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INNOVATIVE MANDARIN PEEL EFFERVESCENT TABLET as Antioxidant and Anticarcinogen Food Supplement: Bioactive Flavanones and Phenolic Acids By HPLC-DAD and LC-ESI-QTOFF-Mass Spectrometry

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Abstract

Recently, the utilization of the potential bioactive phenolics has been the focus of attention owing to their consumption imparts health benefits including various cancer types, reduced risk of coronary heart diseases. Dietary supplements, food tablets, capsules and fortified foods based on food by-product may be alternative for healthy public nutrition. In this research content, the preliminary data was obtained; the detailed chemical

properties of innovative mandarin peel effervescent tablet was determined. Bioactive flavanones and phenolic acids of mandarin peel tablet by HPLC-DAD and LC-ESI-QTOFF-Mass Spectrometry has been put forwarded.

Keywords: Mandarin, effervescent, tablet, bioactive, phenolic, antioxidant, food supplement

Introduction

The citrus production is estimated at 80 million tones per year, making it an important source for useful to human health components. The main waste of the citrus fruits after processing is the citrus peel (Tokusoglu,2018).

Citrus by-products are a good source of phenolic compounds, especially the characteristic flavanone glycosides which mainly include naringin, hesperidin, narirutin, and neohesperidin. Their extraction from citrus peels has attracted considerable scientific interest to use them as natural antioxidants mainly in foods to prevent the rancidity and oxidation of lipids (Tokusoglu,2018; Anagnostopoulou et.al.,2006; Peschel et.al.,2006). Flavonoids, a group of polyphenols, possess potent cardioprotective efficacy and significantly reduce the risk of cardiovascular disease (Bast et al., 2007; Du et al., 2007). Some research groups

have reported that flavonoids exhibit protective effects against cardiomyopathy and cardiomyocyte apoptosis induced by doxorubicin (Bagchi et al., 2003). It is investigated that the effects of naringenin-7-O-glucoside on cardiomyocyte apoptosis induced by doxorubicin has been reported. The results demonstrated that naringenin-7-O-glucoside was able to attenuate doxorubicin-induced H9C2 cell apoptosis, having an effect comparable to that of quercetin.

Recently, the utilization of the potential bioactive phenolics has been the focus of attention owing to their consumption imparts health benefits including various cancer types, reduced risk of coronary heart diseases. Dietary supplements, food tablets, capsules and fortified foods based on food by-product may be alternative for healthy public nutrition.

The aim of this research paper is to study the extraction of polyphenolic compounds from Seferihisar mandarin peels, to determine the phenolic profiles by mass spectrometric analysis, and to determine the compositional structure and antioxidant activity of mandarin peel based innovative effervescent tablet.

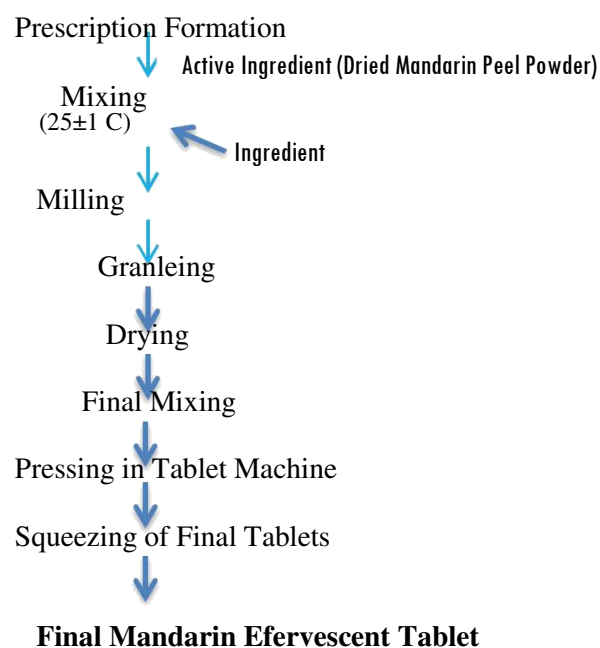
Material and Methods

Plant materials

Fresh mandarins, (*Citrus reticulata* Seferihisar cv.) were harvested at 2018 for two further harvestings in January and late February 2018, as total of 3 replicates from the same area in Seferihisar district, Izmir Province, Turkey. The mandarin peels were cleaned with tap water and cut into 1 cm² pieces which were dried by geothermal system in Seferihisar Doğanbey (as hot air tunnel at 50 °C until reaching a moisture content of 5%). In geothermal drying, peels has been submitted to drying with warm air at relatively low temperature (35 to 75 C). The dried peels were ground with a blade mixer, sieved through a 200 µm sieve and the powder kept in an amber glass bottle at -18 °C until utilized.

