Comparative Analysis of Phenolic Content and Chemical Composition of Agro-industrial By-products of Citrus Species

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Abstract

Comparative analysis of phenolic content and nutritive value for agro-industrial by-products (peel and pomace) of *Citrus aurantıım* (Bitter orange), *Citrus paradisi* (Grapefruit), *Citrus reticulata* (Mandarin), *Citrus limon* (Lemon), and *Citrus sinensis* (Sweet orange) was done. All samples for phenolic content were extracted with 70% ethanol and absorbance reading taken at 765nm and nutritive value was also assessed by chemical analysis. The phenolic content of the five citrus peels significantly differed at P<0.01 from pomaces. Phenolic content from highest to lowest for peels was grapefruit > mandarin > lemon > bitter orange > orange while for pomaces, bitter orange > grapefruit > mandarin > lemon >orange. The principal component analysis showed that the phenolic content of citrus species had no correlation with the nutritive value hence they are non-dependent parameters. In addition, the dry matter of the citrus species was the most important component of the nutritive value. This study showed the high variation of the quality parameters (phenolics content and nutritive value) of citrus species among varieties and countries. Meta-analysis of quality parameters of citrus species is recommended to underpin the broad effects of fruit sourcing, maturation, genetics, sample preparation, extraction solvents and laboratory techniques on the agro-industrial by-products.

Keywords: Agro-industrial; Chemical Composition; Citrus; Phenolics; Principal Component Analysis

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INTRODUCTION

Citrus agro-industrial by-products

Citrus seeds, peels and pulps constitute mainly citrus agro-industrial by-products obtained from the about 50% industrially processed citrus fruits (Zema, 2018). All in the Rutaceae family, orange, lemon, grapefruit and mandarin are industrially important citrus species (Rafiq et al., 2018; Satari and Karimi, 2018) among the world citrus producing countries, and Turkey is included (FAO, 2016; Uzun and Yesiloğlu, 2012).

Citrus agro-industrial by-products are produced in significantly large quantities that pose a major burden on the environment and management cost to the industries hence the need for economically viable and sustainable waste management options such as utilization for animal feeds (Zema, 2018).

Improving Animal Nutrition

Using citrus agro-industrial by-products for improved nutrition and production of animals is growing interest (Volanis and Zoiopolous, 2003) although this is currently being sub-optimally utilized considerably due to low economic capacity, skill, and infrastructure particularly in the low to middle-income countries (Tayengawa and Mapiye, 2018). In addition to these, there are knowledge gaps in the phenolic and nutritive value as it relates to improving animal nutrition.

Knowledge Gap

Fruits contain many phenolic compounds- flavonoids, lignans, stilbenes, and phenolic acids (Manach et al., 2004) and some of these are found in the citrus peels and less in the pulp (Singh et al., 2020). Most studies focus on either peels or pulps of citrus however, their agro-industrial wastes are neither peels nor pulps alone but pomaces, a mix of peels and some pulps. Table 1 shows the phenolic content variations across five different species as this study focused. Reporting the phenolic content is a function of the calibration standard (Valencia-Avilés, et al., 2018) and extraction solvent plays a vital role in the phenol content (Hegazy and Ibrahim 2012). It’s noteworthy that agro-climatic conditions of the environments where that citrus species are sourced are an important factor in the phenolic content in the citrus species (Singh et al., 2020). What remained unknown was if citrus species obtained for this study had different phenolic contents as previously reported and how the peels differed from the pomaces in the five citrus species examined.

Some studies of citrus species showed that dry matter for peels and pomaces ranged between 87 to 97%. Reported crude ash content was 1-10%, crude protein (2.8-9.5%), crude fiber (6-14%), and ether extract (0.5-5%) (Atta and El Shenawi, 2012; Beyzi et al., 2018; Castrica et al., 2019; El-ghfar et al., 2016; Figuerola et al., 2005; Ghanem et al., 2012; Gorinstein et al., 2001; Bejar et al., 2011; Lashkari and Tagizadeh, 2013; Marin et al., 2007; Magda et al., 2008; M’hiri et al., 2015; Nagarajaiah and Prakash, 2016; Özkan et al., 2017; Palangi et al., 2013; Vlaicu et al., 2020). The nutritive value variations may be associated with fruit source and maturation in addition to analytic techniques (Ammerman and Henry, 1991; Olowu and Yaman Fırıncıoğlu, 2019).

