

Physicochemical and functional properties of the cold press lemon, orange, and grapefruit seed meals

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Abstract

The aim of this study was to evaluate physicochemical and functional properties of the defatted cold press meals of lemon, orange and grapefruit seeds. Proximate composition, viscosity, colour, flavonoid and phenolic acid compositions, and functional properties (water and oil holding capacity, emulsion and foaming properties and least gelling concentration) were determined. It was shown that the meals are high in protein, but low in phytic acid. Their colour is usually light yellow-red, and compatible for most food products. These meals were very good sources of citrus flavonoids (eriocitrin, rutin, naringenin, etc.) and phenolic acids. Hence, they can be used in functional food products or be utilised as source for flavonoid extraction. Furthermore, the meals were very good in water holding capacity, and moderately good in emulsification and foaming properties to be used as food ingredients. Citrus seed press meals could be valorised in food products or in extracting fine chemicals.

Keywords: citrus seed, press meal, composition, flavonoid, functional property

1. Introduction

World food processing is constantly increasing due to the exponentially growing human population. While on the one hand, it is necessary to process foods to make their shelf-life extended, on the other hand each processing yields various by-products and wastes which create environmental challenges as well as huge losses of biomaterials. Hence, food processing by-product and waste utilisation and bio-refinery seem a necessary route for sustainability (Matharu *et al.*, 2016). For example, in the 2010-2011 season, major citrus producing countries produced around 82 million metric tonnes of citrus fruits, and around 30-40% of the product was processed (Pfaltzgraff *et al.*, 2013). Large quantities of by-products and wastes were generated since they comprise around 50% of fresh fruit weights. It has been discussed that citrus processing wastes are abundant, inexpensive and undervalued bio-resources (El-Adawy *et al.*, 1999a,b; Russo *et al.*, 2015).

Among citrus processing by-products, citrus peels and pulps are usually utilised for the recovery of essential oils,

aromas, pectin, dietary fibre, molasses, juice sacks, and other products, while valorisation of the citrus seeds is fairly limited. Literature pointed out that citrus seeds are potentially rich sources of oil, protein, fibre, limonoids and phenolic compounds (Pfaltzgraff *et al.*, 2013; Russo *et al.*, 2015). In one study (El-Adawy *et al.*, 1999a) orange seeds have been shown to contain 17.01% protein, 2.01% non-protein nitrogen, 42.59% oil, 3.17% ash, 22.53% fibre, 14.70% total carbohydrate and 8.70% moisture. In another study (Anwar *et al.*, 2008) lemon, grapefruit, sweet orange, and mandarin seeds were reported to contain 3.90-9.56% protein, 27.0-36.5% oil, 5.0-8.5% fibre, and 4.6-5.6% ash on dry weight basis.

There are fairly limited numbers of studies on the utilisation of citrus seeds. To extract the oil present in the seeds, cold pressing technique was applied, and cold pressed citrus seed oils were analysed (Aydeniz Güneşer and Yılmaz, 2017a,b; Yılmaz, 2017; Yılmaz and Aydeniz Güneşer, 2017). The same studies generated a solid by-product called press meal (or press cake) to be utilised, as well. As stated in those studies, cold pressing was preferred since it was good for

rare seeds and kernels, and yields specialty oils with full aroma and bioactives retained. Likewise, the press meals of cold pressing would be more valuable since the cold pressing process is carried out under milder conditions to avoid any damage to the extracted oil (Ghazani *et al.*, 2014; Grajzer *et al.*, 2015).

Industrial oilseed processing meals are usually utilised as animal feed after heat treatment to inactivate antinutritional factors. Minor quantities of the meals are also processed as flour or grits for food applications, or in preparation of protein concentrates and isolates. There are also some other but limited industrial uses such as production of biodegradable plastics, films, fibres, glues and adhesives, dyes, composts, etc. (Pickard *et al.*, 1996). There is no study reporting the properties and utilisation of citrus seed press meals, with the exception of citrus seed flours (Akpata and Akubor, 1999; El-Adawy *et al.*, 1999b; El-Safy *et al.*, 2012; Lima *et al.*, 2014). El-Adawy *et al.* (1999b) presented the functional properties like water and fat absorption, emulsion and foam capacity and gelation of the seed flours. Proximate composition, mineral content and some functional properties of sweet orange seed flour were also published (Akpata and Akubor, 1999). The centesimal composition of six fruit seeds including orange seed was determined (Lima *et al.*, 2014). Furthermore, phenolic and flavonoid composition of different fruit parts including the seeds of lemon have been reported (Xi *et al.*, 2017).

As for press meals, properties of hazelnut meal (Xu and Hanna, 2011), pumpkin seed meal (Rodriguez-Miranda *et al.*, 2012), cold press poppyseed meal (Yilmaz and Dündar Emir, 2017) and capia pepperseed meal (Yilmaz *et al.*, 2017) have been reported.

The objectives of this study were to determine common physico-chemical properties, flavonoids and phenolic acids compositions, and functional properties of the cold press defatted meals of lemon, orange and grapefruit seeds in order to find possible application and utilisation areas for these materials. While compositional and functional data for some citrus seed flours exist in literature, this study is novel for being the first one for cold press meals as well as the first report on flavonoid compositions of the meals.