Tablet Formation

Mandarin efervescent tablets were man



The manufacturing strategies were applied according to Tokusoglu (2017).

Total phenolic content

The total phenolic content of the freeze dried mandarin peel was determined according to the Folin-Ciocalteu colorimetric method (Anagnostopoulou et al.,2006). The total phenolic content was expressed as mg gallic acid equivalent/100 g dried weight (DW).

Quality Analysis

Basic component analyses (total moisture, total protein, total ash, total fat, total sugar and invert sugar, total fiber were done according to TSE Standard methods (TSE,2018). Total fiber, acidity, pH and mineral analysis [calcium (Ca), potassium (K), magnesium (Mg), aluminium (Al), phosphorous (P)] and vitamin C (ascorbic acid) were done according to AOAC international methods (AOAC,1999). DPPH antioxidant activity was done by Tokusoglu and Yildirim (2012). LC-ESI-QTOFF-Mass Spectrometry was performed by Cai et.al. (2005).

Extraction Methodology for HPLC analysis of Naringenin and Phenolic Acids

2 g of mandarin peel tablet sample was weighted and added 20 ml of H₂O and homogenized with ultra turrax (Ika T25) during 5 min, agitated at 180 rpm for 30

min, then centrifuged at 4000 rpm for 8 min; the supernatant was taken and filtered with 0.45 PTFE syringe, injected to HPLC.

HPLC Conditions of Naringenin

HPLC Apparatus : Agilent 1260 infinity HPLC system with quat pump vacuum degas unit (with Autosampler G1329B, with column oven G1316A, with Diode Array Detector (DAD) G4212B, with Agilent lab advisor chemstation software program)

Detector : DAD-Diode Array Detector

Column : Agilent C18 ODS column, 250 x 4,6 mm 5µm

Column Temperature : 30 °C

Elution Duration : 35 min

Wavelength : 289 nm

Injection volume : 30 µl

Flow Rate : 1 ml/min.

Elution : Gradient

Mobile Phase : A: Distilled Water + Formic acid (99.9 : 0.1)

B: % 95 Acetonitril

Naringenin HPLC Analysis Elution Profile

Duration (Min)	A%	B%
0	75	25
25	55	45
30	55	45
35	75	25

HPLC Conditions of Phenolic Acids

HPLC Apparatus : Agilent 1260 infinity HPLC system with quat pump vacuum degas unit (with Autosampler G1329B, with column oven G1316A, with Diode Array Detector (DAD) G4212B, with Agilent lab advisor chemstation software program)

Detector : DAD-Diode Array
 Detector :
 Column : Agilent C18 ODS
 column, 250 x 4,6 mm 5µm
 Column Temperature : 40 °C
 Elution Duration : 70 min
 Wavelength : 280 nm and 320 nm
 Injection volume : 10 µl
 Flow Rate : 0.8 ml/min
 Elution : Gradient
 Mobile Phase : A: Distilled Water
 + Formic acid (99.8 : 0.2)
 B: Methanole

Phenolic Acid HPLC Analysis Elution Profile

Duration (Min)	A%	B%
0	100	0
3	95	5
18	80	20
20	80	20
30	75	25
40	70	30
50	60	40
55	50	50
70	0	100

Results and Discussion

The preliminary data was obtained from mandarin peel effervescent tablet. We aimed to obtain potential healthy components from Seferihisar mandarin peel and Seferihisar mandarin peel based food tablet and also we identified in detail as quantitatively by HPLC-DAD and LC-ESI-QTOFF-Mass Spectrometry.

In mandarin peel tablet, subsequent to fundamental chemical analysis (moisture, protein, ash, fat as

3.44%;5.09%; 29.65%; 0.40%, respectively whereas dried mandarin peel powder includes moisture, protein, ash, fat as 5.24%;4.55%; 3.41%; 0.00% ,respectively. In our mandarin peel tablet; sucrose, invert sugar and total sugar was found as 10.97%; 8.30%,; 11.54%,respectively whereas dried peel powder contained 17.71%; 10.02; 18.64% of level for mentioned sugars . Total fiber, acidity (as citric acid equivalent), pH of mandarin peel tablet was found as 3.03%, 2.74%, 5.96, respectively whereas in dried peel powder, 9.24%, 1.06% and 5.52, respectively ($p < 0.05$). It was found that calcium (Ca), potasium (K), magnesium (Mg), aluminium (Al), phophorous (P) (mg/kg) of efervescent tablet was 4616.0; 2988.4; 417.2; 4.0; 367 mg/kg,respectively whereas 21916.9; 10204.0; 3459.6; 9.7; 572 mg/kg level was determined in dried mandarin peel powder, respectively. Potassium and magnesium were major minerals in innovative tablet ($p < 0.05$).

