Scientific Journal of King Faisal University:
Basic and Applied Sciences

Identification of the Bioactive Components of Dried Citrus Medica Peels and Their Incorporation in biscuits

Latifah A Alhamdan [1], Sahar M Kamel [2] and Hala H. Al-Otaibi [2]

[1] Physical Education Department, College of Education, King Faisal University, Al Ahsa, Saudi Arabia
[2] Food and Nutrition Sciences Department, College of Agriculture and Food Sciences, King Faisal University, Al Ahsa, Saudi Arabia

LINK	RECEIVED	ACCEPTED	PUBLISHED ONLINE	ASSIGNED TO AN ISSUE
https://doi.org/10.37575/b/agr/230044	20/09/2023	16/01/2024	16/01/2024	01/06/2024

NO. OF WORDS	NO. OF PAGES	YEAR	VOLUME	ISSUE
3828	4	2024	25	1

ABSTRACT

The present work aimed to identify the constituents of the dried whole peel (WP) and albedo layer (AL) of citrus medica (Etrog), as well as their antioxidant activity. The GC-MS analysis of the hexane extract revealed that limonene was the most abundant component (93.9% and 38.09% in WP and AL, respectively), followed by l-(+)-Ascorbic acid 2, 6-dihexadecanoate (1.7% and 21.67% in WP and AL, respectively). The antioxidant activity of WP was 91.98% and 93.14% in AL. The appearance, crispiness, flavour, taste, texture, and overall acceptability of biscuits fortified with WP and AL at 3% or 5% were evaluated using 27 panellists. The statistical analysis of sensory evaluation revealed no significant difference in the appearance and flavour characteristics of fortified biscuits with 3% and 5% AL or 3% WP compared to the control. The current findings suggested that fortifying the biscuit with dried Etrog (WP) peel could be done without affecting the taste or sensory acceptance of the product.

KEYWORDS

albedo peel, antioxidant activity, etrog, fortification, phytochemicals, whole peel

CITATION

Alhamdan, L.A, Kamel, M.S. and Al-Otaibi, H.H. (2024). Identification of the bioactive components of dried citrus medica peels and their incorporation in biscuit. *Scientific Journal of King Faisal University: Basic and Applied Sciences*, **25**(1), 21–24. DOI: 10.37575/b/agr/230044

1. Introduction

The peels obtained from various *Citrus* species are a major source of phenols and flavonoids, coumarins, carotenes and terpenes which exhibit several health-related benefits, making them potential candidates for medicine. These peel-derived compounds showed remarkable antibacterial activity against the pathogenic bacteria that cause food poisoning. (Koolaji *et al.,* 2020; Maqbool *et al.,* 2023; Saleem *et al.,* 2023). *Citrus* peels have been examined for their antioxidant and antimicrobial effects and several studies suggest that these peels should be included in the preparation of various breakfast cereals, dietary supplements and in the formulation of nutraceuticals (Andrade *et al.,* 2022). *Citrus medica L.*, commonly known as citron or Etrog, is a large, lean citrus fruit with little juice; its pulp is either acidic or sweet and rarely consumed (Venturini *et al.,* 2014). Citron peel is the more useful part of the fruit. It's thick albedo, or the inner peel layer, is usually consumed after being processed, whereas the lemon-scented flavedo, or the outer peel layer, contains the essential oils. Etrog is used for medicinal and pharmacological purposes in many countries, including Saudi Arabia. Menichini *et al.,* (2011) showed the antioxidant, hypoglycaemic and anticholinesterase of *Citrus medica L.* Sesquiterpenes and monoterpenes, especially limonene and γ-terpinene, were the predominant constituents of hexane extract of Diamante citron peel (Venturini *et al.,* 2014; Benedetto *et al.,* 2023). Mohd *et al.* (2005), identified the essential oil of the *C. medica var. sarcodactyl* fruit. Limonene, γ-terpinene, (Z)-citral and (E)-citral were the main components. However, citron peel oils contained limonene and α-terpineol. Limonene is a cyclic hydrocarbon, coloured liquid at a room temperature with a strong lemon scent (Kvittingen *et al.,* 2021). Some important bitter limonoids present in citrus fruits are limonene, nomilin, and nomilinic acid, and some important non-bitter limonoids present are calamine, deoxylimonin, limonoic acid, deoxylimonoic acid, limonol, deoxylimonol, limonilic acid, ioslimonic acid, etc. Ascorbic acid 2, 6-dihexadecanoate has been reported to have antioxidant, anti-inflammatory, antibacterial, antitumor, and wound healing properties (Akinmoladun *et al.,* 2007; Okwu and Emenike, 2006; Shi *et al.,* 2020). Gómez-Mejía *et al.* (2019) suggested that citrus peel by-products could be used as a value-added ingredient in food products. Biscuits have become one of the popular snacks among bakery products for most people due to their low price, convenience, long shelf life, and nutritive value (Shi *et al.,* 2020; Binou *et al.,* 2022). The current work aimed to identify the constituents of the Etrog peel and albedo layer, as well as their antioxidant activity. The acceptability of biscuits enriched with dried WP and AL was evaluated in order to introduce Etrog peel to food manufacturers as a sustainable source of *Citrus* flavour.

