Orange seeds as juice solid waste: microwave and oven roasting, composition, bioactive properties, fatty acid profiles and principal component analysis

Mehmet Musa Özcan[1]✉
Nurhan Uslu[1]
Tijana Banjanin[2]

[1]Department of Food Engineering
Faculty of Agriculture
Selcuk University
Konya, Turkey

[2]Department of Horticulture
Faculty of Agriculture
University of East Sarajevo
East Sarajevo, Bosnia and Herzegovina

One of the important waste materials of the citrus juice industry, its seeds are a potentially valuable resource and are also a cause of major environmental problems. Evaluation of the parts of herbal products other than the edible part is one of the current paramount issues. However, for oilseeds to be used effectively and beneficially in various fields, it is considered useful to determine the bioactive properties, phenolic components, fatty acids and mineral contents of seeds and oils as a result of heat treatment. The oil contents of unroasted and roasted-orange seeds were determined between 42.55 (unroasted) and 45.56% (oven), respectively. While total phenol amounts of the orange seeds are found between 115.79 (control) and 133.89 mg GAE/100g (microwave), total flavonoid contents of orange seeds were recorded between 22.02 (control) and 150.83 mg/100g (oven). Also, antioxidant activities of seeds were measured between 3.42 (control) and 3.87 mmol/kg (microwave). The relationship between the antioxidant activity of the seeds and their bioactive components was linear. In general, an increase was observed in the amount of phenolic compounds in microwave and oven-roasted orange seeds compared to the control (except for catechin, rutin and quercetin). Gallic acid and 3,4-dihydroxybenzoic acid contents of orange seeds were identified between 5.33 (control) and 45.92 (oven) to 10.01 (control) and 15.14 mg/100g (oven), respectively. While oleic acid contents of the oils obtained from unroasted and roasted orange seeds are identified between 24.44% (microwave) and 24.81% (oven), linoleic acid results of oils were detected between 39.00% (oven) and 39.23% (microwave). The amount of fatty acids of orange seed oils fluctuated depending on the type of roasting and statistically significant differences were monitored between the amounts of fatty acids ($p<0.05$). K, Cu, Ni, Zn and B contents of orange seeds roasted in microwave were higher than those roasted in control and oven. In addition, P, K, Na and Ni contents of oven-roasted orange seeds were found to be higher when compared to the control.

Keywords: orange seed, waste, roasting, bioactive compounds, antioxidant activity, polyphenols, fatty acids, elements, HPLC, GC, ICP-AES

✉ CORRESPONDING AUTHOR:
M.M.Özcan,
email: mozcan@selcuk.edu.tr

Received: May 17, 2023
Accepted: July 14, 2023

1. INTRODUCTION

The agricultural industry creates a serious waste problem in the food and agriculture field as wastes such as seeds, fruit and peels, roots, bark, and leaves, which are generated as waste from fruit and vegetable processing, are mostly discarded [7]. Citrus seeds waste is a potentially valuable resource, and also cause major environmental problems [1-3]. Significant amounts of citrus seeds emerge as waste in citrus processing plants, making it difficult to dispose of them [1,2,4,5]. Solid waste, which is one of the most important environmental problems of today, is left randomly to the environment and its type is increasing day by day. Evaluation of the parts other than the edible part of plant products is one of the important issues of today [5,6]. Many physical and chemical properties of some seed and plant oils

consumed as edibles are close to one another. But, it is considered that it may be beneficial to determine the minor components of the oil extracted from the seeds in order to use oilseeds effectively and beneficially in various fields [1,13,19-21]. Most common fats or oils are a mixture of very small amounts of lipid components and triglycerides. Fatty acids in different lipid molecules have different nutritional importance. The fatty acid profile of citrus seed oil was found to be similar to that of most edible oils [1,12-15,19,20]. Citrus seed is a waste product that can be used after being separated from the shell and pulp [14]. The most important citrus fruits are orange, mandarin, lemon and goldentop fruits, which are used especially in fruit juice production, as well as citrus and citrange species, which are important rootstocks of these species. One of the important waste materials of the citrus juice industry is the seeds. The use of wastes produced in large quantities from fruits daily by the agricultural industry is limited to the animal feed industry or these wastes thrown into the environment cause significant environmental damage. Studies have focused on the determination of bioactive properties, nutritional values, vitamins, minerals, and other beneficial components of such wastes [22, 23]. Recently, the focus has been on the evaluation of waste materials and by-products from food processing. This allows for highly available resources and ultimately the production of a variety of new foods. It is getting more and more difficult to solve the problems arising from industrial wastes. Therefore, more efforts are needed to develop by-products and wastes with nutritional and industrial potential [14]. Apart from the edible part of citrus fruits, the waste part, which is mostly composed of peels and seeds, is used in the treatment of various diseases among the public [8]. Fruit and vegetable peels are rich in bioactive components such as polyphenols and carotenoids, which are called phytochemicals and have various positive effects on health [9,10]. Plant seeds are usually the source of essential oils for nutritional and industrial fields [12]. Today, the waste of one factory can be the raw material of another industry. Therefore, due to the nutritional properties of by-products such as shells and seeds, which are produced as waste in food processing industries, they can be a valuable source of by-products [14,16-18]. Phenolic substances are important for human health due to its effects on taste and odour formation, participation in colour formation and change, antimicrobial and antioxidative effects. Phenolic compounds have positive effects on nutritional physiology [11]. In order to evaluate waste materials and by-products such as orange seeds from food processing, it is necessary to increase the shelf life by removing the water in the seeds, to add flavour and aroma to the seeds, and to destroy some harmful substances in its composition with the effect of heat. As a result of these processes, the changes in the phytochemical contents of the orange seeds of the microwave and oven processes were observed. Therefore, it is necessary to develop by-products and wastes with nutritional and industrial potential and to allow them to be used as food supplements. The disposal of by-products in food processing has become one of the most common problems in the industry [16,17]. Therefore, investigating the use of citrus seeds in human and/or animal diets has become an important issue. There are many studies that determine the bioactive components in the seeds of citrus fruits, which are grown at a significant level in Turkey, reveal their comparative advantages, and reveal the effect of different heat treatments on the determination of the amounts of phenolic compounds with antioxidant properties. The aim of this study: 1- determination of the physico-chemical properties, total phenol, total flavonoid amounts, antioxidant capacity, phenolic and fatty acids of orange seed; 2- the effect of roasting in microwave and oven was compared on the bioactive properties and phytochemicals of seeds.