2. Materials and methods

Materials

The cold press meals of lemon seed (*Citrus limon* L.), orange seed (*Citrus sinensis* L.) and grapefruit seed (*Citrus paradisi* L.) were used in this study. The seeds were provided by the following fruit juice processing factories: Limkon Food Industry and Trade Inc. (Adana, Turkey), Anadolu Etap Penkon Co. (Mersin, Turkey) and Frigo-Pak Food Co. (Bursa, Turkey). The flavonoid standards eriocitrin

(≥98%), rutin hydrate (≥94%), naringin (≥95%), hesperidin (≥80%), neohesperidin (≥90%), and naringenin (≥98%) were purchased from Sigma Chem. Co. (St. Louis, MO, USA). The phenolic acid standards gallic acid (97%), 2-*trans*-hydroxybenzoic acid (97%), vanillic acid (97%), caffeic acid (≥98%), syringic acid (analytical), *p*-coumaric acid (≥98%), *trans*-ferulic acid (99%), hydroxycinnamic acid (97%) were bought from Sigma-Aldrich and Fluka Chemicals (Sigma Chem Co.). All other chemicals used were of analytical grade and purchased from Merck Co. (Darmstadt, Germany) and Sigma Chem. Co. (St. Louis).

Cold pressing and meal defatting

Each type of the citrus seeds was cold pressed by applying different prior treatment against its control to produce six types of the press meals. Different treatments for each seed were preferred to observe the effects of each treatment type both on oil and meal characteristics (Aydeniz Güneşer and Yilmaz, 2017a,b; Yilmaz, 2017; Yilmaz and Aydeniz Güneşer, 2017). Briefly, control group of the lemon seed was roasted at 150 °C for 30 min in an oven (Inoksan PFE, Bursa, Turkey), before cold pressing. The same procedure was applied for orange and grapefruit seeds as the control groups. The treatment group of lemon seed was oil extraction by hexane. Lemon seeds were dried (at 120 °C for 1 h in an oven) and then finely grinded by using Retch Grindomix GM 300 (Haan, Germany), followed by hexane extraction of oil (1:2.5 = seed:hexane, w/v) at 45 °C in a water bath with a constant stirring at 140 rpm for 12 h. The extraction process was repeated for 3 times. The treatment group of orange seeds was the application of microwave heating at 360 Watt for 30 min in a microwave oven (Beko MD 1505, Istanbul, Turkey), before cold pressing. The treatment group of grapefruit seeds was the enzyme application. The enzyme treatment was done by incubation of the grinded seeds with naringinase (Rham 142) 0.06 U/g seed and hesperidinase (Rham 143) 0.033 U/g seed in 100 mM KH₂PO₄/K₂HPO₄ buffer solution (pH 7.5) at 65 °C for 4 h. All cold presses were accomplished with a lab scale press machine (Koçmaksan ESM 3710, İzmir, Turkey, 12 kg seed/h capacity, single head, 1.5 kW power) in two separate batches with 30 rpm screw rotation speed, 10 mm exit die and max 40 °C exit temperature as operation parameters.

Defatting of the collected cold press meals was carried out by hexane extraction (1:4, w/v, 2 h, 190 rpm stirring at room temperature) 3 times, followed by drying first in a forced air-oven at 60 °C for 1 h, and then under a fume hood for overnight. The defatted press meals were grinded (Retch Grindomix GM 300), placed into zipped bags and kept at -20 °C until further analysis. The cold pressed lemon seed meal (CPLM), solvent extracted lemon seed meal (SELM), cold pressed orange seed meal (CPOM), microwave-treated cold pressed orange meal (MTOM), cold pressed grapefruit seed meal (CPGM) and enzyme-

treated cold pressed grapefruit seed meal (ETGM) were the names and the abbreviations of the samples used in this study (Figure 1).

Physico-chemical properties of the meals

As for proximate compositional analyses, the moisture content of meals were determined by placing 1 g sample into Ohaus MB45 analyser (Parsippany, NJ, USA) at 110 °C for 30 min, and calculating the weight difference as % moisture. Total ash of meals was measured by AOCS method Ba 5a-49 (AOCS, 1984), remaining oil by Soxhlet technique of AOAC 920.39 (AOAC, 2002), and protein content using the Kjeldahl method of Aa 5-38 (AOCS, 1984).

Phytic acid contents of the meals was determined by AOAC method 986.11 (AOAC, 1986). Briefly, 2 g of each meal sample was extracted with 40 ml of 2.4% (v/v) HCl, and the extract was diluted with 25 ml of 1:1 (v/v) Na₂EDTA-NaOH solution, before transferring into ion-exchange column prepared with Dowex® AG 1×4 chloride resin (100-200 µm; Sigma) previously washed with 15 ml of 0.7 M NaCl solution and 15 ml of water. Two elutions with 15 ml of water and 15 ml of 0.1 M NaCl were discarded, and final elution with 15 ml of 0.7 M NaCl was collected. Upon this collection, 0.5 ml H₂SO₄ (97%) + 3 ml HNO₃ (65%) was added and wet ashing by Kjeldahl was completed. After adding 10 ml distilled water, the phytate salts were dissolved at 100 °C, and 2 ml molybdate (2.5%) and 1 ml

sulfonic acid solutions (0.16 g 1-amino-2-naphthol-4-sulfonic acid, 1.92 g Na₂SO₃, and 9.60 g NaHSO₃ dissolved in 100 ml water) were added before completion of reaction in 15 min. Finally, spectrophotometric (Agilent 8453 UV-Visible Spectrophotometer, Waldbronn, Germany) determination was done at 640 nm with standard curve prepared with potassium acid phosphate. The phytic acid content of samples was given as mg/g sample.

Viscosity of the meals was measured by the method of Khalid *et al.* (2003). Briefly, 10% (w/v) dispersions were prepared by ultratoraxing (Yellow line D125; IKA Werke, Staufen im Breisgau, Germany) meals at 8,000 rpm for 1 min before adjusting the pH to 7.0 by 1 N HCl or NaOH solutions. Then, the viscosity of the meal dispersions was measured with Brookfield DV II. Pro viscosimeter and Rheocalc software (Brookfield Eng. Lab., Inc., Middleborough, MA, USA) equipped with no. 18 spindle at 25 °C by circulating water around the sample holder. The apparent viscosities were recorded as centipoise (cP) values.