2. Materials and Methods

2.1. Samples:

In March 2021, about 10kg of mature Etrog fruits (*Citrus medica L.*) were purchased at a local market in Al-Ahsa, Saudi Arabia. All fruits were washed and dried with tissues before the WP and AL were manually isolated and weighed and the yield was calculated. WP and AL were cut into small pieces (0.5cm^2) and dried to constant weight in a convection oven at 35°C (Garau *et al.,* 2007). The dried WP and AL were ground and stored at -20°C for the biscuits' fortification.

2.2. Moisture Content:

Etrog fruits, WP and AL were analysed in triplicate for their moisture content at 105°C for 3h, (Latimer, 2023).

2.3. DPPH Free Radical Scavenging Ability:

The free radical scavenging ability of Etrog fruits, WP, AL and flavedo layer samples against DPPH (1, 1-diphenyl-2 picrylhydrazyl) were estimated as described by Qu *et al.* (2010). The same weight (dry base) of all samples was used to compare the antioxidant activity of the samples.

Corresponding Author: Latifah Alhamdan 00966540858118, Lalhamdan@kfu.edu.sa

2.4. GC-MS Analysis:

For 24 hours at 27°C, 50gm dried WP and AL were mixed with 500ml hexane. Whatman No.42 filter paper was used to filter the extracts. The solvents were evaporated using a vacuum rotary evaporator at 30°C. Shimadzu GC/MS-QP2010 Plus (Japan) Gas Chromatography/Mass Spectrometer system with DB-5MS capillary column (30m length, 0.25mm thickness, 0.25m diameter) was used to identify the WP and AL constituents. Injections (250°C) were made in split mode (50:1) with Helium as the carrier gas at a flow rate of 1ml/min. The isothermal program was started at 50°C for 2 min, 7°C/min raised to 200°C and 5°C/min to 220°C and held for 20 min. All compounds reported were confirmed by comparison of their fragmentation patterns with the authentic standards and those cited in literature. The temperature program was isothermal for 2 minutes at 50°C, 7°C/min to 200°C and 5°C/min to 220°C and held for 20 minutes. All reported compounds were validated by comparing their fragmentation patterns to those of authentic standards and those cited in the literature.

2.5. Preparation of Etrog Peel Fortified Biscuits:

According to the method described by Takeungwongtrakul and Benjakul (2017), five types of biscuits (control, 3% WP, 5% WP, 3% AL, and 5% AL) were made (Table 1).

Table 1. Ingredients (gm) of Etrog peel fortified biscuits

Component (gm)	Control	5%WP	3%WP	5%AL	3%AL
Flour	39	34	36	34	36
Sugar	10	10	10	10	10
Butter	20	20	20	20	20
Salt	0	0	0	0	0
Baking powder	2	2	2	2	2
Milk	29	29	29	29	29
WP	0	5	3	0	0
AL	0	0	0	5	3

2.6. Sensory Characteristics of Etrog Peel Fortified Biscuits:

Overall acceptability, texture, taste, flavour, appearance and crispiness of the biscuits fortified with 3% WP, 5% WP, 3% AL and 5% AL were evaluated using 27 panellists. The biscuits were assessed using a 7-point hedonic scale (one = dislike extremely, two = dislike moderately, three = dislike, four = neither like nor dislike, five = like, six= like moderately and seven = like extremely) (Igbabul *et al.,* 2018).

