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Chemical Profiling and Identification of Phytochemicals from Microwave-assisted Extract of Kinnow Peel Using LC-MS and its Fortification in Biscuits

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ABSTRACT

The purpose of the current study was to analyze the phytochemicals using LC-MS extracted from kinnow peel and its fortification in biscuits. The extract was made utilizing environmentally friendly methods, like the microwave. The identification of phytochemical extracts through liquid chromatography-mass spectrophotometry revealed six flavones, five flavon-3-ol, four flavanones, four lignans, one isoflavonoid, four anthocyanins and anthocyanidin dimers, one dihydrochalcone, seven hydroxycinnamic acids, two hydroxybenzoic acids and one stilbene in the extract. The extract also had phytochemicals such as α-carotene, vitamin C (ascorbic acid), fatty acid (linolenic acid) and fatty amide (docosanamide). Furthermore, by substituting kinnow peel for wheat flour, biscuits with varying peel concentrations of 0 (control), 5, 10 and 15% were developed. The sensory evaluation found that adding peel increased the strength of aroma, taste and colour. However, adding more than 10% was not acceptable in terms of taste. According to proximate studies, supplementation significantly boosted dietary fibres, ash and protein content in the biscuits. As a result, kinnow peel extracts can be used as valuable components in a variety of foods and medications. Kinnow peels, which are generally discarded as garbage, could be added to biscuits to make them more flavourful. According to the study, adding kinnow peels could enrich biscuits and had positive effects on their nutritional and sensory qualities. It is possible that the effective use of this fruit waste as a potent natural functional food may help to solve issues like industrial waste management and nutritional stability. In addition, utilizing the microwave process can help the environment by consuming less time, energy, and solvent.

Key words: Antioxidants, kinnow, LC-MS, phytochemicals, microwave extraction

INTRODUCTION

Kinnow, a widespread fruit cultivar in the mandarin family, is a part of the Rutaceae family. In the early 1940s, kinnow was introduced in India after being produced in 1935 at the University of California Citrus Experiment Station. It is a cross between the King and Willow Leaf mandarin citrus varieties (Mahawar *et al.*, 2020). 2.33 million tonnes of citrus are produced annually, with kinnow accounting for nearly 90% of the total. Kinnow peel makes up roughly 35 to 40% of the fruit's weight and is the main source of processing waste (Safdar *et al.*, 2017). Considering the high moisture content and microbial load of peel, researchers are looking into ways to lessen environmental concerns as well as potential advantages (Kumar *et al.*, 2020). However, the waste is not waste because kinnow peel is the largest source of bioactive phenolic components, particularly flavonoids, with a relatively greater polyphenol concentration than the edible sections. Because of these bioactive components, kinnow seeds and peel have anti-inflammatory, anti-microbial and anti-cancer properties, and hence have piqued the curiosity of many people (Purewal and Sandhu, 2020).

The extraction procedure is crucial for extracting natural bioactives from plant materials. Due to the recent increase in energy prices, emissions of carbon dioxide, and other global pollution, research and progress in creating effective extraction processes for numerous industries have gained particular focus (Gómez-Mejía *et al.*, 2019). Traditional solvent extraction techniques, such as Soxhlet, hot continuous extraction, infusion and maceration, have disadvantages such as a long extraction time and temperature, a low extraction yield, the usage of a significant number of solvents, mass transfer resistance, and health risks. Thus, interest in non-traditional green technologies is growing due to their effectiveness and excellent reliability, low power and solvent consumption, and minimal heat used, reducing bioactive chemicals' loss (Picot-Allain *et al.*, 2021).

While kinnow peel is a rich source of numerous bioactive components, its powder or extract can be used in the development of foodstuffs, and those functional foods can meet the demands of health-conscious individuals, as fast-paced life has diverted focus away from health due to poor lifestyle choices (Yaqoob *et al.*, 2021). Kinnow peel-based functional foods have improved the antioxidant and technological properties of foods (Rafiq *et al.*, 2022). Along with antioxidants, the peel also enhanced the dietary fiber content of food products (Singla *et al.*, 2019). According to the literature, peel extract contains some antinutritional chemicals. Antinutrients are chemicals that limit nutrient absorption, ingestion, transport, and use while also having additional negative consequences. Tannin, oxalate and phytic acids have been detected in kinnow peel in the literature; therefore, removing these compounds will open the door to waste in the food sector and directly impact the health of the economy and the environment. Secondly, exposing antinutrients to heat renders them inert (Ani and Abel, 2018). When it comes to extracting bioactives from plant materials, microwave extraction (MAE) outperforms other green technologies considerably (Vázquez-Espinosa *et al.*, 2019).