2. MATERIAL AND METHODS

2.1. MATERIAL

Orange (*Citrus sinensis* L.) fruits (50 kg) obtained from Mersin (Büyükeceli-Gülnar) in Turkey in 2021 were used. The seeds obtained from the orange fruit were cleaned with tap water and dried in atmospheric weather conditions. The seeds were dried by arranging them in a single layer on a cloth. The seeds were mixed at regular intervals during the drying process. The dried seeds were powdered in a laboratory mill and stored in hermetically sealed coloured glass jar at 4°C until analysis.

2.2 METHODS

2.2.1 Heat treatment

Ground orange seeds were subjected to a heat treatment prior to analysis (except Control). The orange seeds were heated in an oven at 120°C for 50 min; in a microwave at 900W for 7 min.

2.2.2 Moisture content

The moisture contents of the orange seeds were determined by the KERN & SOHN GmbH infrared moisture analyser [24].

2.2.3. Oil content

After grinding the roasted and unroasted orange seeds, 10 g were weighed into the Soxhlet cartridge and placed in the Soxhlet apparatus. Petroleum ether was used for oil extraction. The extraction process continued for 5 hours at 50°C. This period was completed, the petroleum ether was evaporated in the evaporator. After oil extraction, possible particles were removed by filtering, the crude oil content (%) was determined. [24].

2.2.4 Extraction procedure

Extraction was made according to the method determined by Garcia-Salas et al [25]. 2 g samples were taken from the grinded orange seeds, mixed with 10 ml of methanol, and the mixture was stirred by vortex for 1 minute. Then, after the solution was sonicated for 30 min, it was centrifuged at 4500 rpm for 10 min. The resulting supernatants were collected and concentrated at 37°C. After the volume of the extracts was made up to 10 ml, they were filtered. All analyses were performed in 3 replications.

2.2.5 Total phenolic content

The total phenolic results of the orange seeds were recorded by the Folin-Ciocalteu reagent according to study stated by Yoo et al. [26]. FC (1 ml) and Na_2CO_3 (10 ml) were added to extract and mixed with vortex. The deionised water was added until the final volume was 25 ml and kept in the dark for 1 h. The absorbance was measured at 750 nm in a spectrophotometer. A calibration curve was prepared with gallic acid (0-200 mg/ml) as the standard. The results are shown as mg gallic acid equivalent (GAE)/100 g.

2.2.6 Total flavonoid content

The orange seed extract (1 mL) was mixed with 0.3 ml of $NaNO_2$, 0.3 ml of $AlCl_3$ and 2 ml of NaOH, respectively, and kept in the dark for 15 min. The absorbance of mixture was measured at 510 nm using spectrophotometer. The results are given as mg quercetin (QE)/100g [27].

2.2.7 Antioxidant activity

1.1-diphenyl-2-picrylhydrazyl (DPPH) was used for the antioxidant capacity of orange seed extracts [28]. The extract was added to 2 ml of a methanolic solution of DPPH, followed by vortexed and kept in the dark for 30 min. The absorbance was read at 517 nm. The results obtained was stated as mmol trolox (TE)/kg.

2.2.8 Determination of phenolic compounds

HPLC (Shimadzu) equipped with a PDA detector and an Inertsil ODS-3 (5 µm; 4.6 x 250 mm) column was used for chromatographic separation of phenolic compounds of seed extracts. The mobile phase was a mixture of 0.05% acetic acid in water (A) and acetonitrile (B) with the flow rate of 1 ml/min at 30 °C. The injection volume was 20 µl. The peaks were taken at 280 using a PDA detector. The elution programme was employed: 0-0.10 min 8% B; 0.10-2 min 10% B; 2-27 min 30% B; 27-37 min 56% B; 37-37.10 min 8% B; 37.10-45 min 8% B. The total running time per sample was 60 min.

2.2.9 Fatty acid composition

The orange seed oil was esterified according to the ISO-5509 (1978) method. Fatty acid methyl esters of samples were analysed using gas chromatography (Shimadzu GC-2010) equipped with a flame-ionisation detector (FID) and capillary column (Tecnocroma TR-CN100, 60 m x 0.25 mm, film thickness: 0.20 µm). The temperature of the injection block and detector was 260ºC. The mobile phase was nitrogen with 1.51 ml/min flow rate. The total flow rate was 80 ml/min and split rate was also 1/40. The column temperature was programmed 120ºC for 5 minutes and increased 240ºC at 4ºC/min and held 25 minutes at 240ºC.

2.2.10 Determination of mineral

After orange seeds were dried at 70ºC until reaching a constant weight, they were ground in a laboratory-type mill. About 0.5 g of ground seeds was burned by using 5 ml of 65% HNO_3 and 2 ml of 35% H_2O_2 in a microwave system. A 40-cell microwave was used to ensure the reliability of the analysis. After the volumes of the dissolved samples were made up to 20 ml with deionised water, the element concentrations in the samples were analysed by Inductively coupled plasma optical emission spectrometry (ICP-AES; Varian-Vista Model) equipment [30].

Working conditions of ICP-AES:

Instrument: ICP-AES (Varian-Vista)
RF Power: 0.7-1.5 kw (1.2-1.3 kw for Axial)
Plasma gas flow rate (Ar): 10.5-15 L/min. (radial) 15 " (axial)
Auxiliary gas flow rate (Ar): 1.5 "
Viewing height: 5-12 mm
Copy and reading time: 1-5 s (max.60 s)
Copy time: 3 s (max. 100 s)

2.3 Statistical Analyses

After averaging the triple analysis data for all treatments, the mean values were exposed to analysis of variance. Significant differences between raw (control), roasted kernels and roasting type results were calculated by Duncan's Multiple Range test ($p<0.05$).