Despite these reports on phenolics and chemical composition, there is a gap of knowledge on how the chemical composition of many citrus species correlate with the phenolics as Rehman et al. (2020) is one of the very few to have comparatively assessed the total phenolics within different varieties of certain citrus species using the principal component analysis.

Table 1. Reports of Phenolic Contents Based on Ethanol Extract

SN	Citrus Species	Variety	Sample	Country	Phenolic content	Reference
1	*Citrus sinensis*	Hamlin	Pulp	Pakistan	222.3 (mgGAE/g)	Rehman et al (2020)
	Citrus sinensis	Red blood Succuri	Pulp		207.0 (mgGAE/g)	
	Citrus sinensis		Pulp		243.3 (mgGAE/g)	
2	*Citrus sinensis*		Peel	Sudan	35.6 (mgGAE/g)	Sir Elkhatim et al. (2018)
3	*Citrus sinensis*	Baladi	Peel	Egypt	169.50 (mgGAE/g)	Hegazy and Ibrahium (2012)
4	*Citrus sinensis*	Novel	Peel	Egypt	559.32(mgTAE/100g FW)	El-aal and Halaweish (2010)
5	*Citrus paradise*	Macfed	Pulp	Pakistan	165.6 (mgGAE g)	Rehman et al (2020)
6	*Citrus paradise*		Peel	Sudan	77.3 (mgGAE/g)	Sir Elkahatim et al. (2018)
7	*Citrus aurantium*		Pulp	Pakistan	158.9 (mgGAE/g)	Rehman et al (2020)
8	*Citrus aurantium*		Peel	Turkey	487 (mgGAE/10g)	Ersus and Can (2007)
9	*Citrus reticulata*		Pulp	Pakistan	180.6 (mgGAE/g)	Rehman et al (2020)
10	*Citrus limon*		Peel	Sudan	49.8 (mgGAE/g)	Sir Elkahatim et al. (2018)
11	*Citrus limon*		Peel	Israel	190 (mgChA/100g FW)	Gorinstein et al. (2001)
12	*Citrus reticulata*		Peel	Israel	179 (mgChA/100g FW)	Gorinstein et al. (2001)
13	*Citrus paradisi*		Peel	Portugal	155 (mgChA/100g FW)	Guimarães et al. (2010)

Study Objectives

This study had three clear objectives. Firstly, phenolic content of the peels and pomace samples of *Citrus sinesis* (sweet orange), *Citrus limon* (lemon), *Citrus reticulata* (mandarin), *Citrus paradisi* (grapefruit), and *Citrus aurantium* (bitter orange) were evaluated. Secondly, chemical composition assessments were carried out, and lastly, a comparative analysis was done on the phenolic content and chemical composition of the *Citrus* species.

MATERIAL and METHOD

Commercially mature *Citrus sinesis* (sweet orange), *Citrus limon* (lemon), *Citrus reticulata* (mandarin), *Citrus paradisi* (grapefruit), and *Citrus aurantium* (bitter orange) were obtained. Peels and pomaces samples were cut into pieces, oven-dried at 50^{o}C for 48 h, and finely grounded using a 1mm sieve in a Retsch ZM 200 laboratory mill. (AOAC, 1995). All procedures were carried out in the animal nutrition laboratory of the Faulty of Agricultural Science and Technologies in Niğde Ömer Halisdemir University, Turkey.

Phenolic Content Assessment

Folin-Ciocalteu's reagent method (Waterhouse, 2001) for the assessment of phenolic content was adapted in this study. Sample extracts (2.5ml) and 70% ethanol was used to prepare 25ml stocks and sample dilutions were done. In duplicates, 100µl of the sample dilutions were marked up with distilled water (900µl), Folin-Ciocalteu reagent (5ml), and sodium carbonate (4ml). These samples were vortexed, stored in the dark for 2 h and at 765nm, a UV spectrophotometer was used to measure the absorbance for each *Citrus* sample. Gallic acid calibration curve was determined (R^2 =0.9959) and phenolic contents of the samples were expressed as mg GAE/g.