The colour values of the meals was determined with a Minolta CR-400 colourmeter (Minolta Camera Co., Osaka, Japan) previously calibrated against white tile, and the values of L, a* and b* colour coordinates were recorded (Yilmaz *et al.*, 2017).

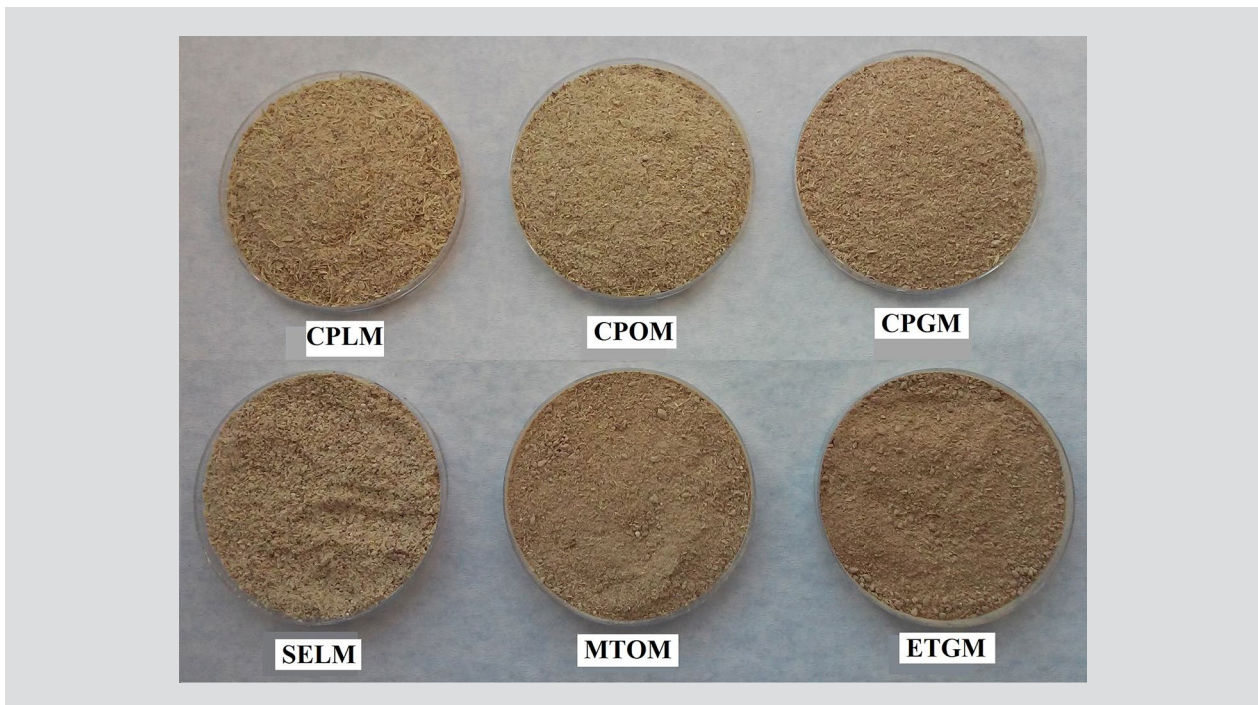


Figure 1. The defatted cold press meals of the citrus seed samples (CPLM = cold pressed lemon seed meal; SELM = solvent extracted lemon seed meal; CPOM = cold pressed orange seed meal; MTOM = microwave treated orange seed meal; CPGM = cold pressed grapefruit seed meal; ETGM = enzyme treated grapefruit seed meal).

Flavonoid and phenolic acid compositions of the meals

Extraction of these components was followed by the method of Challacombe *et al.* (2012) with some modifications. Briefly, 1 g of each meal sample extracted with 10 ml of methanol:water (80:20, v/v) for 1 h at room temperature by shaking vertically at 150 rpm. The slurry was centrifuged at $6,797\times g$ for 15 min, and the supernatant was collected. This procedure was repeated and all extracts were collected together. Under nitrogen flush, solvents were evaporated and the extract was reconstituted to 2 ml in deionised water. Before chromatography, the extract was passed through an $0.45\ \mu\text{m}$ membrane filter.

The analysis of phenolic composition was accomplished by the modified method of Moulehi *et al.* (2012). A reverse-phase-HPLC system equipped with a SPD-M20A diode array detector (Shimadzu Corporation, Kyoto, Japan) was used. The Zorbax Eclipse Plus C18 (Agilent Technologies) column ($250\times 4.6\ \text{mm}$, $5\ \mu\text{m}$) was used for compounds separation at $25\ ^\circ\text{C}$. The 0.2% sulphuric acid (solvent A) and acetonitrile (solvent B) were the mobile phase, and the flow rate of mobile phase was 0.5 ml/min. The gradient program was as follows: 0-0.1 min 0% A/100% B, 0.1-18 min 80% A / 20% B, 18-24 min 70% A / 30% B, 24-30 min 67.5% A / 32.5% B, 30-36 min 45% A / 55% B, 36-40 min 0% A / 100% B, 40-45 min 60% A / 40% B, 45-47 min 80% A / 20% B. The injection volume was 20 μl , and the peaks were monitored at 280 nm. All compounds were identified according to the retention times of commercially available standards.

Functional properties of the meals

Water holding capacity (WHC) of the meal samples was measured according to Moure *et al.* (2002) and Manamperi *et al.* (2007). First, 20 ml of deionised water and 5.0 g of meal sample were rigorously mixed for 30 min, and then centrifuged at $2,291\times g$ for 15 min. After drainage of the supernatant, the tubes inverted and waited for 30 min to release all free water. Finally, the tubes weighed, and from initial and final weight difference, the WHC value was calculated as g water held per g sample.

