruits. Higher temperatures (80 °C) can weaken the tissue structure of both peels and albedo, compromising tissue integrity during subsequent blanching, osmotic dehydration, and drying processes [36]. Additionally, the 4 blanching and cooling cycles, combined with osmotic dehydration and drying, may contribute to the lower limonin content observed.

Hesperidin content

The hesperidin content in osmodehydrated lemon products is notably higher than that of naringin and limonin, ranging from 0.014 to 0.044 mg/g. Particularly, the sample from Day 0, which was not treated with NaCl and heated to 80 °C, showed the highest hesperidin content ($p < 0.05$). Despite this, all osmodehydrated lemon samples had lower hesperidin content compared to fresh lemon fruits. Higher temperatures (80 °C) can weaken the tissue structure of both peels and albedo, affecting tissue integrity during subsequent blanching, osmotic dehydration, and drying processes [36]. Furthermore, the 4 blanching and cooling cycles, along with the osmotic dehydration and drying, likely contributed to the observed reduction in hesperidin content. **Figure 1** illustrates the chromatograms of naringin, hesperidin and limonin in the 8 osmodehydrated lemon samples, as well as the corresponding standard

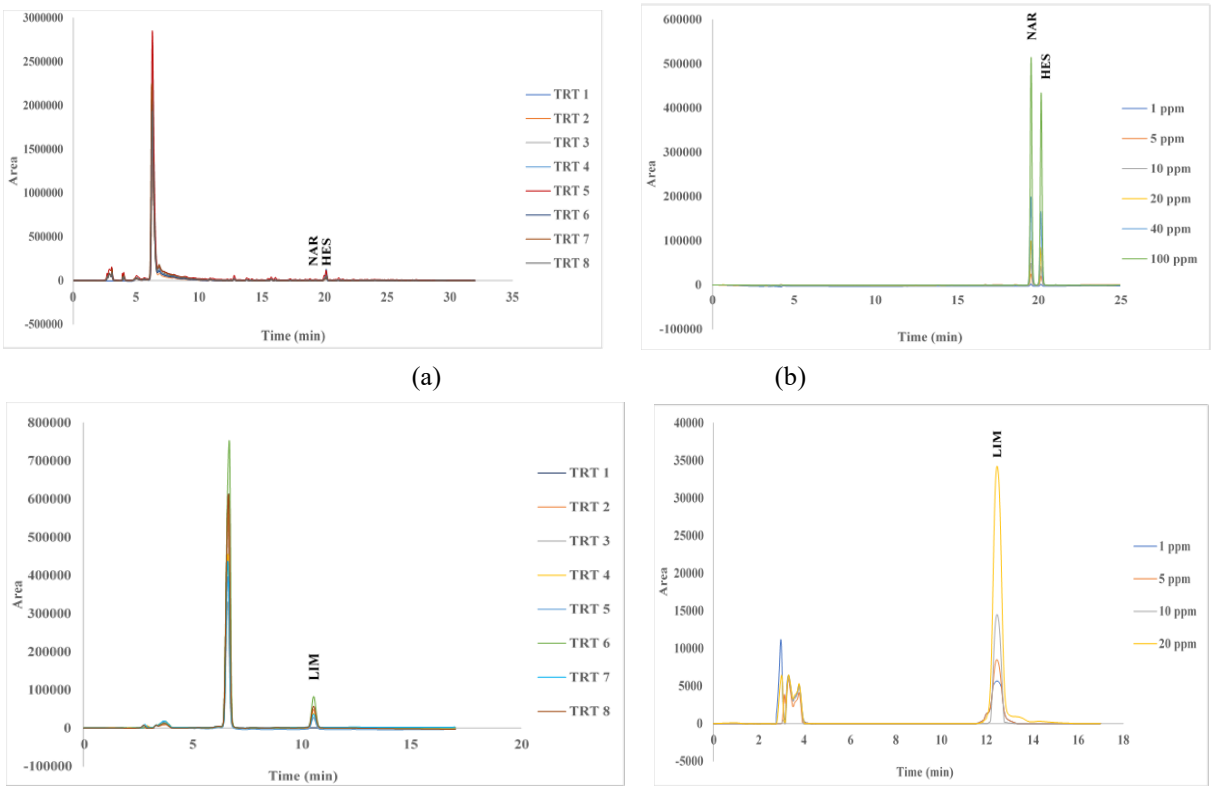

Figure 1 Chromatograms for naringin and hesperidin in osmodehydrated lemon samples (a), in standard (b) and for limonin in osmodehydrated lemon samples (c) and in standard (d).