3. RESULTS AND DISCUSSION

3.1 BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY OF ORANGE SEEDS

The results of bioactive compounds and antioxidant activities of orange seeds roasted in the oven and microwave are shown in Table 1. Results obtained with the bioactive compounds and antioxidant activity values of orange seeds exhibited some changes depending on roasting types. The moisture and oil results of unroasted (control) and roasted-orange seeds were calculated between 2.93% (oven) and 7.66% (control) to 42.55% (control) and 45.56% (oven), respectively. While the total phenolic results of the orange seeds change between 115.79 (control) and 133.89 mg GAE/100g (microwave), total flavonoid results of unroasted and roasted-orange seeds were determined between 122.02 (control) and 150.83 mg/100g (oven). Antioxidant activities of seeds were

Table I - Some chemical and bioactive compounds of orange seeds roasted in microwave and oven

Process	Moisture content (%)	Oil content (%)	Total phenolic content (mg/100 g)	Total flavonoid content (mg/100 g)	Antioxidant activity (mmol/kg)
Control	7.66 ± 0.63a*	42.55 ± 2.55c	115.79 ± 2.65c	122.02 ± 4.68c	3.42 ± 0.02c
Microwave	3.68 ± 0.14b	43.47 ± 0.10b	133.89 ± 2.14a	143.45 ± 4.75b	3.87 ± 0.00a
Oven	2.93 ± 0.33c	45.56 ± 0.73a	121.03 ± 8.95b	150.83 ± 3.79a	3.56 ± 0.14b

* values within each column followed by different letters are significantly different at P < 0.05.

Table II - Phenolic compounds of orange seeds roasted in microwave and oven

Phenolic compounds (mg/100 g)	Control	Microwave oven	Oven
Gallic acid	5.33 ± 0.45c*	38.18 ± 2.79b	45.92 ± 4.78a
3,4-Dihydroxybenzoic acid	10.01 ± 2.49c	11.47 ± 1.31b	15.14 ± 0.12a
Catechin	18.73 ± 0.61b	17.96 ± 4.23c	25.48 ± 4.67a
Caffeic acid	2.08 ± 0.67c	4.18 ± 0.60a	3.79 ± 1.16b
Syringic acid	3.37 ± 1.20c	4.28 ± 0.22ab	4.37 ± 0.57a
Rutin	10.15 ± 0.08b	10.40 ± 0.71a	9.83 ± 3.33c
p-Coumaric acid	1.60 ± 0.42c	3.59 ± 0.78b	3.86 ± 0.65a
Ferulic acid	2.13 ± 0.59bc	3.74 ± 0.95a	2.78 ± 0.92b
Resveratrol	0.75 ± 0.10c	1.32 ± 0.38a	1.27 ± 0.25b
Quercetin	4.02 ± 1.98b	6.23 ± 1.40a	3.06 ± 0.82c
Cinnamic acid	0.73 ± 0.29a	0.53 ± 0.07b	0.45 ± 0.17c
Kaempferol	0.92 ± 0.51c	2.40 ± 0.71a	1.76 ± 0.55b

* values within each row followed by different letters are significantly different at P < 0.05.

measured between 3.42 (control) and 3.87 mmol/kg (microwave). The moisture amount of orange seeds decreased with microwave and oven roasting, while the oil content of seeds partially increased. This increase is probably due to the evaporation of water in the seed during roasting and the increase in dry matter content. In addition, bioactive compounds and antioxidant activities of orange seeds increased with the roasting process compared to the control. The highest total phenol and antioxidant activities were established in orange seeds roasted in the microwave. A linear relationship between the antioxidant activity results of the seeds and their bioactive components was monitored. Statistically significant changes among the chemical properties and antioxidant activity results of orange seeds were monitored depending on the type of roasting ($p<0.05$). Microwave and oven roasting increased both the bioactive component amounts and antioxidant activity values of orange seeds. The oil results of orange and tangerine seeds were reported as 17.01% and 15.87%, respectively [14]. In another study, the oil results of citrus seeds obtained from Turkey and Vietnam were determined to be 45.1-58.8% and 32.1-54.8%, respectively [31]. The oil content of citrus seeds was determined between 34.92 and 41.66% [32]. The total phenolic content of oil extracted from orange seeds was 1152.88 mg GAE/kg [32]. The oil contents of citrus seeds growing in Egypt changed between 40.2 and 45.5% [13]. The rate of oil result of citrus seeds varied between 33.4% and 41.9% [33]. The oil results of mandarin and bitter orange seeds were recorded as 27.61% and 36.42%, respectively [34]. The total phenolic contents of orange seed oils were 1152.88 mg GAE/kg [32]. Moulehi et al. [35] reported that the total polyphenol result of Mandarin (*Citrus reticulata*) seed was established between 0.68 and 2.11 mg GAE/g DW. The total phenolic results of orange seed was determined between 10.9 - 39.4 mg GAE/g (DW) [36]. While total phenolic results of *Citrus* seeds change between 411.43 (lemon) and 814.84 mg GAE/100 g (bitter orange), total flavonoid results of *Citrus* seeds were monitored between 97.84 (grapefruit) and 126.48 mg/100 g (lemon), respectively [34]. Antioxidant activity of orange fruit seed was determined as 94.10 µmol Trolox/100 g [37]. Also, Özcan et al. [34] determined between 53.27 (mandarin) and 74.21% (lemon) antioxidant activity in *Citrus* seeds. Kumar and Sharmai [38] measured between 94.87 (*C.limetta*) and 97.82 µmol/g (*C.sinensis*) antioxidant activities in few Citrus seeds. The results we obtained regarding the bioactive components of orange seeds showed some changes when compared to the values of previous works. These differences could be probably attributed to harvest time, ripeness, growing conditions, and climatic factors.

3.2 PHENOLIC COMPOUNDS OF ORANGE SEEDS

The quantitative results of phenolic constituents of unroasted and roasted orange seeds are given in Table 2. The predominant phenolics of unroasted and roasted orange seeds were gallic acid and 3,4-dihydroxybenzoic acid (Fig.1). The phenolic constituent results of the seeds differed according to the roasting type compared to the control. Gallic acid and 3,4-dihydroxybenzoic acid contents of orange seeds were established between 5.33 (control) and 45.92 mg/100g (oven) to 10.01 (control) and 15.14 mg/100g (oven), respectively. Also, while catechin results of orange seeds vary between 17.96 (microwave) and 25.48 mg/100g (oven), syringic acid results of seed samples were monitored between 3.37 (control) and 4.37 mg/100g (oven). Rutin and quercetin results of