In light of the aforementioned information, a study project was developed to improve the environment, nutrition, and economic sectors in a single experiment by extracting phytochemicals from kinnow peels using microwave technology, identifying them using LC-MS and fortifying biscuits.

MATERIALS AND METHODS

Kinnow fruits were purchased from fruit market in Ludhiana and transported to the Lovely Professional University's Chemistry Lab in Phagwara (India). To remove dust, dirt and chemical residue (pesticide) from the surface of the fruit, it was adequately washed under running water. Kinnow was peeled; the peels were chopped into pieces using a stainless steel knife and shade-dried for two weeks. The dried peels were ground into a fine powder using a grinder. Kinnow peel powder was packaged in sealed polyethylene zip bags and refrigerated for later use.

A laboratory-scale microwave extraction device operating at barometric pressure with a microwave frequency of 2450 MHz was used for the extraction. The device was outfitted with a digitally controlled temperature, timing and power system. Extraction was carried out following the experimental design with minor modifications (Nayak *et al.*, 2015). Five g of peel powder was extracted with 50 ml of ethanol for 3 min at 480 W at 60°C and the supernatant was filtered through Whatman filter paper No. 1 before being evaporated under reduced pressure at 30°C using a rotary evaporator until dry. The extract yield was calculated in percentage as :

$$\text{Yield (\%)} = \frac{\text{Mass of extract (g)}}{\text{Mass of dried powder (g)}} \times 100 \quad ...(1)$$

The extract was then sealed in a petri dish, and the LC-MS technique was used to determine the phytochemicals.

The bioactives in the extracts were detected and analyzed using liquid chromatography-Mass Spectrometry (LC-MS) with minor changes (Safdar *et al.*, 2017). Kinnow peel extract samples were loaded onto a reversed-phase C 18 column with internal diameters of (4.6 x 250 mm, 5 µm). The mobile phase was a linear gradient of solvent A (acetonitrile) and solvent B (distilled water/acetic acid, 99 : 1, v/v, pH 2.30±0.1). For the bioactive compounds, the following gradient protocol was used: 20% A (5 min), 80% A (10 min) and 20% A (5 min). The experiments were carried out at a flow rate of 1 ml/min, with the UV detector set at 280 and 370 nm to detect polyphenols and a sample injection volume of 20 µl. For the MS analysis, the positive mode of ESI was used. The instrument was calibrated and configured according to the manufacturers' guidelines.

Ingradients required for making biscuit were : wheat flour, kinnow peel powder, corn syrup, eggs, baking powder and ghee. Biscuits with varying concentrations of peel, particularly B_0 (control), B_1 (5% of peel powder), B_2 (10% of peel powder) and B_3 (15% of peel powder) were prepared by substituting wheat flour.

The ingredients were accurately weighed and added to the container, ghee and corn syrup were combined, and eggs were put one at a time. The wheat flour was uniformly combined with varying amounts (Table 1) of peel and mixed with baking powder and filtered. The batter was rolled to a consistent thickness. Biscuits were given shape using a cutter and placed in trays. Baked at 175°C for 20 min. Biscuits were allowed to cool at room temperature before being stored in airtight glass jars for subsequent examination.

Table 1. Wheat flour replaced with kinnow peel powder

Ingredients	B_0	B_1	B_2	B_3
Wheat flour (g)	100	100	100	100
Kinnow peel (%)	-	5	10	15

On a 9-point Hedonic scale, peel-supplemented biscuits were rated for sensory features such as taste, colour, aroma, texture and overall acceptability. Twenty-five unskilled sensory participants were chosen. The participants were instructed on the study's aims. The judges were given a sensory ballot form to fill out in order to record their views.

Using the AOAC approach, the nutritional profile of the final product was assessed. To calculate the concentrations of moisture, ash, crude fiber and crude protein, standard analytical methods were employed. Weight loss was recorded as a moisture percentage in dried peel powder, and moisture was measured in a hot air oven at 115°C up to a constant weight. Samples of burnt peel powder were ashed at 650°C for 8 h to get a fixed value. The crude fiber content was estimated using acid and alkali digestion, followed by ashing, and the crude protein content was calculated using the Kjeldahl method. The total carbohydrate content of a sample was determined. Total carbohydrates were calculated by subtracting from 100 the sum of the values for moisture, protein, fat, ash and fiber.