Sensory evaluation of osmodehydrated lemon products

Sensory evaluation was conducted to assess panelists' liking scores and preference levels for the osmodehydrated lemon products. Significant differences ($p < 0.05$) were found across all 8 attributes (**Table 3**). A notable trend emerged where most samples blanched at 80 °C received higher liking scores, especially for attributes such as palatability and color, compared to those blanched at 35 °C. Regarding bitterness, only the samples from Day 0, treated with 2 % NaCl and blanched at both 35 and 80 °C, showed no significant difference in bitterness scores. For other samples, those blanched at 80 °C generally had higher liking scores for bitterness compared to those blanched at 35 °C, indicating a significant blanching effect on bitterness.

The sample from Day 3, which was soaked in 2 % NaCl and blanched at 80 °C, received notably higher liking scores across all 8 attributes. Its flavor and overall liking scores were rated between like moderately and like very much, whereas other samples ranged from dislike slightly to like slightly. Consequently, this sample was selected for further studies to calculate production costs. These sensory results were corroborated by physical and chemical analyses, which showed that the Day 3 sample soaked in 2 % NaCl and blanched at 80 °C had the lowest hardness, and lower total phenolic content and bitter compounds contents.

Table 3 Liking score of osmodehydrated lemon product from different pre-treatment process.

Treatment	Palatability	Color	Size	Odor	Texture	Bitterness	Flavor	Overall
Day 0, 0 %, 35 °C	4.37 ± 1.40^{d}	4.43 ± 1.79^{d}	6.00 ± 1.34^{bc}	5.47 ± 1.43^{cd}	5.00 ± 1.58^{d}	3.87 ± 1.53^{d}	4.57 ± 1.96^{c}	4.77 ± 1.76^{c}
Day 0, 0 %, 80 °C	3.37 ± 1.13^{bc}	6.70 ± 1.15^{abc}	6.50 ± 1.14^{ab}	6.37 ± 1.13^{ab}	5.67 ± 1.49^{bcd}	4.97 ± 1.94^{ab}	5.80 ± 1.86^{b}	5.90 ± 1.75^{b}
Day 0, 2 %, 35 °C	6.20 ± 1.24^{bc}	6.30 ± 0.99^{bc}	6.23 ± 1.14^{bc}	5.37 ± 1.30^{d}	5.53 ± 1.36^{cd}	5.00 ± 1.78^{bc}	5.30 ± 1.77^{bc}	5.47 ± 1.61^{b}
Day 0, 2 %, 80 °C	7.07 ± 1.14^{a}	7.20 ± 1.35^{a}	6.43 ± 1.65^{ab}	6.10 ± 1.69^{abc}	6.23 ± 1.61^{ab}	5.70 ± 2.04^{b}	5.93 ± 1.87^{b}	6.20 ± 1.73^{b}
Day 3, 0 %, 35 °C	4.77 ± 1.74^{d}	5.00 ± 1.88^{d}	5.73 ± 1.55^{c}	5.77 ± 4.41^{bcd}	5.63 ± 1.73^{bcd}	4.83 ± 1.66^{c}	5.60 ± 1.69^{b}	5.77 ± 1.36^{b}
Day 3, 0 %, 80 °C	6.30 ± 1.12^{bc}	6.37 ± 1.25^{bc}	5.77 ± 1.57^{c}	6.07 ± 1.08^{abc}	5.87 ± 1.50^{bc}	5.77 ± 1.81^{b}	5.90 ± 1.35^{b}	5.93 ± 1.36^{b}
Day 3, 2 %, 35 °C	5.90 ± 1.21^{c}	6.10 ± 1.47^{c}	5.93 ± 1.46^{bc}	5.77 ± 1.55^{bcd}	5.90 ± 1.45^{bc}	5.37 ± 1.92^{bc}	5.87 ± 1.66^{b}	5.97 ± 1.50^{b}
Day 3, 2 %, 80 °C	6.57 ± 1.10^{ab}	6.83 ± 1.34^{ab}	6.93 ± 1.39^{a}	6.63 ± 1.10^{a}	6.80 ± 1.10^{a}	6.83 ± 1.32^{a}	7.23 ± 1.10^{a}	7.37 ± 1.03^{a}

Data is mean ± SD of triplicates, values with different letters within a same column present significant difference ($p < 0.05$).
Liking scores: Dislike extremely (1); Dislike very much (2); Dislike moderately (3); Dislike slightly (4); Neither like nor dislike (5); Like slightly (6); Like moderately (7); Like very much (8); and Like extremely (9).