2.7. Statistical Analysis:

The replicates of dataset were statistically subjected to one-way analysis of variance ANOVA using SPSS package version 23. The least significant difference (LSD) value at $p < 0.05$ was carried out.

3. Results and Discussion

3.1. Moisture Content, Yield and Antioxidant Activity:

Etrog fruit was purchased in March 2021 from local markets in Al-Ahssa, Saudi Arabia. The fruit weighed about 600gm. The moisture content in fresh WP and AL was 81.74 and 82.30%, respectively, according to the results in Table 2. Whole peel and albedo yields were 59.87 and 26.29% of the fresh fruit, respectively, these yields were higher than other citrus fruits like, mandarin (28%), sweet orange (25%), lemon (50%), and grapefruit (45%) peel yield that obtained by (Hakim and Harris, 2001). The data revealed that fresh fruit, WP and AL had high antioxidant activity, with 92.31, 92.31and 90.02%, respectively. The drying process used in this study (Table 2) had no significant effect on the antioxidant activity of WP (from 92.31 to 91.98%); however, the antioxidant activity of AL increased significantly after drying (from 90.02 to 93.14%). This increase in antioxidant activity in AL could be attributed to higher moisture levels than whole peel. The high content of limonene and Ascorbyl palmitate (l-(+)-Ascorbic acid 2, 6-dihexadecanoate) and other terpenes may contribute to the antioxidant activity. D-limonene has been shown to have strong antioxidant and anti-inflammatory properties (Yu *et al.,* 2017; Wei and Shibamoto, 2007). Ascorbyl palmitate is a reducing agent that also acts as an oxygen scavenger in food (Younes *et al.,* 2020).

Table 2. Moisture and antioxidant activity of Etrog

Part	Moisture content %	Antioxidant activity %
Fresh fruit	98.66± 0.76a	93.00 ±0.17a
Fresh WP	81.74± 0.87b	92.31± 1.04b
Dried WP	14.36± 0.97c	91.98 ±0.23b
Fresh AL	82.30± 0.57b	90.02± 0.91c
Dried AL	13.4± 0.89c	93.14 ± 0.31a

Whole peel WP; Albedo layer AL; Means±SD; Different letters within a column indicate significant difference ($p < 0.05$).

3.2. GC-MS Analysis:

The GC-MS analysis of the WP hexane extract (Table 3A, Table 3B, Figure 1 and Figure 2) revealed that limonene was the most abundant component (93.9%), followed by l-(+)-Ascorbic acid 2, 6-dihexadecanoate (1.7%) and beta – pinene (1.29%). Other components detected included lemonol, citral, and alpha pinene. As in WP, the results in Table 3A revealed that the main constituent in the albedo layer was limonene (38.09%), followed by l-(+)-Ascorbic acid 2,6-dihexadecanoate (21.61%). Some fatty acids, including Oleic and Myristic Acids, were found mostly in albedo layer (Table 3B). Limonene was identified to be the major component (54%) in Corsican fruit from France (Venturini *et al.,* 2014). Verzera *et al.* (2005), concluded that *Citrus medica* flavedo oil contains D-limonene and other terpenes such as linalool, geraniol, citronellol, α-terpineo, α-pinene, etc.

Table 3A. GC-MS analysis of Etrog whole peel and albedo layer Presented as Area %

No	Compound name	WP	AL
1.	Limonene	93.9	38.09
2.	l-(+)-Ascorbic acid 2,6-dihexadecanoate	1.70	21.61
3.	Beta- Pinene	1.29	-
4.	Linalool	0.74	-
5.	Lavandulol	0.68	-
6.	Alpha-Pinene	0.31	-
7.	Tetradecanoic acid	0.31	-
8.	Citral	0.22	-
9.	trans-Citral	0.20	-
10.	alpha-Camphorene	0.16	-
11.	Geraniol acetate	0.15	-
12.	Hexadecamethylcyclooctasiloxane	0.13	-

Table 3B. GC-MS analysis of Etrog whole peel and albedo layer Presented as Area %