The energy content in kilocalories per gram was calculated using the conversion factors crude protein (16.74 kJ/g), total carbohydrates (16.74 kJ/g) and crude fat (37.66 kJ/g). The results were all expressed in terms of dry matter. One hundred g of dry product was weighed to determine the calorie (Kcal), protein, carbohydrate, fat, ash and fibre content.

For each analysis, three repetitions were done, and the values were represented as mean±standard deviation (SD), and data were statistically evaluated using the software SPSS V.28 through the analysis of variance technique (ANOVA).

RESULTS AND DISCUSSION

In plants, pharmacologically significant compounds are frequently detected in trace amounts. An extraction process that produces high-yielding extracts while needing minimal modification to the extract's functional characteristics is referred to as a high-yielding extraction procedure. Compared to the literature, yield obtained was very high (Safdar *et al.*, 2017). Table 2 shows the extract yields of kinnow peel extracted using the MAE procedures with ethanol as the solvent.

Table 2. Yield of microwave-assisted kinnow peel extract

Compound	% Yield (w/w)
Kinnow peel	32.46

The polarity, solubility and particular extraction parameters, including solvent type, solvent concentration, extraction temperature and extraction duration, are all related to the nature of phytochemicals recovered from plants (Altemimi *et al.*, 2017).

The various phytochemicals isolated from peel were recognized using LC-MS, as indicated in Table 3. By correlating the m/z values from the published data (Wang *et al.*, 2019; Chen *et al.*, 2021; Yang *et al.*, 2022; Yaqoob *et al.*, 2022), the phytochemicals were found and identified. Compared to hydroxybenzoic acids, hydroxycinnamic acids among the phenolic acids were present in lower amounts. Two hydroxybenzoic acids and seven distinct hydroxycinnamic acids were found. Cinnamic acid was shown to be the main phenolic compound in the kinnow peel in hydroxycinnamic acids. In comparison, hydroxyl benzoic acids included ellagic acid and galloyl glucose. Furthermore, six flavones, five flavon-3-ols, four flavanones, four anthocyanidin and anthocyanins, one isoflavonoid, one dihydrochalcone, four lignans, one stilbene and one fatty amide were found. Out of all flavones, Apigenin-7-O-glucoside and Tangeretin were found in higher concentration. Quercetin-3-O-glucosyl-pentoside (Flavon-3-ol) was found in highest concentration among all but lower than flavones and flavanone. A flavanone prunin, which posesses anti-microbial properties, was also found in high concentration. Additionally, vitamin C, α-Carotene, and α-3 fatty acids like linolenic acid were found. A lot of carotene was present. The phytochemicals processed a long history of anti-cancer, anti-inflammtory and anti-oxidant capabilities.

Biscuits were tested to know the effect of the quantity in % addition of kinnow peel powder. All sensory metrics in enriched biscuit

Table 3. LC-MS identification of several phytochemicals in peel

S. No.	Compound	Tentative (m/z)	Concentration (%)
Flavones			
1.	Apigenin-7-O-glucoside	250	100
2.	Trihydroxydimethoxyfavone	331	9
3.	Tangeretin	373	100
4.	Diosmetin 6,8-di-C-glucoside	625	65
5.	Cirsilineol	344	20
6.	Monohydroxytrimethoxyflavone	327	5
Flavon-3-ol			
1.	Quercetin-3-O-glucosyl-pentoside	595	23
2.	Quercetin	301	5
3.	Quercetin-7-O-xyloside-3-O-rutinoside	741	4
4.	Quercetin-glucoside	463	5
5.	3-Methoxysinensetin	403	4
Flavanone			
1.	Hesperetin 7-O-methyl ester	315	19
2.	Liquiritin	417	7
3.	Prunin	433	90
4.	Acacetin	284	6
Isoflavonoids			
1.	5,6,7,30,40- Pentahydroxyisoflavone	302	9
Anthocyanidin and Anthocyanins			
1.	Cyanidin-3-O-rutinoside	287	8
2.	Petunidin 3-O-(60'-acetyl-glucoside)	522	5
3.	Cyanidin 3-O-diglucoside-5-O-glucoside	773	9
4.	Peonidin 3-O-diglucoside-5-O-glucoside	786	11
Dihydrochalcones			
1.	Phloridzin	435	4
Hydroxycinnamic acids			
1.	5-O-Feruloylquinic acid	160	10
2.	Cinnamic acid	150	30
3.	Feruloylquinic acid	366	5
4.	Chlorogenic acid	353	7
5.	3-Sinapoylquinic acid	397	4
6.	Ferulic acid	193	15
7.	Rosmarinic acid	359	20
Hydroxybenzoic acids			
1.	Ellagic acid	229	100
2.	Galloyl glucose	332	100
Lignans			
1.	Episesamin	354	32
2.	Schisandrin C	384	5
3.	Todolactol A	375	20
4.	Matairesino	357	12
Carotenoids			
1.	α-Carotene	457	67
Stilbenes			
1.	Resveratrol 3-O-glucoside	390	18
Omega-3-fatty acid			
1.	Linolenic acid	277	5
Fatty amide			
1.	Docosanamide	338	7
Ascorbic acid			
1.	Ascorbic acid	177	15