Evaluation of production cost of osmodehydrated lemon products

The production cost at the laboratory scale was calculated by summing the material cost, labor cost, and manufacturing overhead. For a package containing 45 g of osmodehydrated lemon, the production cost was 100 Baht for low-lemon-fruit production and 60 Baht for high-lemon-fruit production (data not shown). The higher cost was primarily due to the cost of lemons and sugar. Specifically, the cost of lemons was 150 Baht/kg for low-lemon-fruit production and 60 Baht/kg for high-lemon-fruit production, while sugar cost 25 Baht/kg and nearly 4 kg was used in the formula. The laboratory-scale production costs were elevated due to the small quantities of ingredients purchased, which made them more expensive. However, if production were scaled up to pilot and commercial levels, the cost would significantly decrease.

While the study shows promising results, it has certain limitations. First, the research was conducted at a laboratory scale, and the results may not fully extrapolate to pilot or commercial-scale production. The variety of lemon used and the specific maturity stages limit the generalizability of the findings to other citrus varieties. Additionally, the reproducibility of the results might be affected by varying geographical locations and climate conditions that could influence lemon quality. Last, the stability of the final product under different storage conditions (temperature, humidity) was not tested and requires further exploration. Furthermore, a

notable disadvantage of this study is the high production cost at the laboratory scale, primarily due to the small quantities of raw materials used, which increased the overall expense. Future studies should focus on optimizing the process for commercial production, which is expected to reduce costs.

Moving forward, future research could explore alternative pre-treatment methods or osmotic solutions to further reduce bitterness while preserving quality, prioritize cost reduction for commercial production and further investigate the product's shelf life through additional storage tests. However, scaling up this process may pose challenges, such as optimizing energy consumption and maintaining consistency across different lemon varieties. Moreover, incorporating enzyme-based debittering methods could be promising, though it may introduce complexities in balancing cost and product quality.

Conclusions

The study investigated the effect of pre-treatment, followed by osmotic dehydration and drying processes on the bitterness and quality of osmodehydrated lemon. Fresh lemons kept at room temperature for 3 days and soaked in 2 % NaCl for 3 nights showed similar total phenolic and naringin contents compared to fresh lemons, although differences were observed in total flavonoid content, limonin, and hesperidin.

The blanching and cooling cycles, combined with osmotic dehydration in a 60 °Brix sugar solution and drying at 70 °C until the water activity fell below 0.6, influenced the color, hardness, moisture content, water activity, total phenolic content, total flavonoid content, and levels of bitter compounds, including naringin, hesperidin, and limonin. This study successfully reduced the bitterness of osmodehydrated lemon through a natural process involving pre-treatment, osmotic dehydration, and drying which was a chemical-free and suitable for small-scale or small enterprise production. The optimal production conditions for osmodehydrated lemon were found to be fresh lemons kept at room temperature for 3 days, soaked in 2 % NaCl, and blanched at 80 °C, followed by osmotic dehydration and drying, resulting in the reduction of naringin, limonin, and hesperidin contents by 34, 24 and 10 times, respectively, compared to fresh lemon. Additionally, sensory evaluations demonstrated a high consumer acceptance level, with liking scores ranging from 6.57 to 7.37 on a 9-point scale. This method produced a final product with moisture content and water activity meeting the standards of Thai Community Product Standard No. 136/2546 for dried fruits. It indicated that the final product not only meets the moisture and water activity standards but also achieves significant reductions in bitter compounds. These findings suggest that the process is highly applicable for lemon processing, offering a sustainable solution for producing consumer-acceptable products.

The production cost of osmodehydrated lemon at the laboratory scale was 150 Baht/kg for low-lemon-fruit production and 60 Baht/kg for high-lemon-fruit production.

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