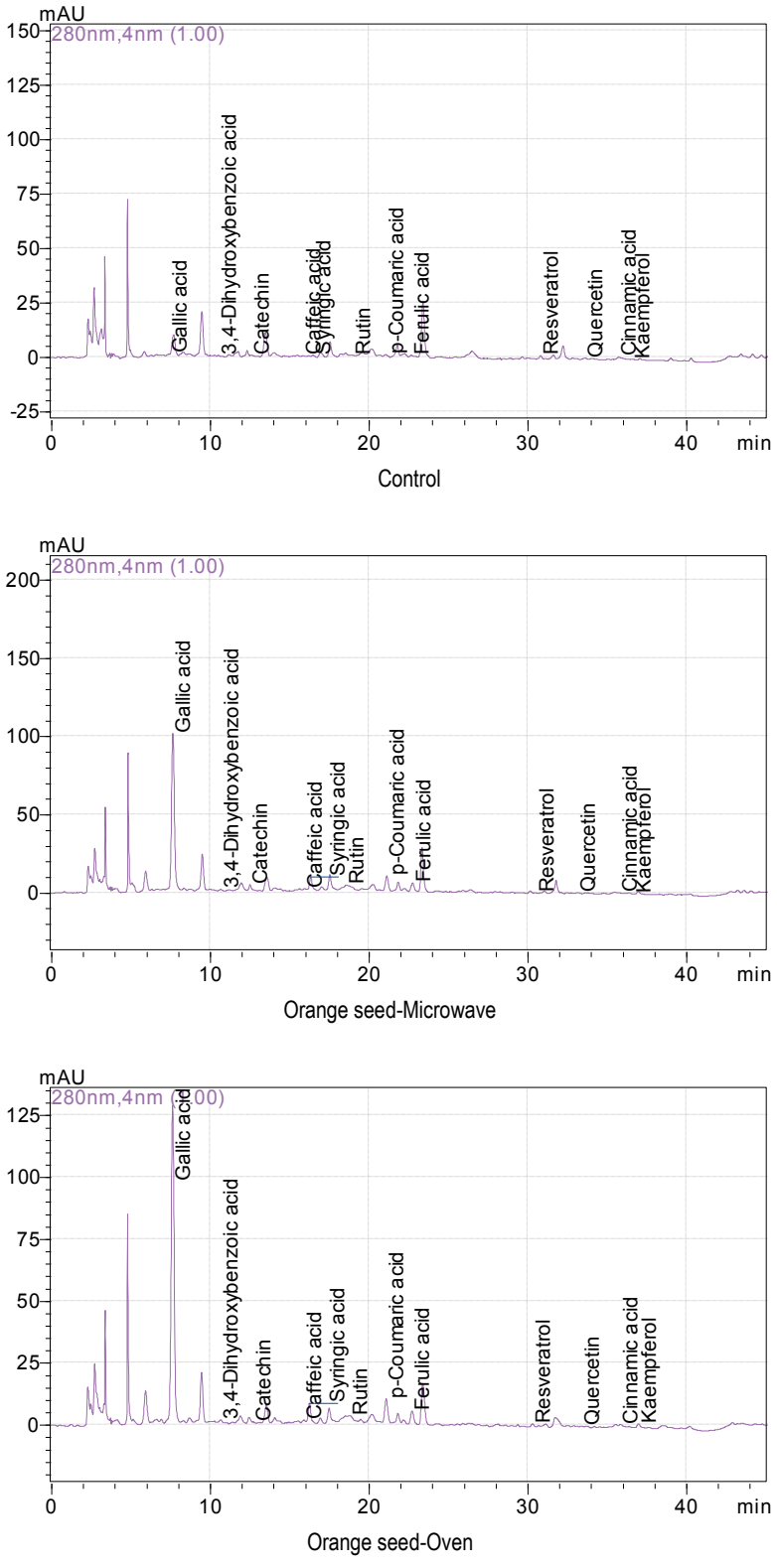

Figure 1 - Phenolic chromatograms of orange seeds

unroasted and roasted orange seeds were recorded between 9.83 (oven) and 10.15 mg/100g (control) to 3.06 (oven) and 6.23 mg/100g (microwave), respectively. Also, while caffeic acid results of seeds are monitored between 2.08 (control) and 4.18 mg/100g (microwave), ferulic acid results of the orange seeds were established between 2.13 (control) and 3.74 mg/100g (microwave). *p*-Coumaric acid values of the seeds changed between 1.60 (control) and 3.86 mg/100g (oven). The highest kaempferol and resveratrol (2.40 mg/100g and 1.32 mg/100g) were identified in orange seed roasted in microwave. In general, an increase was observed in the amount of phenolic constituents in microwave and oven-roasted orange seeds compared to the control (except for catechin, rutin and quercetin). However, the routine and resveratrol contents of microwave roasted seeds were higher when compared to the results of control and oven roasted seeds. The amounts of most phenolic compounds roasted in the microwave were higher than those roasted in the oven. The higher phenolics of roasted orange seeds compared to the control may have been caused by Maillard reaction products during roasting, oil oxidation and caramelisation in the seed. Statistically significant differences were monitored between the results of phenolic compounds depending on the type of roasting ($p<0.05$). Silva and Jorge [37] reported that the predominant phenolic compounds of the citrus fruit seed oils were salicylic acid, quercetin and *p*-coumaric acid. It has been reported that genotype and environmental factors have a significant effect on the phenolic profile of citrus seeds, which are rich in *p*-coumaric, ferulic acid and caffeic acid [35,39]. When the results are compared with the previous studies, although the dominant phenolic component is the same, the amounts may differ. These changes may be due to variety, genetic structure, climatic factors, maturation, and harvest time.