Oil holding capacity (OHC) was determined by the method of Manamperi *et al.* (2007). 2.0 g of meal sample put into previously weighed tubes, and 10 ml sunflower oil was added. After vortexing 1 min, the sample rested for 30 min at room temperature, before centrifugation at $2,291\times g$ for 15 min. Once the upper oily phase decanted, the tubes turned over to drain all unbound oil for 1 h. Finally, the tube was weighed, and from the weight difference, the OHC was calculated as g oil bound per g sample.

Emulsion activity (EA) and emulsion stability (ES) were accessed according to Wu (2001). Each meal, water and

sunflower oil were mixed at 7:100:100 (w:v:v) ratio, and homogenised for 1 min at 10,000 rpm. Finally the tubes were centrifuged at $2,291\times g$ for 5 min. The formula below was used for calculation:

$$EA = 100 \times \frac{\text{height of emulsion layer}}{\text{total height of mixture in tube}} \quad (1)$$

The same emulsion was kept 30 min at $80\ ^\circ\text{C}$ in a water bath, and then rapidly cooled on ice. Finally tubes were centrifuged at $2,291\times g$ for 5 min, and ES was calculated by the formula:

$$ES = 100 \times \frac{\text{height of remaining emulsified layer}}{\text{total height of mixture in tube}} \quad (2)$$

The foaming capacity (FC) and foam stability (FS) of the samples were determined by the method of Cano-Medina *et al.* (2011). 100 ml deionised water and 3.0 g meal sample were mixed and pH was adjusted to 7.0 with either 1 N HCl or NaOH solution. The slurry was shaken at high speed in a Waring blender for 3 min at room temperature, and the mixture was put into a 250 ml graduated cylinder. FC was calculated using Equation 3.

$$FC(\%) = 100 \times \frac{\text{volume after agitation-volume prior to agitation}}{\text{volume prior to agitation}} \quad (3)$$

To find the FS values, the tubes were kept 30 min at room temperature in the graduated cylinders, and then the height of remaining foam was read to calculate the value by:

$$FS(\%) = 100 \times \frac{\text{residual foam volume}}{\text{total foam volume}} \quad (4)$$

The least gelation concentrations (LGC) of the meals was determined by modified method of Moure *et al.* (2002). Stock meal dispersions were prepared in deionised water at 20% (w/v) and pH was adjusted to 7.0 with acid/alkali solutions. Then each stock was serially diluted to 16, 14, 12, 10, 8, 6, 4, and 2% (w/v) solutions. The pH of each dilution was checked and adjusted to 7.0, if needed. The liquid fraction of each dispersion was put into tubes (5 ml each), and kept for 1 h in a water bath at $100\ ^\circ\text{C}$. Finally, the tubes were cooled under tap water, and visually examined for solid gel structure. If the gel is fixed to tube wall, it was called solid gel; if the gel remained inside tube, it was called the clot; if no gel formed it was called liquid.

Statistical analysis

This study was replicated two times for the meal production, and analyses for each replicate meal sample were done at least two times. The data were reported as mean±standard deviation. Two-way analysis of variance (ANOVA) and Tukey's multiple comparison tests were applied to separate the means. Data comparison in the tables were done for each seed pairs, namely the meals extracted from lemon seeds compared with each other (its control and treatment group), and not with the meals extracted from the orange seed or grapefruit seed, since the applied treatment groups prior to cold oil pressing of the three seeds were different. The software programs Minitab (2010) and SPSS (1994) were used. The level of confidence was at least 95% for all tests.

3. Results and discussion

Physico-chemical properties

The physico-chemical properties of the six citrus seed meals prepared by different methods were presented in Table 1. There were significant differences between the control and treatment groups of each seed meal samples for the proximate composition values. In all meals, the treatment groups had lower levels of moisture than their control samples. The lowest moisture content (6.54%) was in the SELP sample, and it was probably caused by previous hexane extraction of oil, during which most of the water was removed. Moisture of the other meals was around 8-12%. Total ash contents of each sample pairs were not significantly different, but ash content of the lemonseed meals was lower than those of the orange and grapefruit seed meals. Ash content of meals ranged from 3.23 to 5.65%. The remaining fat content of the SELP

sample was significantly higher (3.20%) than its control sample (1.41%). Since no defatting process was applied to this solvent extracted meal, it is clear that some fat had remained from the oil extraction process. For other meals, the remaining fat contents in both treated and control samples were below 1.0%, and hence, the defatting process could be accepted as fairly effective. The protein contents of the sample pairs were significantly different. Protein contents of the control sample for orange and grapefruit seed meals were lower than their treated pairs, while the contrary was observed for the lemonseed meals. Among all samples, the highest protein (29.41%) was in the MTOM, and the lowest (20.98%) one was in the SELM samples. In general, protein contents of the meals ranged between 20-30%. Hence, the citrus seed meals can be accepted as high crude protein containing ingredients as compared to other seed meals or flours in literature (Akpata and Akubor, 1999; El-Adawy *et al.*, 1999b; El-Safy *et al.*, 2012). In general, the proximate composition of these citrus seed meals are in accordance with other citrus, poppy, pumpkin and hazelnut seed or kernel meals (Rodriguez-Miranda *et al.*, 2012; Xu and Hanna, 2011; Yilmaz and Dündar Emir, 2017; Yilmaz *et al.*, 2017).

