

***Citrus sinensis* essential oil: chemical characterization and preliminary application in baking**

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Abstract

The byproducts generated during citrus processing are a potential source of bioactive compounds with food applications. The study aimed to chemically characterize the essential oil obtained from *Citrus sinensis* byproducts from commercial fresh-juice establishments and to preliminarily evaluate its incorporation into a bakery product. The essential oil was extracted by steam distillation, yielding a gravimetric yield of 0.31% (w/w, fresh basis) relative to the fresh weight of the peel. Gas chromatography-mass spectrometry (GC-MS) analysis showed a profile dominated by limonene (55.47%), followed by β -myrcene (8.28%) and (R)-3,7-dimethyl-6-octenal (2.93%), consistent with the typical composition of sweet orange. Flours derived from the peel and bagasse were incorporated into the formulation of an experimental cookie, while the essential oil was added after baking as jelly made with orange juice and pectin. The product was evaluated using a hedonic sensory test (n= 28), showing intermediate to favorable overall acceptability (mean= 3.32 on a five-point scale). The results suggest the preliminary technological feasibility of the integral use of *C. sinensis* byproducts as a source of essential oil rich in limonene and dietary fiber, contributing to sustainable revalorization strategies for agrifood waste.

Keywords:

Citrus sinensis, fortified biscuit, limonene, valorization of citrus byproducts.



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Introduction

The food industry generates large volumes of byproducts during fruit processing, particularly peels and bagasse, which contain bioactive compounds of interest with potential food applications. In citrus, these residues can account for 20-60% of the processed material, constituting a significant source of essential oils, dietary fiber and other functional metabolites (Castro *et al.*, 2020; Suri *et al.*, 2021; Selvaprasad *et al.*, 2025).

The use of these byproducts aligns with circular economy and sustainability strategies aimed at reducing waste and valorizing agrifood materials (Aguirre-Forero *et al.*, 2022; Andrade *et al.*, 2023; Xia *et al.*, 2024; Cinardi *et al.*, 2025). The recovery of functional compounds from citrus residues has gained interest in the development of food ingredients and nutraceuticals (Ben-Hsouna *et al.*, 2023).

Citrus essential oils are complex mixtures of secondary metabolites, dominated by monoterpenes such as limonene, linalool and citral, with aromatic and functional properties (Yang *et al.*, 2017; Guo *et al.*, 2018; Sousa *et al.*, 2023). In particular, limonene is the major component of *C. sinensis* essential oil and can vary with origin and extraction method (Bouabdallah *et al.*, 2022). Given their GRAS status, these compounds represent an alternative to synthetic additives (Sun, 2007; Guo *et al.*, 2018).

In addition, peel and bagasse contain dietary fiber with potential applications in bakery products, where their impact on technological and sensory properties has been evaluated (Romero *et al.*, 2011; Olajumoke and Sade, 2018; Guerra *et al.*, 2020). However, the chemical profile of the essential oil can vary significantly depending on factors such as plant variety, geographical origin, degree of fruit ripeness, raw material storage conditions, and the extraction technique used, which makes it necessary to characterize it using GC-MS (Guo *et al.*, 2018; Bhandari *et al.*, 2024).

Steam distillation remains one of the most widely used methodologies for obtaining citrus essential oils because of its operational simplicity, reproducibility and broad applicability in chemical characterization studies (Brah *et al.*, 2024). Although there are emerging technologies aimed at increasing extraction efficiency, the present research focused on the chemical characterization of the oil obtained using a widely documented conventional technique (Mahato *et al.*, 2019; Costa *et al.*, 2022; Brah *et al.*, 2024).

The objective of this study was to chemically characterize *C. sinensis* essential oil obtained from the peel of byproducts of the orange juice industry and to preliminarily evaluate its application in a bakery product.