3.3 FATTY ACID PROFILES OF THE OILS EXTRACTED FROM ORANGE SEEDS

The quantitative amounts of fatty acid profiles of the oils obtained from unroasted and roasted orange seeds are illustrated in Table 3. Stearic, palmitic, oleic and linoleic acids are the key fatty acids of orange seed oils (Fig. 2). Palmitic and stearic acid results of the orange seed oils were detected between 26.67% (oven) and 26.84% (unroasted) to 6.02% (unroasted) and 6.04% (oven), respectively. While oleic acid contents of the oils extracted from unroasted and roasted orange seeds are identified between 24.44% (microwave) and 24.81% (oven), linoleic acid results of orange seed oils were monitored between 39.00% (oven) and 39.23% (microwave). Also, linolenic acid results of the orange seed oils varied between 3.04% (control) and 3.17% (microwave). Arachidic acid contents were found below 0.41% in all oil samples. In general, the amount of fatty acids of the unroasted orange seed oils was slightly increased compared to the control. While the results of some fatty acids were different, some were found to be similar. The amount of fatty acids of orange seed oils differed according to the type of roasting, and these differences in fatty acids were found to be statistically significant ($p<0.05$). The seed oils of orange contained 38.26% linoleic, 24.89% oleic, 28.12% palmitic, 4.34% stearic, 2.58% linolenic and 0.55% arachidic acids [14]. Citrus seed oil contained 76.19% linoleic, 13.87% oleic, 6.76% stearic and 2.40% palmitic acids [40]. Park et al. [41] determined that palmitic, stearic, oleic, linoleic and linolenic acid contents of lemon (*Citrus limon*) seed oil changed between 11.68-16.86%, 1.95-3.41%, 11.10-18.65%, 15.51-27.03% and 1.50-5.54%, respectively. The orange seed oil contained 26.42% palmitic, 5.20% stearic, 23.04% oleic, 40.19% linoleic, 3.92% linolenic and 0.38% arachidic acid [32]. Orange seed oil contained 26.2% palmitic, 5.8% stearic, 26.5% oleic, 37.4% linoleic, 3.1% linolenic and 0.4% arachidic acids [37]. Citrus seed oils contained 33.2% to 36.3% linoleic, 24.8% to 29.3% oleic and 23.5% to 29.4% palmitic acids as the key fatty acid found [33]. Oleic and linoleic acid results of some *Citrus* seed oils varied between 21.84% and 27.58% to 33.94% and 38.67%, respectively [34]. Saidani et al. [21] verified that oils extracted from Tunisian citrus seeds are mostly constituted of triacylglycerols that are rich in unsaturated fatty acids. Valencia seed oil contained palmitic acid (47.33%), stearic acid (7.50%), oleic acid (18.40 %) and linoleic acid (l 8.39%) as the major fatty acids and less amount of myristic (1.55%), palmitoleic (5.04%) acids were the minor fatty acids

Table III - Fatty acid composition of the oils extracted from orange seed roasted in microwave and oven

Fatty acids (%)	Control			Microwave oven			Oven		
Palmitic	26.84	±	0.25a*	26.73	±	0.48b	26.67	±	0.08c
Stearic	6.02	±	0.02c	6.03	±	0.07b	6.04	±	0.01a
Oleic	24.67	±	0.09b	24.44	±	0.16c	24.81	±	0.05a
Linoleic	39.03	±	0.13b	39.23	±	0.22a	39.00	±	0.04b
Arachidic	0.40	±	0.00b	0.41	±	0.01a	0.40	±	0.00b
Linolenic	3.04	±	0.01c	3.17	±	0.01a	3.09	±	0.01b

* values within each row followed by different letters are significantly different at $P < 0.05$.

[42]. The fatty acid composition of seed oils can be affected by climatic factors such as location, precipitation and temperature, and harvest time [43]. The results showed some fluctuations depending on the processing conditions. These differences are likely due to agricultural factors, physiological factors, fruit maturity, temperature, other climatic factors and harvest time and agriculture applications.

3.4 ELEMENT CONTENTS OF ORANGE SEEDS ROASTED IN MICROWAVE AND OVEN

The effects of microwave and oven roasting on the element contents of protocol seeds, which are produced as industrial fruit juice production waste, are given in Table 4. Depending on the roasting type, differences were observed in the macro and micro element contents of the orange seeds when compared to the control. P and K amounts of orange seeds were recorded between 2939.01 (microwave) and 3012.91 mg/kg (oven) to 9477.58 (control) and 10265.58 mg/kg (microwave), respectively. In addition, while Ca contents of seed samples are found between 2.49 (Oven) and 2.75 mg/kg (control), Mg amounts of orange seeds were reported between 321.85 (Oven) and 357.68 mg/kg (control). The highest S amount was determined in control seed sample. Although the Fe contents of orange seeds were statistically different ($p<0.05$), the results were close to each other. But, there was no statistically significant difference between the Fe contents of the control and microwave

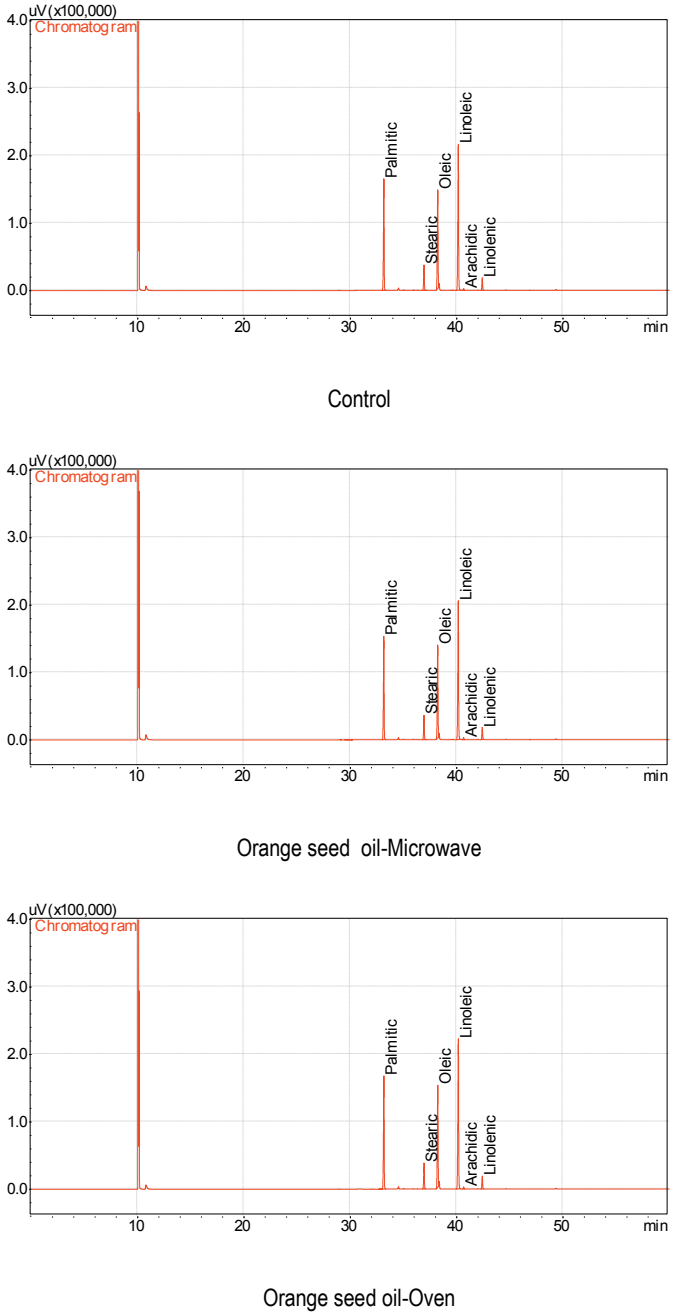

Figure 2 - Fatty acid chromatograms of the oils extracted from orange seeds

Table IV - Mineral contents of orange seeds roasted in microwave and oven (mg/kg)