Phytic acid contents of the meal sample pairs were not significantly different from each other, and all samples ranged from the lowest of 3.34 mg/g in CPLM to the highest of 5.06 mg/g in ETGM (Table 1). Clearly, seed pre-treatments had not changed phytate content of the corresponding meals, unlike seed type. Lemonseed meals had lower level of phytates than those of the orange and grapefruit seed meals. In a previous study (El-Adawy *et al.*, 1999a), phytic acid contents of citron, orange and mandarin seeds were reported as 0.17, 0.26 and 0.23%, respectively. In another study (El-Safy *et al.*, 2012), phytic acid amount in orange seed flour was given as 10.71 mg/100 g dry weight

Table 1. The physico-chemical properties of citrus seed meals prepared by different methods.^{1,2}

	CPLM	SELM	CPOM	MTOM	CPGM	ETGM
Moisture (%)	11.29±0.09 ^a	6.54±0.13 ^b	12.04±0.02 ^a	10.55±0.07 ^b	9.13±0.05 ^a	8.84±0.11 ^b
Ash (% dw)	3.62±0.08 ^a	3.23±0.23 ^a	5.09±0.08 ^a	5.55±0.06 ^a	4.96±0.13 ^a	5.65±0.06 ^a
Fat (% dw)	1.41±0.06 ^b	3.20±0.11 ^a	0.68±0.09 ^a	0.31±0.07 ^b	0.46±0.04 ^a	0.36±0.00 ^a
Protein (% dw)	27.27±1.06 ^a	20.98±0.93 ^b	23.21±1.66 ^b	29.41±0.61 ^a	26.07±0.76 ^b	28.49±0.28 ^a
Phytic acid (mg/g)	3.34±0.01 ^a	3.57±0.31 ^a	4.03±0.33 ^a	4.90±0.23 ^a	4.99±0.19 ^a	5.06±0.32 ^a
Viscosity (cP)	130.50±1.50 ^a	125.35±1.35 ^b	166.9±14.2 ^a	146.75±3.25 ^b	198.45±1.35 ^a	147.20±2.00 ^b
Colour L*	72.22±0.92 ^b	74.25±0.61 ^a	70.57±0.21 ^a	66.60±0.19 ^b	71.50±0.65 ^a	65.21±0.87 ^b
a*	2.70±0.45 ^a	2.70±0.36 ^a	2.62±0.05 ^b	4.76±0.21 ^a	4.30±0.27 ^b	5.87±0.20 ^a
b*	20.49±1.52 ^a	21.92±0.64 ^a	20.71±0.55 ^a	21.76±0.53 ^a	21.39±0.09 ^a	22.64±0.33 ^a

¹ CPGM = cold pressed grapefruit seed meal; CPLM = cold pressed lemon seed meal; CPOM = cold pressed orange seed meal; dw = dry weight; ETGM = enzyme treated grapefruit seed meal; MTOM = microwave treated orange seed meal; SELM = solvent extracted lemon seed meal.

² Values are given as mean ± standard deviation (n=4). Means denoted by different letters in a row for each pairs of the samples are significantly different (P<0.05).

flour. These results are in accordance with our findings. Phytate content of various oilseeds, including rapeseed, sunflowerseed and nigerseed were reported as 6.89, 8.80, and 8.35 mg/g, respectively (Eklund, 1975). Phytate content of hazelnut meals were ranged between 18.5 to 33.0 mg/g (Xu and Hanna, 2011), while phytates in capia pepperseed meals were found around 10.34-11.51 mg/g (Yilmaz *et al.*, 2017). The phytate content of citrus seed meals can be considered as low compared to the other meals. Since phytates can chelate some mineral nutrients to lower their bioavailability, their low content would be an advantage in terms of the nutritional value (Eklund, 1975).

Viscosity values of the meal dispersions are presented in Table 1. In all three seed meals, pre-treated (solvent extracted, microwaved and enzyme treated) samples showed lower viscosity values than their control pairs. The highest value (198.45 cP) was in CPGM sample, while the lowest value (125.35 cP) was measured in SELM sample. Furthermore, lemon seed meals had lower viscosities than the other seed meals. Prior seed treatments might have caused some starch gelatinisations or partial protein denaturations to yield lower viscosities in their meals. Inyang and Nwadike (1992) reported 2.5 to 7.0 cP viscosity values for 1 and 10% dispersions of sesame seed flour, respectively. Viscosity values ranging from 958 to 1093 cP were reported for 20% dispersions of capia pepperseed flours (Yilmaz *et al.*, 2017). Clearly, viscosity of a meal

dispersion changes with the type and concentration of the meal.

Colour is an indispensable part of any material for food or other consumer utensils. Colour of the citrus seed meals are presented in Table 1. In CIE colour system, the L value indicates luminosity (L =0, black to L=100, white), a* value (+ a* = red, -a* = green) and b* value (+ b* = yellow, -b* = blue) indicate the colour components (Yilmaz *et al.*, 2017). The citrus seed meals had usually yellow-red colour (Figure 1). Microwave (MTOM) and enzyme (ETGM) treated samples lost brightness (decreased L value) and became more reddish (enhanced a* value). The colours of these meals could be compatible for most bakeries and formulated food products. Since cold pressing were carried out under milder conditions, there were no excessive darkening of the meals, and this situation could be credited as an advantage for product applications.

Flavonoid and phenolic acid composition

Six flavonoids and eight phenolic acids were quantified in the citrus seed meals (Table 2). Solvent extraction of lemonseeds, microwave pre-treatment of orange seeds and enzyme pre-treatment of grapefruit seeds prior to oil pressing were found effective on the specific flavonoids and phenolic acids quantified. Eriocitrin content in the treated samples of lemonseed and orange seed were lower than their controls, while enzyme treatment was ineffective for

Table 2. The flavonoid and phenolic acid compositions of citrus seed meals prepared by different methods.^{1,2}