No	Compound name	WP	AL
1.	Oleic Acid	-	7.69
2.	Myristic acid	-	3.14
3.	Nerol acetate	-	3.03
4.	Trans-Carveol	-	2.90
5.	Lemonol	-	2.54
6.	4-vinylguaiacol	-	2.21
7.	Limetin	-	1.97
8.	3,3-Dimethylbutane-1,2-diol	-	1.57
9.	Acetic acid, chloro-, octadecyl ester	-	1.33
10.	Linolenic acid	-	1.23
11.	Lauric acid	-	1.07
12.	Unknown	-	7.25

Figure 1. GC-MS chromatogram of Etrog whole peel extracted with hexane

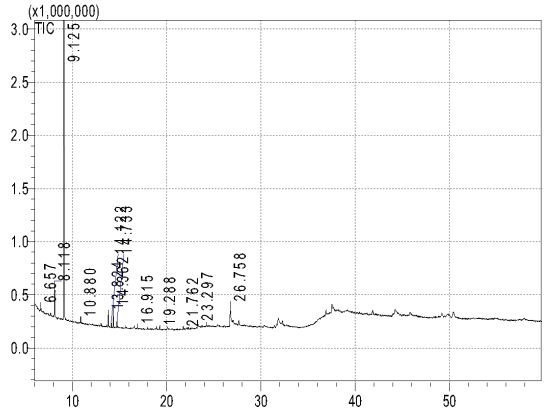
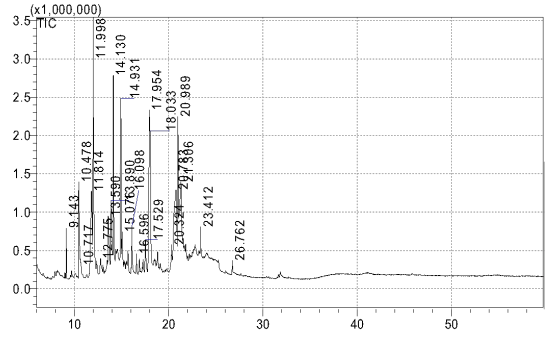


Figure 2. GC-MS chromatogram of albedo layer extracted with hexane



The current limonene content is higher than the results obtained by Bhuiyan, *et al.* (2009), who found that the major components are isolimonene (39.37%), citral (23.12%) and limonene (21.78%). Hexane was found to be an effective solvent for extracting limonene (Nguyen, 2012). He tested four different solvents (n-hexane, dichloromethane, ethyl acetate and diethyl ether) and found that hexane was the most effective in extracting D-limonene from orange peels in a traditional solid–liquid extraction.

3.3. Sensory Properties:

The appearance, crispiness, flavour, taste, texture and overall acceptability of fortified biscuits were evaluated. The statistical analysis of sensory evaluation (Table 4) revealed no significant difference in the appearance and flavour characteristics of biscuits fortified with WP and AL at 3% or 5% compared to the control. The texture of all fortified biscuits was improved and preferred by panellists (5.1-5.2) over the control (4.6). When the crispiness of fortified biscuits was compared to the control, the results revealed a significant improvement at WP 5% (4.3), AL 5% (5) and AL 3% (4.3) compared to the control (3.1). Biscuits fortified with 3% whole peel and 3% and 5% albedo, on the other hand, did not exhibit this unfavourable taste effect in comparing to control. The treatments with 5% WP fortification had a significant negative effect on taste (moderate dislike) and overall acceptability (dislike). The increase of limonene concentration in whole peel resulted in increasing the bitterness in taste. Okpala and Akpu (2014) indicate that orange peel powder at 3% enhanced the quality and general acceptance of the bakery products as well as increasing the nutritional value.