Samples	P	K	Ca	Mg	S	Na	Fe	Cu	Mn	Ni	Zn	B
Control	2955.46 ±28.16b*	9477.58 ±189.12c	2.75 ±0.01a	357.68 ±0.44a	319.14 ±1.18a	51.22 ±0.20ab	7.75 ±0.30a	2.08 ±0.03b	1.46 ±0.02a	0.20 ±0.02	2.72 ±0.16b	6.54 ±0.09b
Microwave (900 W/7 min)	2939.01 ±36.21c	10265.58 ±839.77a	2.71 ±0.20ab	349.79 ±27.10b	311.75 ±30.34b	52.01 ±4.93c	7.74 ±0.70a	2.35 ±0.09a	1.38 ±0.09b	0.35 ±0.02	2.87 ±0.43a	6.77 ±0.69a
Oven (120°C/50 min)	3012.91 ±30.62a	9863.12 ±1354.47b	2.49 ±0.21c	321.85 ±28.91c	289.59 ±20.19c	52.91 ±7.11a	7.29 ±0.58b	1.96 ±0.09c	1.28 ±0.17c	0.32 ±0.03	2.47 ±0.18c	6.04 ±0.39c

* values within each row followed by different letters are significantly different at $P < 0.05$.

roasted orange seeds. Cu and Zn amounts of orange seeds were measured between 1.96 (Oven) and 1.46 mg/kg (control) to 2.47 (Oven) and 2.87 mg/kg (microwave), respectively. As seen in Table 4, K, Cu, Ni, Zn and B contents of seeds roasted in microwave were higher than those roasted in control and oven. In addition, P, K, Na and Ni contents of oven-roasted orange seeds were found to be higher when compared to the control. The probable reason why the element contents of the orange seeds roasted in microwave and oven differ compared to the control may be due to the applied heat treatment parameters (temperature/time) and some analytical conditions. Orange seeds contained 40 mg/100g P, 45 mg/100g Ca, 66 mg/100g K and 18 mg/100g Na [13]. Orange seed cake contained 0.006 ppm Ca, 1.53 ppm Mg, 0.02 ppm Na, 7.33 ppm K, 0.02 ppm Fe and 0.01 ppm Cu [44] [45] reported that The seeds of "late Valencia" and "Blood orange" orange varieties contained 22.80 and 25.20 mg/100g K, 13.45 and 11.76 Na, 93.85 and 82.60 Ca, 33.86 and 27.74 P, 0.78 and 0.87 Fe. The Mn, Cu, Ni, Cd, Cr, Zn, Ca, Mg, Na, K contents of the orange seeds were 0.13 mg/100g, 0.27 mg/100g, 0.03 mg/100g, 0.04 mg/100g, 0.15 mg/100g, 0.63 mg/100g, 31.00 mg/100g, 1.02 mg/100g, 55.56mg/100g and 57.50mg/100g [46]. The sweet orange seed contained 132.53 ± 0.2 mg/100 g Ca, 375.23 ± 0.2 mg/100 g P, 85.63 ± 0.2 mg/100 g Mg, 2.53± 0.1mg/100 g Zn, 51.43 ± 0.2 mg/100 g Na and 12.46 ± 0.2 mg/100 g Fe [47]. Özcan and İnan [48] determined 451.60 mg/kg-2739.30 mg/kg Ca, 886.99-1708.00 mg/kg Mg, 2443-3939 mg/kg P, 23.43-35.87 mg/kg Zn in the seeds of several orange varieties. The element contents obtained from orange seeds differed from the results of previous studies. These differences may be due to the orange variety, harvest time, applied heat treatments and types.

3.5 PRINCIPAL CONSTITUENTS ANALYSİS (PCA) OF PHENOLIC COMPOUNDS OF ORANGE SEEDS

The Principal Component Analysis (PCA) was applied to assess the effect of heating on phenolic components of orange seeds, which are given in Fig 3. PC1 explained about 67.166% of variability. PC2 exhibited about 32.834% of variability. PC1 was identified with gallic acid (0.993), caffeic acid (0.972), syringic acid (0.999), *p*-coumaric acid (0.999), resveratrol (0.990) and kaempferol (0.875). Moreover, rutin (0.991) and quercetin (0.987) were the main variables on PC2.

4. CONCLUSION

The results regarding the bioactive compounds, antioxidant activity values, phenolics and fatty acids profiles of orange seed and oils showed some changes depending on the type of roasting. In addition, bioactive compounds, antioxidant activity values of orange seeds increased with the roasting process compared