Vitamin C (ascorbic acid) was determined as 89.3 mg/100 g in mandarin peel effervescent tablet while 216.4 mg/100 g in dried peel powder. The avg.141.22 mg gallic acid equivalent phenolics [mg gallic acid equivalent (GAE) phenolic /100g] in mandarin peel effervescent tablet whereas avg.128.15 mg GAE /100 g in dried peel powder of

Seferihisar mandarin ($p < 0.05$). DPPH antioxidant activity (%) was found as 27.10% in innovative efferescent tablet and it was found 26.56% was in dried mandarin peel powder ($p < 0.05$).

Majorly L-ascorbic acid, citric acid, malic acid, succinic acid, galactaric acid, glucaric acid (*Saccharic acid*), glucaric acid lactone, *p*-salicylic acid as organic acids; (+)-naringenin, hesperedin, naringenin-7-O-glucoside, nobiletin, tangeretin, eupatorin (3',5-dihydroxy-4',6,7-trimethoxyflavone), gallic acid, *p*-coumaric acid, chlorogenic acid, caffeic acid, ferulic acid, quinic acid, rutin, diosmin flavone, casticin (methoxylated flavonol) were determined as phenolics; also sucrose, , trehalose sugars and DL-phenylalanine, D-Tryptophan aminoacids were found by LC-ESI-QTOFF-Mass Spectrometry as qualitative and quantitatively (Figure 1). Major antioxidant phenolic was naringenin in mandarin efferescent tablet ($p < 0.05$).

Scientific evidence shows that manufactured mandarin peel tablet can be used as dietary supplement and is beneficial for overall health and for managing some health conditions. By utilizing of Seferihisar mandarin peel, mandarin peel tablet was produced at DEPART Technopark Spil Innova LLC as industrial health innovative. We revealed the chemical characterization, functional properties, its unique bioactive features and its comprehensive antioxidative,

anticarcinogenic reports of new manufactured mandarin peel efferescent tablet.

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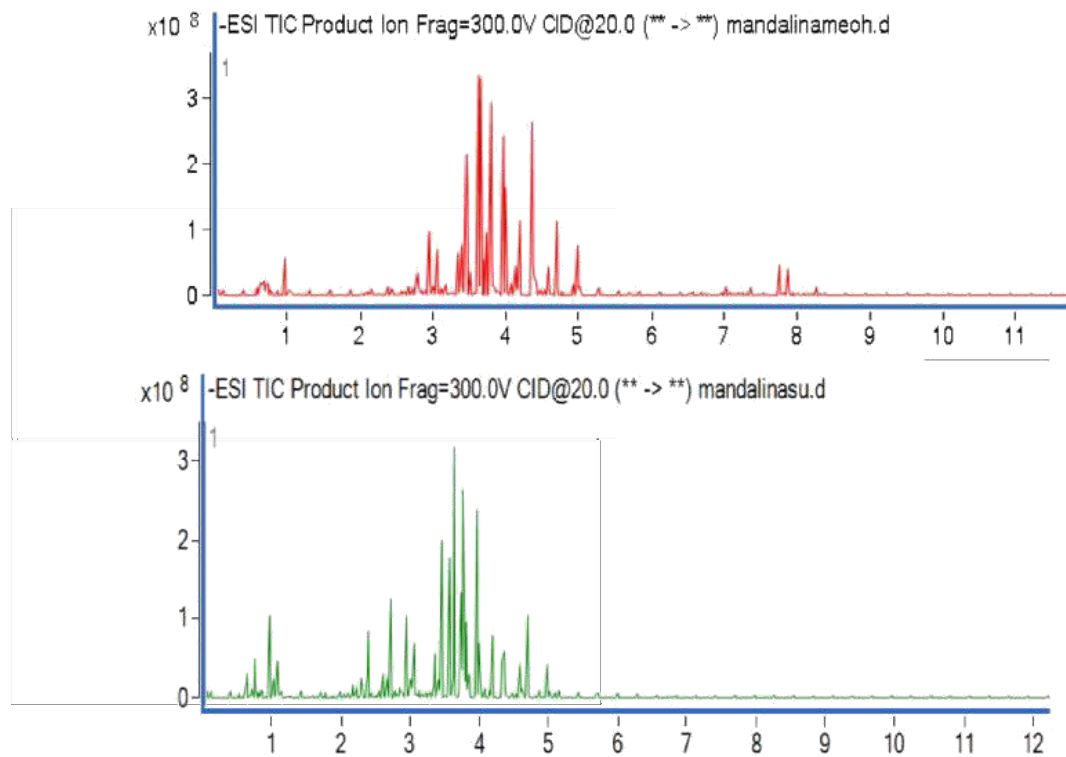


Figure.1.LC-ESI-QTOFF-Mass Spectrometry Analysis Chromatograms of Mandarin Tablets as methanol and water utilizing.