	CPLM	SELM	CPOM	MTOM	CPGM	ETGM
Flavonoids (mg/g)						
Eriocitrin	147.2±5.2 ^a	131.5±10.9 ^b	4.9±0.03 ^a	3.9±0.02 ^b	3.6±0.04 ^a	3.3±0.5 ^a
Rutin	42.7±2.1 ^a	40.0±3.2 ^b	29.4±0.3 ^a	30.1±0.4 ^a	72.8±0.6 ^a	37.1±0.5 ^b
Naringin	3.1±0.7 ^a	3.4±0.5 ^a	2.4±0.02 ^a	2.0±0.9 ^a	35.4±0.9 ^a	22.9±2.3 ^b
Hesperidin	9.2±0.3 ^b	12.1±2.3 ^a	8.4±0.01 ^a	8.5±0.2 ^a	4.5±0.01 ^a	2.3±0.5 ^a
Neohesperidin	4.7±0.2 ^a	2.1±0.2 ^b	5.3±0.02 ^a	5.7±0.8 ^a	6.6±0.01 ^a	1.9±0.7 ^b
Naringenin	13.4±1.2 ^a	5.7±0.5 ^b	6.2±0.01 ^a	6.3±0.7 ^a	2.2±0.01 ^a	1.02±0.9 ^a
Phenolic acids (mg/g)						
Gallic	109.5±10.2 ^a	97.9±3.5 ^b	53.8±0.8 ^a	53.0±0.2 ^a	53.6±0.5 ^a	50.6±1.1 ^a
3,4-hydroxybenzoic	13.0±0.9 ^a	10.9±0.9 ^b	12.6±0.4 ^a	11.5±0.4 ^b	15.0±0.3 ^a	15.0±0.8 ^a
Vanillic	0.5±0.01 ^a	0.01±0.0 ^b	0.5±0.01 ^a	0.2±0.01 ^b	0.3±0.01 ^a	0.4±0.1 ^a
Caffeic	5.6±0.8 ^a	4.6±0.7 ^a	4.4±0.02 ^a	1.2±0.01 ^b	4.3±0.02 ^a	4.1±0.5 ^a
Syringic	4.1±0.1 ^a	3.9±0.2 ^a	4.0±0.01 ^a	2.0±0.01 ^b	3.9±0.01 ^a	3.5±0.5 ^a
p-coumaric	2.1±0.1 ^a	2.3±0.01 ^a	5.2±0.01 ^a	5.1±0.01 ^a	5.0±0.2 ^a	4.3±0.7 ^a
trans-ferulic	7.4±0.9 ^a	5.8±0.1 ^b	20.2±0.6 ^a	17.5±0.9 ^b	37.6±0.2 ^a	35.9±0.9 ^a
trans-2-hydroxycinnamic	4.0±1.5 ^a	4.1±0.1 ^a	16.1±0.3 ^a	13.2±0.8 ^b	17.3±0.1 ^a	18.1±0.9 ^a

¹ CPGM = cold pressed grapefruit seed meal; CPLM = cold pressed lemon seed meal; CPOM = cold pressed orange seed meal; dw = dry weight; ETGM = enzyme treated grapefruit seed meal; MTOM = microwave treated orange seed meal; SELM = solvent extracted lemon seed meal.

² Values are given as mean ± standard deviation (n=4). Means denoted by different letters in a row for each pairs of the samples are significantly different (P<0.05).

grapefruit seed meal. Furthermore, eriocitrin content of lemon seed meals was significantly (30-40 fold) higher than others meals. Rutin content of solvent extracted lemonseed meal and enzyme treated grapefruit seed meal were lower than their controls samples. The highest rutin was quantified in the CPGM meal. Except for enzyme treated grapefruit seed meal, there was no significant difference between treated and control samples for naringin. Naringin concentration was significantly higher in the grapefruit seed meals, and ranged between 2.0 and 35.4 mg/g for all meals. Hesperidin concentration was lower in the control sample of lemonseed meal (CPLM) than in its treated sample (SELM), and there was no significant difference between the other two meal pairs. Neohesperidin content was significantly decreased after solvent extraction and enzyme treatment, but not by microwave treatment. The highest neohesperidin content was found in CPGM and the lowest in ETGM. Naringenin content decreased significantly in SELM, while there was no significant difference between other meal pairs. From these results, it was clear that hesperidinase/naringinase treatments of crushed grapefruit seeds significantly lowered the flavonoids rutin, naringin and neohesperidin in this meal. Similarly, also a decrease in SELM was observed. Contrarily, microwave treatment did not affect on flavonoids except eriocitrin.

Gallic acid content of SELM was significantly lower than its control sample (CPLM), while there was no significant difference for the other meal sample pairs. There were some differences for 3,4-hydroxybenzoic acid content, and its concentration decreased in solvent extracted lemonseed meal and microwave treated orange seed meal. Vanillic acid concentrations in treated lemon and orange seed meals were significantly lower than their control samples. Usually quantified vanillic acid contents were fairly lower in all samples. Caffeic acid content was between 1.2 and 5.6 mg/g among all meals, and only microwave treatment decreased it significantly. A similar trend was evident in the syringic acid contents (Table 2). Concentrations of *p*-coumaric acid were not changed by the previous seed treatments, and found usually lower in lemon seed meals than in the rest. Both solvent extraction and microwave treatment caused decreases in the *trans*-ferulic acid contents. The highest amounts of *trans*-ferulic acid were observed in grapefruit seed meals. While *trans*-2-hydroxycinnamic acid levels were lower in lemonseed meals, only microwave treatment caused a decrease in orange seed meals. When the cumulative amounts were considered, CPLM had the highest (220.3 mg/g) flavonoid and phenolic acid (146.2 mg/g) contents, while MTOM had the lowest flavonoid (56.5 mg/g) and phenolic acid (103.7 mg/g) contents. In general, the order of cumulative concentration was lemon seed meals > grapefruit seed meals > orange seed meals. Hence, especially lemon seed meals could be suggested as an important and rich source of citrus flavonoids for extraction and purification for commercial purposes.