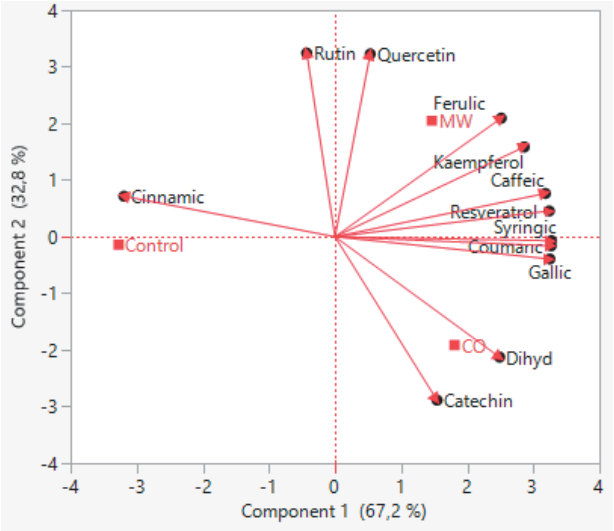

Figure 3 - Biplot graph drawn with results of PCA

to the control. The highest total phenol and antioxidant activities were established in microwave roasted orange seeds. The relationship between the antioxidant capacity of the seeds and their bioactive components was linear. In general, an increase was observed in the amount of phenolic constituents in microwave and oven-roasted orange seeds compared to the control (except for catechin, rutin and quercetin). In general, an increase was observed in the amount of phenolic constituents in microwave and oven-roasted orange seeds compared to the control (except for catechin, rutin and quercetin). However, the routine and resveratrol contents of microwave roasted seeds were higher when compared to the results of the control and oven roasted seeds. The amounts of most phenolic compounds roasted in the microwave were higher than those roasted in the oven. The higher phenolics of roasted orange seeds compared to the control may have been caused by Maillard reaction products during roasting, oil oxidation and caramelisation in the seed. Statistically significant differences were monitored between the results of phenolic compounds depending on the type of roasting ($p<0.05$). The amount of fatty acids of orange seed oils differed according to the type of roasting, and these differences in fatty acids were found to be statistically significant ($p<0.05$). In general, the amount of fatty acids of the unroasted orange seed oils was slightly increased compared to the control. While the amounts of some fatty acids were different, some were found to be similar. The result of fatty acids of orange seed oils fluctuated depending on the type of roasting and statistically significant differences were determined between the results of fatty acids. K, Cu, Ni, Zn and B contents of seeds roasted in microwave were higher than those roasted in control and oven. In addition, P, K, Na and Ni contents of oven-roasted orange seeds were found to be higher when compared to the control.

Acknowledgements We wish to thank the laboratory staff for their technical assistance.

Funding No funding

Competing interests The authors declare no competing interest.

BIBLIOGRAPHY

[1] E.S. Lazos, D.C. Servos. Nutritional and chemical characteristics of orange seed oil. Grasas Aceites, 39, 232-234 (1988).

[2] F.Al-Juhaimi, B. Matthaus, M.M. Özcan, K. Ghafoor, The physico-chemical properties of some citrus seeds and seed oils. Zeits Naturforsch C, 71(3–4), 79-85 (2016).

[3] Ö. İnan, M.M. Özcan, F. Aljuhaimi. Effect of location and Citrus species on total phenolic, antioxidant, and radical scavenging activities of some Citrus seed and oils. Journal of Food Processing and Preservation 42(3), 1-6 (2018).

[4] Ö. Gültekin, M.M. Özcan, F. Aljuhaimi, Some physicochemical properties, fatty acid composition, and tocopherol contents of *Citrus* seed oils**.** Riv. Ital. Sostanze Grasse –XCIII - Gennaio/Marzo:47-51 (2016).

[5] A.Y. Abdel-Rahman, A study on some Egyptian *Citrus* seed oils. Grasas y Aceites 31(5), 331-333 (1980).

[6] E.Alkaya, T.H. Ergüder, G.N. Demirer. Effect of operational parameters on anaerobic co-digestion of dairy cattle manure and agricultural residues: A case study for the Kahramanmaraş region in Turkey. Engineering Life Science 10(6), 552–559 (2010).

[7] I.S. Ashoush, M.G.E. Gadallah. Utilization of mango peels and seed kernels powders as sources of phytochemicals in biscuit. World Journal of Dairy & Food Science 6 (1),35-42 (2011).

[8] G. Oboh, A.O. Ademosun. Characterization of the antioxidant properties of phenolic extracts from some citrus peels. J Food Sci Technol 49(6): 729–736 (2012).

[9] M.M. Özcan,K. Ghafoor, F. AlJuhaim, UN.slu N, Babiker, I.M.A. Ahmed, I.A. Almusallam. Influence of drying techniques on bioactive properties, phenolic compounds and fatty acid compositions of dried lemon and orange peel powders. Journal of Food Science and Technology 58(1),147–158 (2021).

[10] O.S. Omoba, R.O. Obafaye, S.O. Salawu, A.A. Boligon, M.L. Athayde. HPLC-DAD phenolic characterization and antioxidant activities of ripe and unripe sweet orange peels. Antioxidants 4, 498–512 (2015).

[11] M. Burak, Y. Çimen. Flavonoidler ve antioksidan özellikleri. Türkiye Klinikleri Tıp Bilimleri Dergisi 19, 296-304 (1999).