Materials and methods

Experimental design

The study corresponded to experimental, descriptive and exploratory research conducted in two stages. The first stage consisted of extracting and chemically characterizing the essential oil obtained from *Citrus sinensis* byproducts using gas chromatography-mass spectrometry (GC-MS). The second stage consisted of the preliminary evaluation of its application in a bakery product made with flours derived from orange peel and bagasse.

Collection and preparation of raw materials

Samples of *C. sinensis* (Valencia and Veracruz varieties) were obtained between March and May 2024 from juice establishments in Tijuana, Baja California, Mexico. The peels and bagasse, after removing the seeds, were collected immediately after mechanical squeezing, transported in airtight containers, and stored at 4 °C until processing. Before extraction, the peels were separated from the bagasse and cut into pieces (~1 cm²) to improve the efficiency of the process.

Essential oil extraction

The essential oil of *C. sinensis* was obtained by steam distillation. For each run, approximately 250 g of previously segmented fresh peel was used. The plant material was placed in a distillation system equipped with a Liebig-type condenser, and distilled water was added in sufficient volume to maintain constant boiling. Distillation was carried out for 45 minutes after boiling began.

The distillate was collected as an oil-water mixture and the essential oil was separated by a decanting funnel. It was then dehydrated with analytical-grade anhydrous sodium sulfate and stored in amber glass bottles at 4 °C until analysis. Three independent extractions ($n=3$) were performed under the same experimental conditions.

The gravimetric yield was calculated as a weight/weight percentage (% w/w) with respect to the initial fresh mass of peel used in each extraction, according to the equation:

$$\text{Yield}(\%) = \frac{\text{mass of oil obtained (g)}}{\text{fresh peel mass (g)}} \times 100$$

The average yield obtained was $0.31 \pm 0.02\%$ (w/w) ($n=3$). Each extraction was performed using approximately 250 g of fresh peel.

Gas chromatography-mass spectrometry (GC-MS analysis)

The chemical characterization was performed on a Thermo Scientific Trace 1310 chromatograph coupled to an ISQ LT mass spectrometer operating in electron-impact ionization mode (70 eV) and full-scan acquisition. A TG-SQC capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 μm) was used with helium as the carrier gas (1.2 ml min^{-1}). The injection was carried out in split mode (16.7:1), with injector, transfer line and source temperatures set at 300 °C.

The oven temperature program was: 50 °C (2 min), 7 °C min^{-1} increments to 85 °C (5 min), followed by 10 °C min^{-1} increments to 250 °C, with maintenance times in between. The samples were diluted in hexane (1:100, v/v) and analyzed in triplicate, including blank runs.

Compound identification was performed by comparison with the NIST library (R match ≥ 800) and verification by Kovats retention indices using n -alkanes (C_8 - C_{20}), according to the literature (Babushok *et al.*, 2011; Bizzo *et al.*, 2023). Relative abundances were calculated based on the areas of the peaks of the total ion chromatogram (TIC), without the use of an internal standard.

Obtaining peel and bagasse flours

After extracting the essential oil, the peels and bagasse were dehydrated in an Excalibur® food dehydrator at 40-47 °C for 6-8 h. The dehydrated material was ground in a blade mill to a fine powder and then manually sieved through a 500 μm metal mesh to homogenize the particle size.

The fraction obtained was stored in airtight containers, protected from light and moisture, at 25 ± 2 °C until use. In addition, oat and rice flour were prepared by grinding following the same procedure and were used as part of the product's base mixture.

Formulation of the bakery product

The base formulation of the product was prepared using a total mixture of 325 g of flour, distributed as follows: 100 g oat flour, 80 g whole wheat flour, 50 g rice flour, 45 g *C. sinensis* peel flour and 50 g *C. sinensis* bagasse flour. Peel and bagasse flours accounted for 13.8% and 15.4% of the total mixture, respectively.

The dough was prepared by homogeneously mixing the dry ingredients, followed by incorporating the liquid components, in accordance with the standard baking procedure (Jose *et al.*, 2022). The cookies were manually molded and baked in a conventional oven at 180°C for 15 min until they reached a firm texture and uniform color.