There is limited data in literature about flavonoid and phenolic contents of citrus seed meals or flours. In one study (Russo *et al.*, 2015), 13 phenolic compounds and flavonoids were quantified in different residues of orange processing, including seeds. Values of 153.6, 84.7, 747.0 and 40,399.7 mg/kg were reported for *p*-hydroxybenzoic acid, caffeic acid, eriocitrin and hesperidin, respectively. Except for hesperidin, values measured for the meals in this study are higher than those mentioned above. In another similar study (Russo *et al.*, 2014), the flavonoid and phenolic composition of lemon seeds have been published, and 28 compounds were identified. Eriocitrin and hesperidin concentrations in this study were significantly higher than that reported in Russo *et al.* (2014). Lately, Xi *et al.* (2017) studied phenolic compositions of different fruit parts in lemon cultivars. They reported 3.50-11.95 µg/g gallic acid, 10.11-52.51 µg/g caffeic acid and 9.31-32.43 µg/g ferulic acid in the seeds. It seems that values reported in this study were around 1000-fold higher than these reported values. Similar results were observed for eriocitrin and rutin, while Xi *et al.* (2017) did not detect rutin. Overall, flavonoid and phenolic acid contents of these meal samples were generally higher than those reported in the literature. These differences might be due to the difference of the source materials or analytical procedures. In general, the defatted citrus seed meals in this study have a high potential as sources of flavonoids and phenolic acids. Mir and Tikou (2015) discussed some possible anti-inflammation, anti-oxidation, anticancer, cardiovascular protection, anti-diabetes, renal protection, protection against Alzheimer's disease and antihyperuricemic activities for naringenin. Likewise, naringenin in a rat feeding study showed improvements for renal failure and platelet alteration (Chtourou *et al.*, 2016). Lei *et al.* (2016) fed hamsters with nobiletin and tangeretin and found significantly reduced plasma lipids and weight gain. Citrus flavonoids and phenolics also pose functional food properties. Hence, defatted citrus seed meals can be a candidate source to extract these compounds or can be used in food enrichments to provide these bio-active compounds to consumers. More studies in these aspects are needed.

Functional properties

Functional properties of the defatted citrus seed meals were given in Table 3. There was no significant difference for the WHC values between each treatment and control pairs of the lemon, orange and grapefruit seed meals. Clearly, WHC values of lemonseed meals seem lower than the rest. The highest value (4.25 g/g) was measured in the ETGM sample. Water absorption capacity of citron, lemon, orange, and mixed seed flours were reported between 329.2 and 368.8 g/100 g (El-Adawy *et al.*, 1999b), within the same range of the values measured in this study. In another study (El-Safy *et al.*, 2012), water absorption capacity of orange seed flour was reported as 1.20 g/g. It is fairly lower than the values for orange seed meals measured, but their flour was prepared

directly from grinded seed, which includes its inherently present oil. The WHC of swell-dried soybean flour was reported between 1.429 and 2.150 g/g sample (Nguyen *et al.*, 2015). As literature suggest WHC is dependent on material type and treatments applied. Hence, citrus seed meals could be credited as good WHC materials. It was indicated that WHC could be an important property in processed foods like communitated meats, bakery, sauces and soups (Moure *et al.*, 2006). Further studies of the citrus seed meals in similar products could be suggested.

OHC of the samples ranged from 1.24 to 1.79 g/g (Table 3). Except for microwave treated orangeseed meal, there was no significant difference between the meal pairs. Compared to the reported OHC values in El-Adawy *et al.* (1999b) and El-Safy *et al.* (2012), these measured values were lower. The differences could originate from source material and measurement techniques. Adebowale *et al.* (2005) reported the OHC of 6 mucuna beans between 2.1 and 2.6 g/g, and for linseed flour samples between 2.36 and 1.37 g/g (Madhusudhan and Singh, 1985), respectively. Likewise, OHC of swell-dried soybean flours ranged from 0.698 to 1.122 g/g sample (Nguyen *et al.*, 2015). Compared with the literature, citrus seed meal samples can be accepted as moderate OHC samples. This property is very important in emulsion food products like frankfurters, sausages and mayonnaise (Foegeding and Davis, 2011). Hence, utilisation of citrus seed meals in emulsion foods to provide citrus flavonoids as bio-active functional components could be a future research area.

EA and ES values of the meals were also presented in Table 3. Compared with their controls, solvent extracted lemonseed meal and enzyme treated grapefruitseed meal had significantly higher EA values. The highest EA value (51.46%) was in the ETGM sample. While for the ES values, significant differences between their control and treatment samples of orangeseed meal and grapefruitseed meal were

deducted, there was no difference between lemonseed meal samples. Clearly, microwave treatment and enzyme treatment of the seeds resulted in higher ES values in corresponding meals. The partial unfolding of proteins or starch gelatinisation might have caused this situation (Moure *et al.*, 2006). El-Adawy *et al.* (1999b) reported the emulsion capacity of citrus flour samples between 140 and 190 ml oil/g flour. Similarly, El-Safy *et al.* (2012) reported 50.96 ml oil/g protein for orange seed flour emulsion capacity. ES of the same sample was given as 36.60%. Since the unit of measurements were different, it is not possible to directly compare the findings, but, clearly the meal samples in this study were moderate emulsion forming materials. It has been reported (Madhusudhan and Singh, 1985) that heat treatment of linseed reduced the EC of its flour. Overall, prior microwave and enzyme treatment of citrus seeds in this study resulted in enhanced ES values, while only enzyme treatment caused an increase in EC value. Generally the type and extent of previous seed or meal treatments might change their emulsification properties.