[12] F.S. El-Safy, R.H. Salem, M.E. Abd El-Ghany. Chemical and nutritional evaluation of different seed flours as novel sources of protein. World Journal of Dairy Food Science 7(1),59–65 (2012)
[13] M.A. Habib, M.A. Hammam, A.A. Sakr, Y.A. Ashoush. Chemical evaluation of Egyptian citrus seeds as potential sources of vegetable oils. Journal of American Oil Chemist Society 63, 1192–1196 (1986).
[14] T.A. El-Adawy, E.H. Rahma, A.A. El-Bedawy, A.M. Gafar, Properties of some citrus seeds Part 3, Evaluation as a new source of protein and oil. Nahrung, 43, 385-391 (1999).
[15] M.M. Özcan, Ö. İnan. Physico-chemical properties, fatty acid composition and tocopherol contents of Mandarin, Orange and Lemon Seed Oils. Erwerbs Obstbau 64, 445-453 (2022).
[16] M.I. Akpata, P.I. Akubor, Chemical composition and selected functional properties of sweet orange (*Citrus sinensis*) seed flour. Plant Foods for Hum Nutrition 54, 353-362 (1999).
[17] A.Schieber, F.C. Stintzing, R. Carle. By-products of plant food processing as a source of functional compounds-recent developments. Trends Food Science and Technology 12(11), 401-413 (2001).
[18] F. Anwar, R. Naseer, M.I. Bhanger, S. Ashraf, F.N. Talpur, F.A. Aladedunye, Physico-chemical characteristics of citrus seeds and seed oils from Pakistan. Journal of American Oil Chemist Society 85, 321–330 (2008)..
[19] B.S. Kamel, J.M. Demna, B. Blackman. Nutritional, fatty acid and oil characteristics of different agricultural seeds. Journal of Food and Technology 17, 263-269 (1982).
[20] K. Aitzetmüller, G.L.C. Capillary. Fatty acid fingerprints of seed lipids – A tool in plant chemotaxonomy. Journal of High Resolution Chromatograph16,488-490 (1993).
[21] M. Saidani, W. Dhifi, B.J. Marzouk. Lipid evaluation of some Tunisian citrus seeds. Food Lipids 11:242-250 (2004).
[22] C.O.B. Okoye, C.N. Ibeto, J.N. Ihedioha, Preliminary studies on the characterization of orange seed and pawpaw seed oils. American Journal of Food Technology 6, 422–426 (2011),
[23] N. Babbar, H.S. Oberoi, D.S. Uppal, R.T. Patil, Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues. Food Research International 44, 391-396 (2011).
[24] AOAC, Official Methods and recommended practices (Vol.1, 4th ed.). American Oil Chemists`Society, Champaign, IL (1990).
[25] P. Garcia-Salas, A.M. Gomez-Caravaca, Arraez-Roman D, Segura-Carretero A (2013) Guerra-Hernandez E, Garcia-Villanova B, Fernandez-Gutierrez A. Influence of technological processes on phenolic compounds, organic acids, furanic derivatives, and antioxidant activity of whole-lemon powder. Food Chemistry 141:869-878.
[26] K.M. Yoo, K.W. Lee, J.B. Park, H.J. Lee, I.K. Hwang. Variation in major antioxidants and total antioxidant activity of Yuzu (*Citrusjunos* Siebex-Tanaka) during maturation and between cultivars. Journal of Agriculture and Food Chemistry 52, 5907-5913 (2004).
[27] S. Hogan, L. Zhang, J. Li, B. Zoecklein, K. Zhou. Antioxidant properties and bioactive components of Norton (*Vitis aestivalis*) and Cabernet Franc (*Vitis vinifera*) wine grapes. LWT - Food Science and Technology 42,1269–1274 (2009).
[28] S.K. Lee, Z.H. Mbwambo, H.S. Chung, L. Luyengi, E.J.C. Games, R.G. Mehta. Evaluation of the antioxidant potential of natural products. Combinatorial Chemistry & High Throughput Screening 1, 35-46 (1998).
[29] ISO-International Organization for Standardization, Animal and vegetable fats and oils preperation of methyl esters of fatty acids. Method ISO 5509. ISO, Geneve, pp 1–6 (1978)
[30] S. S. Skujins. Handbook for ICP-AES (Varıan-vista). A short guıde to vista series ICP- AES operation. Varian int. AG Zug.version 1.0., p 29, (1998).
[31] B. Matthaus, M.M. Özcan. Chemical evaluation of citrus seeds, an agro-industrial waste, as a new potential source of vegetable oils. Grasas y Aceites 63: 313–320 (2012).
[32] C.R. Malacrida, M. Kimura, N.Jorge. Phytochemicals and antioxidant activity of *Citrus* seed oils. Food Science and Technology Research 18(3), 399-404 (2012).
[33] M. Reazai, I. Mohammadpourfard,S. Nazmara,M. Jahanbakhsh, L.Shiri. Physico-chemical characteristics of citrus seed oils from kerman, Iran. Journal of Lipids 2014, 1–3 (2014).
[34] M.M. Özcan, Ö.Öztürk, V. Lemiasheuski. Quality properties, fatty acid composition, and mineral contents of some *Citrus* Sseds and oils extracted by solvent extraction. Erwerbs-Obstbau 65,127–132 (2022).
[35] I. Moulehi, S. Bourgou, I. Ourghemmi, M.S. Tounsi. Variety and ripening impact on phenolic composition and antioxidant activity of mandarin (*Citrus reticulate* Blanco) and bitter orange (*Citrus aurantium* L.) seeds extracts. Industrial Crops and Products 39, 74-80 (2012).
[36] A. Molan, M.H. Ismail, R.H. Nsaif. Phenolic contents and antioxidant activity of peels and seeds of orange (*Citrus sinensis*) cultivated in Iraq. World Journal of Pharmacy and Pharmaceutical Science 5(4), 473-482 (2016).
[37] A.C. Silva, N. Jorge. Bioactive properties and antioxidant capacity of oils extracted from citrus fruit seeds. Acta Alimentaria 48 (2), 196–203 (2019).

[38] I. Kumar, R.K. Sharma. Antioxidant properties in methanol extract of kernels of commonly marketed Indian fruits. International Journal of Fruit Science 21, 334–343 (2021).
[39] A. Bocco, M.E. Cuvelier, H. Richard, C. Berset. Antioxidant activity and phenolic composition of citrus peel and seed extracts. Journal of Agriculture and Food Chemistry 46,2123-2129 (1998).
[40] F. Saloua, N.I. Eddine, Z. Hedi. Chemical composition and profile characteristics of Osage orange *Maclura pomifera* (Rafin.) Schneider seed and seed oil. Industrial Crops and Products 29, 1–8 (2009).
[41] Y.S. Park, II.D. Kim, S.K. Dhungana, E.J. Park, J.J. Park, J.H. Kim, D.H. Shin. Quality characteristics and antioxidant potential of lemon (*Citrus limon* Burm. f.) seed oil extracted by different methods. Frontiers Nutrition 8, 1-10 (2021).
[42] S. Mahmud, S. Akbar, A. Sattar. The fatty acid composition of seed oıls of citrus sinensis varietıes: Valencia and Salustiana. Pakistan Journal of Science Ind Research 44(2),93-95 (2000).
[43] M.M. Özcan, Ö. İnan. Physicochemical properties and nutrition element contents of *Citrus* seeds and agroindustrial waste. Erwerbs-Obstbau 65,133–142 (2023)
[44] C.O.B. Okoye, C.N. Ibeto, J.N. Ihedioha. Preliminary studies on the characterization of orange seed and pawpaw seed oils. American Journal of Food Technology 6,422–426 (2011).
[45] J. Adubofuor, Y.G. Akyereko, V. Batsa, O.D. Apeku, I. Amoah, C. Diako, Nutrient Composition and physical Properties of two orange seed varieties. International Journal of Food Science, Article ID 6415620, 11 pages (2021).
[46] D. Uzama, S.C. Okolo, A.B. Adebiyi, R.U. Okoh-Esene, A.T. Orishadipe. The study of phytochemicals, proximate and mineral contents of sweet orange seeds and peels. Uzama, Okolo, Adebiyi, Okoh-Esene and Orishadipe; njcr 26(1), 25-32 (2021).
[47] E.T. Williams, K.Y. Wayas, N. Timothy. Assessment of nutritional composition of Citrus Sinensis (Sweet Orange) Seed in Madagali Admawa Nigeria. IJISET - International Journal of Innovative Science, Engineering & Technology, 8, 43-54 (2021).
[48] M.M. Özcan, Ö. İnan. Physicochemical properties and nutrition element contents of citrus seeds and agroindustrial waste. Erwerbs-Obstbau 65,133–142 (2023)