The essential oil was not incorporated directly into the dough or subjected to heat treatment. For its application, a jelly was prepared from fresh orange juice squeezed in the laboratory, with pectin added as a gelling agent.

Once the jelly had formed and reached room temperature, the essential oil was incorporated at a ratio of 0.5313 g per 16 g of jelly, carefully homogenizing for uniform distribution. This preparation was then applied to the already baked cookies, thereby avoiding exposure of the essential oil to high temperatures.

This strategy was selected in order to minimize the loss of volatile compounds and preserve the essential oil's aromatic characteristics during its application in the product. The cookies were left to rest at room temperature until their sensory evaluation.

Sensory evaluation

The acceptability of the product was evaluated using a hedonic sensory test with a five-point Likert-type scale (1= very low acceptance; 5= very high acceptance), considering the attributes of taste, consistency, shape/presentation and overall acceptance. A total of 28 university students selected through non-probability convenience sampling participated, and they signed informed consent forms prior to their participation.

Each participant evaluated a single formulation of the product, corresponding to a complete cookie weighing 15 g, prepared under the conditions described in this study. Samples were coded with three-digit random numbers and evaluated under controlled conditions of lighting, ambient temperature and sample presentation.

The internal consistency of the instrument was evaluated using Cronbach's α coefficient (0.839). Data was analyzed using descriptive statistics (mean, standard deviation, median and mode) in SPSS Statistics v.24 (IBM Corp., USA).

Results and discussion

Extraction yield

Steam distillation performed on approximately 250 g of fresh peel per run yielded an average of $0.31 \pm 0.02\%$ (w/w), calculated from three independent extractions. This value falls within the range reported for essential oils obtained from previously squeezed citrus byproducts (Agarwal *et al.*, 2022), although it is lower than that reported for intact fresh material.

The plant material used consisted of peels from juice establishments, so previous mechanical handling could have caused partial rupture of the flavedo oleiferous vesicles and, consequently, a decrease in the recovery of the essential oil (Fisher and Phillips, 2008; Costa *et al.*, 2022).

The secretory glands located in the flavedo constitute the main site of essential oil accumulation in citrus fruits (Crişan, 2026). Nonetheless, the yield obtained confirms that agrifood byproducts are a viable source of essential oil, consistent with reports on citrus waste valorization processes (Mahato *et al.*, 2019).

Chemical composition of essential oil

The main components identified by GC-MS are presented in Table 1. GC-MS characterization identified limonene as the major component of the essential oil, followed by β -myrcene, citronellal and pinene. This chemical profile is consistent with reports on *Citrus sinensis* essential oils, in which D-limonene is usually the predominant compound and constitutes a significant fraction of the total essential oil composition (Crişan, 2026).

Table 1. Chemical composition of *C. sinensis* essential oil determined by GC-MS.

Retention time (min)	Compound	Relative abundance (%)
9.32	Limonene	55.47
8.12	#-myrcene	8.28
12.05	Citronellal (R)-3, 7-dimethyl-6-octenal	2.93
6.8	#-pinene	1.6
15.39	Decanal	1.15
8.44	Octanal	0.7
10.82	1-octanol	0.7
17	(E)-3, 7-dimethyl-2, 6-octadienal	0.63
7.69	4-methylene-1-(1-methylethyl)cyclohexane	0.49
16.34	(Z)-3, 7-dimethyl-2, 6-octadienal	0.33
15.13	#-terpineol	0.31
16.66	Geraniol	0.15
17.15	4-(1-methylethenyl)cyclohexene-1-carboxaldehyde	0.13
4.31	Ethyl butanoate	0.07

The differences observed in the relative abundance of the compounds can be attributed to factors such as raw materials' origin, the fruit's state of ripeness, the processing conditions and the characteristics of the plant material used for extraction (Ferrer *et al.*, 2022).

The compounds were identified by comparing the mass spectra with the NIST library. The relative abundance was calculated based on the areas of the peaks of the total ion chromatogram (TIC) without correction factors.