Table 4. Sensory properties of biscuits fortified with dried Etrog peel and albedo

Type	Crispiness	Appearance	Flavour	Taste	Texture	Acceptability
Control	3.1± 1.7 a	5.2± 1.3 a	3.6± 1.9b	4.9± 1.9 b	4.6 ± 1.9 a	4.6± 1.8 b
WP5%	4.3± 1.8 a	4.9± 1.4 a	4.6± 2.0 a	2.8± 2.0 a	5.1± 1.7 a	3.6± 2.0 a
WP3%	3.9±1.9 a	5.2 ± 1.5 a	4.8 ± 1.2 a	4.2± 2.1 ab	5.2 ± 1.8 a	4.1± 2.1 ab
AL 5%	5.0± 1.5 b	5.2 ± 1.4 a	4.7 ± 1.8 a	4.7± 2.1 b	5.2± 1.7 a	5.1± 1.8 b
AL3%	4.3± 1.7 a	5.2 ± 1.5 a	4.5± 2.0 a	4.2± 2.0 ab	5.2± 1.6 a	4.6± 1.9 b

Whole peel WP; Albedo layer AL; Means±SD; Different letters within a column indicate significant difference ($p < 0.05$).

While Ojha and Thapa (2017) showed that fortification of biscuits with mandarin peel powder by 6% was similar to the control in the sensory evaluation with the increase of antioxidants and active ingredients, another study suggested that fortified biscuits with citrus peel (lemon and oranges) evaluated with the addition of 5% did not significantly affect the quality of the product (Kohajdova, *et al.,* 2011). However, no research on Etrog peel fortification in bakery products was found. The current findings suggested that fortifying the biscuit with Etrog peel at 3% could be done without affecting the taste or sensory acceptance of the product. At higher concentrations, the albedo layer may be more appropriate than the whole peel, increasing acceptability while taking advantage of the active compounds and high antioxidant activity.

4. Conclusion

This study concluded that whole Etrog peel contains a high percentage of limonene, the main aromatic compound of citrus, and has a wide array of benefits. The albedo layer analysis revealed a significant amount of ascorbyle palmitate, which has antioxidant activity and is highly recommended for food preservation. Dried peel showed antioxidant capacity as the fresh peel had. The results of this study suggest that the peel and albedo of the Etrog fruit could be used as a value-added ingredient and, therefore, can be used to make biscuits that are both nutritious appealing to consumers.

Biographies

Latifah A Alhamdan

Physical Education Department, College of Education, King Faisal University, Al Ahsa, Saudi Arabia, 00966540858118, lalhamdan@kfu.edu.sa

Latifah A Alhamdan, from Saudi Arabia, Corresponding Author, Graduate of King Faisal University, Master of Nutrition Sciences from the College of Agricultural and Food Sciences, Department of Food and Nutrition Sciences. She is interested in research related to the chemistry and analysis of food, food security, the nutritional habits of society, as well as research related to sports activity and public health. Currently, she is working as a lecturer in the Department of Physical Education at the College of Education, King Faisal University.

ORCID: 0000-0003-2259-4228

Sahar M Kamel

Food and Nutrition Sciences Department, College of Agriculture and Food Sciences, King Faisal University, Al Ahsa, Saudi Arabia, 00966595634095, smsalem@kfu.edu.sa

Prof. Sahar M Kamel, from Egypt, works at King Faisal University, College of Agricultural and Food Sciences, as well as in Food Technology Research Institute, Giza, Egypt. She Graduated from Cairo University, College of Agriculture, Professor of Agricultural Biochemistry, and holds master's and Ph.D. degrees from Cairo University. She is interested in nutrition, food analysis and food security as well as functional food and its impact on public health. She has two filed patents.

ORCID: 0000-0002-2314-2592

Hala H Al-Otaibi

Food and Nutrition Sciences Department, College of Agriculture and Food Sciences, King Faisal University, Al Ahsa, Saudi Arabia, 00966505929224, hhalotaibi@kfu.edu.sa

Prof. Al-Otaibi, Saudi Arabia, is the Vice Dean for Deanship of Scientific Research, and her prior position was Vice Dean for Female Students Affairs in the College of Agriculture and Food Science. Member in the local scientific advisory board (National Nutrition Committee), and the leader of scientific team for assessing the nutritional status of community in the KSA (National Nutrition Committee). Al-Otaibi's research explores health-associated diet and lifestyle with a particular focus on obesity prevention, sustainable healthy diets and food security.

ORCID: 0000-0002-4585-5120

Acknowledgements

The authors would like to thank the Scientific Research Deanship, King Faisal University. The authors thank all members in the Food Science and Nutrition department, Agriculture and Food Sciences, King Faisal University. Moreover, the authors express their appreciation to the Deanship of Scientific Research at King Faisal University for their support for this work, Project No: (182034).

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