Chemical Composition Assessment

Dry matter, crude ash, and crude protein analysis by the Kjeldahl method (AOAC, 1995) were carried out in duplicates on peels and pomaces samples of five Citrus species- (bitter orange, grapefruit, lemon, mandarin, and orange). Van Soest's (1991) method was used to assess the neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents of peels and pomaces.

Statistical Analysis

With the JAMOVI, R-based statistical package (Jamovi project, 2021), analysis of Variance (ANOVA) was done to determine the statistical significance ($P \leq 0.01$) of the phenolics concentrations and principal component analysis (PCA) were done to compare the phenolic concentrations to the chemical composition obtained.

RESULTS and DISCUSSION

Phenolic Content Assessment

Each Citrus species is significantly different from the other ($P <0.01$). Notably, pomaces of bitter orange, grapefruit, mandarin, and orange showed far higher concentrations of phenolics than their peels. Also, all the pomaces samples of the five Citrus species significantly differ from the peels except the lemon (peels and pomaces), mandarin (peels and pomaces), and orange pomace which did not significantly differ from each other ($P> 0.05$). In order of phenolic concentration among the citrus peels, grapefruit > mandarin > lemon > bitter orange > orange and for pomaces, bitter orange > grapefruit > mandarin > lemon >orange. *Citrus sinensis* (orange) had the lowest phenolic content for both peels and pomaces while bitter orange pomaces expressed the highest phenolic contents which significantly differed from the bitter orange peels (Figure 1).

In this study, results had been expressed in mg GAE/ g given that gallic acid was the calibration differently from mg TAE/ 100g (El-aal and Halaweish, 2010) having calibrated with tannin and mg ChA/100g (Gorinstein et al., 2001) with a chlorogenic acid calibration. Calibration standards may be reported with the standard used or re-evaluated with any other standard given that reactions leading to phenolic content estimates are independent, quantitative, and predictable (Singleton et al., 1999).

Citrus sinensis (Orange) peel (277.2 mg GAE/ g) in this study had higher phenolic content than in Hegazy and Ibrahim (2012) and much lower (35.6 mg GAE/ g) was reported by Sir Elkahatim et al., (2018). Similarly, orange pomace had higher phenolic content (462.8 mg GAE/ g) compared to all three orange varieties reported by Rehman et al. (2020).

The phenolic content of *Citrus reticulata* (mandarin) in this study is significantly higher than Gorinstein et al., (2001). Although the phenolic content of the mandarin pomace did not significantly differ from the peel, it was higher than the mandarin pulps reported by Rehman et al., 2020. Citrus reticulata (lemon) peels had a higher phenolic concentration than Gorinstein et al. (2001) who reported 179 mg ChA/ 100g. Similarly, 180.6 mg GAE/g in lemon pulp (Rehman et at., 2020) was lower than both lemon peel and pomace in this study. Prior reports of Citrus paradisi (grapefruit) peel and pomace differed from results obtained in this study as phenolic contents of grapefruit peels and pomaces were found to be significantly higher than as previously reported (Sir Elkahatim et al., 2018; Rehman et al., 2020). Citrus aurantium (bitter orange) in this study stands out differently from the reports of Ersus and Cam (2007) (487 mg GAE/ 10g for peels) and Rehman et al., (2020) (158.9 mg GAE/ g for pulp).

The difference in phenolic contents may be attributed to the effect of agro-climatic conditions on fruit quality (Hussain et al., 2017; Singh et al., 2020) given the difference in the agro-ecological zones of Egypt, Israel, Pakistan, Portugal, Sudan, and Turkey as reported by the previous studies. For instance, Washington navel orange grown in the Mediterranean climate (cool and wet winters; hot and dry summers) similar to Adana, Antalya, and Izmir- the top three provinces with high production of citrus in Turkey (Yesiloğlu et al., 2007) has been reported to have higher fruit qualities compared to those grown in coastal to desert areas (Davies and Albrigo, 1994; Zekri, 2011). In addition, these results further agree with the variations in parameters that have been associated with fruit source and maturation in addition to analytic techniques (Ammerman and Henry, 1991; Olowu and Yaman Fırıncıoğlu, 2019).