The chromatographic profile showed a predominance of limonene (55.47%), followed by β -myrcene (8.28%) and (R)-3,7-dimethyl-6-octenal (2.93%), in accordance with reports for *C. sinensis* (Mahato *et al.*, 2019; Ferrer *et al.*, 2023). Limonene content falls within the typical range (50-90%), depending on factors such as variety, geographic origin and extraction method.

Monoterpenes such as α -pinene and β -myrcene were also identified, along with oxygenated compounds, including α -terpineol, geraniol and aldehydes (decanal and octanal), which contribute to the essential oil's aromatic profile (Dosoky and Setzer, 2018; González-Mas *et al.*, 2019). The predominance of hydrocarbon monoterpenes confirms that the oil retains the typical sweet orange profile, even though the material came from post-processing byproducts. The analysis was performed on a non-chiral column, so the enantiomeric distribution of the compounds was not evaluated (Elshafie and Camele, 2022).

Preliminary application in bakery products

The incorporation of essential oil and flour derived from peels and bagasse allowed us to evaluate the technological viability of the developed product. The results of the sensory evaluation showed favorable acceptance among participating consumers, suggesting that incorporating ingredients derived from citrus byproducts can be done without negatively affecting the overall product acceptance.

These results are consistent with previous studies reporting adequate acceptance of food products enriched with citrus-derived compounds and essential oils used as functional ingredients (Zhang *et al.*, 2022).

Sensory evaluation

The results of the sensory evaluation are presented in Table 2. Overall acceptance was 3.32 ± 0.84 (CV= 25.3%), with a median of 3.5 and a mode of 4, indicating a favorable trend.

Attribute	Mean $\pm$ SD	CV (%)	Median	Mode
Taste	3.36 ± 0.88	26.2	4	4
Consistency	3.64 ± 0.81	22.3	4	4
Shape/presentation	3.93 ± 0.72	18.3	4	4
Overall acceptance	3.32 ± 0.84	25.3	3.5	4
Consumption frequency	2.93 ± 0.91	31.1	3	3

Five-point hedonic scale (1= lower acceptance; 5= higher acceptance).

Similar results have been reported in bakery products enriched with citrus byproducts, where the concentration of the functional ingredient affects consumer acceptance (Sayed-Ahmad *et al.*, 2023). The use of the mean and standard deviation for Likert-type scales is accepted when complemented by robust measures such as the median and mode (Huh and Gim, 2025).

The evaluated formulation corresponded to a preliminary experimental formulation developed to explore the incorporation of *Citrus sinensis* byproducts into a food matrix. Consequently, the present study did not aim to optimize the formulation or apply specific sensory optimization methodologies, so the results obtained should be interpreted as initial evidence of the developed product's acceptability.

The results obtained suggest that incorporating ingredients derived from citrus byproducts can be done without negatively affecting the product's overall acceptance, which aligns with previous studies on bakery products enriched with citrus residues, which reported adequate levels of consumer sensory acceptance (Sayed-Ahmad *et al.*, 2023).

Conclusions

The essential oil obtained from *C. sinensis* byproducts via steam distillation had a yield of $0.31 \pm 0.02\%$ (w/w), confirming that peels from juice establishments are a viable source for recovering volatile compounds.

The GC-MS analysis showed a chemical profile dominated by limonene (55.47%), accompanied by β -myrcene and other characteristic monoterpenes, consistent with reports for *C. sinensis*, even though the plant material corresponded to post-processing byproducts.

Incorporating the essential oil into a baking matrix and applying it after baking allowed its aromatic characteristics to be preserved. Sensory evaluation showed intermediate to favorable acceptability, with no evidence of rejection associated with the inclusion of essential oil and fiber derived from peel and bagasse.

Overall, the results support the comprehensive use of *C. sinensis* byproducts as an agrifood valorization strategy, demonstrating their potential for technological applications in food development.

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