samples exhibited statistically significant variations. The judges found the biscuit supplemented with up to 5% peel powder tasted better than the control product without peel, which received an overall acceptance score of 8.01 out of 9 (Table 4).

B_1 received 8.14, B_2 scored 7.62 and B_3 received 6.90 out of 9 for total acceptance in a rating session where the samples were given scores for each of the evoked aspects such as appearance, scent, texture, taste and overall acceptability. B_1 was better not only in taste, but addition of peel gave a natural golden flavour and a lovely scent to the biscuits. However, adding more than 10% resulted in an unpleasant taste. Thus, 15% peel incorporated biscuits (B_3) scored less among all the formulations. It was discovered that B_1 and B_2 were even better than the control in terms of appearance; however, in terms of flavour, B_1 was better but B_2 was lacking than the control, and the texture of the control was better than all of the formulations. In terms of aroma, B_1 was superior, while B_2 and B_3 were comparable. Except for aroma, formulation B_3 lacked all qualities. The pungent odour and bitter taste grew as the percentage of peel increased. Judges also expressed a wish to purchase such a food product if it was commercially available. Table 5 shows the score for individual attribute.

Table 4. The overall acceptability score of biscuits

Formulations	Overall acceptability score
B_0	8.01±0.05
B_1	8.14±0.12
B_2	7.62±0.10
B_3	6.90±0.06

Table 5. Sensory score for individual attribute

Parameters	Appearance	Taste	Texture	Aroma
B_0	7.91±0.01	8.20±0.01	8.34±0.02	7.60±0.01
B_1	8.19±0.03	8.42±0.04	7.97±0.02	7.98±0.03
B_2	8.01±0.04	7.89±0.04	7.00±0.01	7.59±0.01
B_3	6.98±0.02	6.16±0.02	6.81±0.01	7.76±0.01

Table 6. Nutritional profile of control and supplemented product (g/100 g)

Parameters	Moisture (g)	Ash (g)	Protein (g)	Fat (g)	Total dietary fibre (g)	Carbohydrates (g)	Energy (Kcal)
Control	3.12±0.003	0.42±0.007	11.57±0.001	22.13±0.004	1.34±0.009	62.85±0.002	442±0.765
B_1 (5% kinnow peel)	3.32±0.001	0.99±0.003	12.31±0.004	23.09±0.002	1.72±0.010	61.13±0.006	469±0.202

The biscuits developed in B_1 were yellow in colour, had a pleasant scent, and had higher protein, ash and fiber content (Table 6). In the biscuits, carbohydrates were reduced, while fat and moisture were increased. Calories are the units of energy released by your body when it ferments and intakes food. The higher the calorie content of a food, the more energy it can supply to your body. The energy content of fortified biscuits was higher than that of the control product.

CONCLUSION

To get the maximum value out of byproducts, valuable bioactive phytochemicals must be extracted and evaluated. Advanced technology-based extractions drastically reduced the amount of solvent used and the extraction time to get the most out of these wastes in terms of social, environmental and financial benefits. According to our study, the kinnow peel was a good source of vital elements and phytochemicals. Thus, peel supplemented biscuits were beneficial for consumer consumption to improve flavour and appeal while also increasing nutritional levels. The peel incorporated food products can help to lower the risk of severe disorders and improve biological activities. Lastly, the peel is extremely nutritious and has healing properties.

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