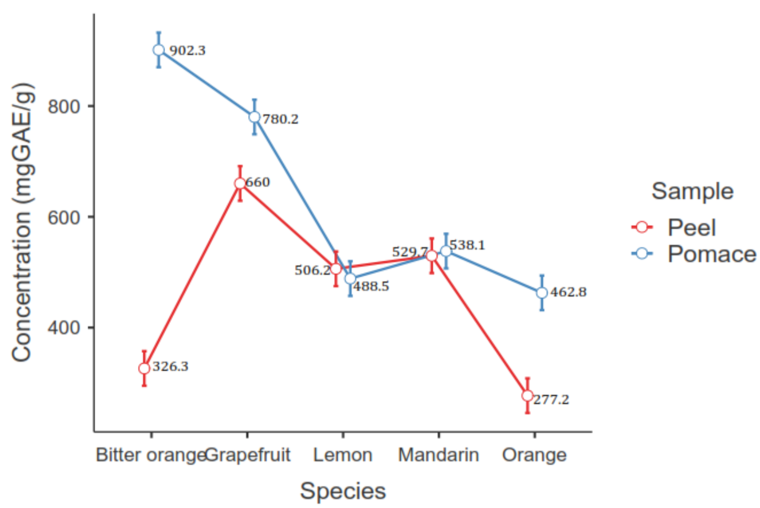

Figure 1. Analysis plot showing from highest (bitter orange pomaces) to lowest (orange peels) the different (P <0.01) phenolic concentrations of the peels and pomaces for the five *Citrus* species- grapefruit peel; grapefruit pomace; lemon peel; lemon pomace; mandarin peel; mandarin pomace; orange pomace.

Comparative Analysis of Phenolic Content and Chemical Composition

The principal component analysis (PCA) showed high variability between the phenolic content and the chemical composition of the citrus species assessed in this study (Figure 2). Dry matter is generally attributed as the most important determinant component of available soluble carbohydrates (Lashkari and Taghizadeh, 2013; Mamma and Christakopoulos, 2014). Although, ash content was positively correlated to dry matter, acid detergent fiber and neutral detergent fiber were less correlated with the dry matter. Ash content is indicative of available minerals for animal nutrition (Shariff et al., 2021) and is as important as the dry matter for the delivery of minerals. Crude protein also showed a low correlation to other chemical components despite being an important parameter in the feed composition.

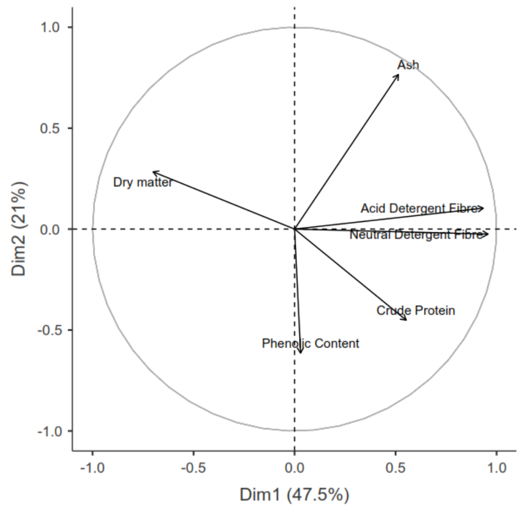

Figure 2. Result of PCA analysis showing three principal components (dry matter; ash, ADF; NDF, CP, and phenolics) that comparatively assesses phenolic content and chemical components (dry matter, ash, acid detergent fiber, neutral detergent fiber, and crude protein) of the *Citrus* species in this study

CONCLUSION

In this study, there is no correlation between chemical composition and phenolics of peels and pomaces of different citrus species. The importance of phenolics and chemical composition has been established by several authors and in this study as well. Noteworthy are the variations in the quality parameters of *Citrus species* by different studies and regions of the world which have mostly been attributed by researchers to effects of fruit sourcing, maturation, genetics, sample preparation, extraction solvents, and laboratory techniques. The limitation, however, is the determination of the major effect driving variation of quality parameters by the conventional experimental methods hence the need to further study this through a meta-analysis approach. Meta-analysis of the effect of quality parameters in *Citrus species* is recommended to provide an in-depth understanding of the variations and perhaps what to do differently when considering citrus agro-industrial by-products for enhanced animal nutrition.

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