FC and FS of the samples were also determined (Table 3). Seed pre-treatments of solvent extraction in lemonseeds and microwave treatment in orangeseeds caused FC to increase, while enzyme treatment in grapefruitseed decreased it. Exactly the opposite effect was observed for the FS values in the meal sample pairs. FC ranged from the highest (95.81%) in CPGM sample to the lowest (30.00%) in the CPLM sample. In general, lemonseed meals had lower values. The highest FS was in the CPLM (70.00%), and the lowest was in the CPGM (4.19%). Clearly, FC and FS occur in converse directions. Around 12-85% of volume increase measured as foam capacity has been reported for citrus seed flours (El-Adawy *et al.*, 1999b). In another study (El-Safy *et al.*, 2012), foam expansion of 11.72% and FS of 59.77% have been reported for orange seed flour. Foaming capacities of almonds, chestnut, Brazil nuts, hazelnuts, macadamia nuts, pine nuts, pistachio nuts, soybeans W82

Table 3. The functional properties of citrus seed meals prepared by different methods.^{1,2}

	CPLM	SELM	CPOM	MTOM	CPGM	ETGM
WHC (g water/g)	3.38±0.04 ^a	2.27±0.05 ^a	4.12±0.15 ^a	4.07±0.11 ^a	3.93±0.09 ^a	4.25±0.37 ^a
OHC (g oil/g)	1.64±0.02 ^a	1.50±0.13 ^a	1.79±0.00 ^a	1.36±0.02 ^b	1.41±0.02 ^a	1.24±0.01 ^a
EA (%)	39.83±1.34 ^b	47.06±1.47 ^a	45.36±1.37 ^a	44.39±1.72 ^a	44.10±0.93 ^b	51.46±1.53 ^a
ES (%)	44.71±0.86 ^a	43.26±0.66 ^a	44.02±0.87 ^b	49.27±0.43 ^a	43.91±1.03 ^b	47.31±1.72 ^a
FC (%)	30.00±5.00 ^b	58.33±8.33 ^a	65.00±5.00 ^b	86.71±2.10 ^a	95.81±0.20 ^a	46.97±1.52 ^b
FS (%)	70.00±5.00 ^a	41.67±8.33 ^b	35.00±5.00 ^a	13.29±2.10 ^b	4.19±0.20 ^b	53.03±1.52 ^a

¹ CPGM = cold pressed grapefruit seed meal; CPLM = cold pressed lemon seed meal; CPOM = cold pressed orange seed meal; EA = emulsion activity; ES = emulsion stability; ETGM = enzyme treated grapefruit seed meal; FC = foaming capacity; FS = foam stability; MTOM = microwave treated orange seed meal; OHC = oil holding capacity; SELM = solvent extracted lemon seed meal; WHC = water holding capacity.

² Values are given as mean ± standard deviation (n=4). Means denoted by different letters in a row for each pairs of the samples are significantly different (P<0.05).

variety flours were found all above 40% and quite dependent on the source (Sharma *et al.*, 2010). Overall, citrus seed meals can be considered as good foaming materials to be applied in foods where foam formation is desired.

LGC of the meals was also measured as another functional property (Table 4). This parameter is mostly measured for extracted seed proteins, but for meals it also provides information about thermal behaviour (Foegeding and Davis, 2011; Moure *et al.*, 2006). Until 16% concentration no meal was gelled. Orange and grapefruit seed meals started clot formation at 8% concentration, while the same effect occurred at 10% for lemon seed meals. According to El-Adawy *et al.* (1999b), citron, orange, mandarin and mixed citrus seed flours were started to gel at 7, 6.5, 6 and 7% concentrations, respectively. In another study (Akpata and Akubor, 1999), sweet orange defatted and undefatted seed flours shown to start gelation at 6 and 8%, respectively. Generally defatted pressed meals in this study showed to start gelling at relatively higher concentrations. In a study (Yilmaz *et al.*, 2017) control and roasted capia pepperseed meals were shown to gel at around 24 and 32% concentrations. Sharma *et al.* (2010) reported the LGCs for almonds, Brazil nuts, chestnuts, hazelnuts, macadamia nuts, pine nuts, pistachios, and soy protein concentrates at around 6, 8, 8, 12, 20, 12, 10, 16%, respectively. Clearly, LGC is heavily dependent on the kind of material and its processing conditions. Citrus seed meals can be utilised in formulations in which gel formation is a goal.

4. Conclusions

Oilseed meals and specifically cold pressed oilseed meals are abundantly available as high quality biomaterials to be utilised more effectively in food, feed and other industries for various purposes. As less common and less known meals, citrus seeds cold press meals were studied. The proximate composition showed that these meals could provide 20-30% protein on dry weight basis. Most importantly they can

provide very important citrus flavonoids and phenolic acids. Recent studies have shown that these citrus flavonoids are bioactive and health beneficial molecules and that can be applied in special functional food formulations. Furthermore, processing functionalities, such as water and OHC, emulsion and foaming properties and gelling abilities of the meals demonstrated their potential for utilisation in processed foods, such as processed meat products, bakery products, mayonnaise, dry soap mixtures, etc. Further studies are recommended on the extraction and purification of the bio-active compounds in the meals, and direct applications of these meals in food products or animal feeds.

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Table 4. The minimum gelling concentrations of citrus seed meals prepared by different methods.¹

Dispersion rate (%)	CPLM	SELM	CPOM	MTOM	CPGM	ETGM
2	liquid	liquid	liquid	liquid	liquid	liquid
4	liquid	liquid	liquid	liquid	liquid	liquid
6	liquid	liquid	liquid	liquid	liquid	liquid
8	liquid	liquid	liquid+clot	liquid+clot	liquid+clot	liquid+clot
10	liquid+clot	liquid+clot	liquid+clot	liquid+clot	liquid+clot	liquid+clot
12	liquid+clot	liquid+clot	liquid+clot	liquid+clot	liquid+clot	liquid+clot
14	liquid+clot	liquid+clot	liquid+clot	liquid+clot	liquid+clot	liquid+clot
16	gel	gel	gel	gel	gel	gel

¹ CPGM = cold pressed grapefruit seed meal; CPLM = cold pressed lemon seed meal; CPOM = cold pressed orange seed meal; ETGM = enzyme treated grapefruit seed meal; MTOM = microwave treated orange seed meal; SELM = solvent extracted lemon seed meal.

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