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MASTER THESIS

T.R.
NİĞDE ÖMER HALİSDEMİR UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF ANIMAL PRODUCTION AND TECHNOLOGIES

ASSESSING NUTRITIVE VALUE AND IN-VITRO DIGESTIBILITY OF PEELS AND POMACES OF DIFFERENT CITRUS SPECIES

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Master Thesis

Supervisor
Asst. Prof. Dr Sema YAMAN FIRINCIOĞLU

February 2021

The study titled **“Assessing nutritive value and in-vitro digestibility of peels and pomaces of different Citrus species”** and presented **by Oyinkansola Olubunmi OLOWU** under the supervision of **Dr. Sema YAMAN FIRINCIOĞLU**, has been recognized as Master thesis by the jury at the Department of Animal Production and Technologies of Niğde Ömer Halisdemir University, Graduate School of Natural and Applied Sciences).

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THESIS CERTIFICATION

It is certified that I have written this thesis by myself. I further confirm that all information included in this thesis is scientific and is in accordance with the university rules and regulations. Any materials that I have used from external sources as well as help received and all sources used in finalizing this research work and preparing this thesis, all have been acknowledged in the thesis.

Oyinkansola Olubunmi OLOWU

SUMMARY

ASSESSING NUTRITIVE VALUE AND IN-VITRO DIGESTIBILITY OF PEELS AND POMACES OF DIFFERENT CITRUS SPECIES

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Peels and pomaces of five citrus species- *Citrus aurantium* (Bitter orange), *Citrus paradisi* (Grapefruit), *Citrus reticulata* (Mandarin), *Citrus limon* (Lemon) and *Citrus sinensis* (Sweet orange) were comparatively assessed for nutritive value and digestibility. The nutritive value of the peels and pomaces of the five citrus species were determined by chemical analysis and in-vitro technique (ANKOM Daisy II Incubator) was used to assess the digestibility. There was no statistical significance ($P>0.5$) in species and samples effects among the five citrus species. However, lemon peel and pomace showed higher crude protein, crude fiber, NDF and ADF compared to other species. There were statistically significant differences ($P<0.001$) among the species and samples for in-vitro true digestibility. The bitter orange pomace had the highest in-vitro true dry matter digestibility and NDF digestibility of the five citrus species. Agro-industrial by-products from *Citrus aurantium* and *Citrus limon* are important to consider for further studies in nutritive value and digestibility improvement to achieve cost-effective and environmentally friendly feed modifications for ruminants.

Keywords: Citrus, species, by-products, peels, pomace, nutritive, digestibility

ÖZET

FARKLI NARENCIYE TÜRLERININ KABUK VE POSALARININ BESIN DEĞERI VE IN-VITRO SINDIRILEBILIRLIKLERININ DEĞERLENDIRILMESI

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Citrus aurantium (Acı portakal), *Citrus paradisi* (Greyfurt), *Citrus reticulata* (Mandarin), *Citrus limon* (Limon) and *Citrus sinensis* (Tatlı portakal) olmak üzere beş turunçgil türünün kabukları ve posaları besin değeri ve sindirilebilirlik açısından karşılaştırmalı olarak değerlendirildi. Beş narenciye türünün kabuklarının ve posalarının besleyici değeri kimyasal analizlerle belirlendi ve sindirilebilirliklerini değerlendirmek için in-vitro teknik (ANKOM Daisy II İnkübatör) kullanıldı. Beş turunçgil türü arasında türler ve örneklerde istatistiksel anlamlılık ($P > 0.5$) yoktu. Bununla birlikte, limon kabuğu ve posası diğer türlere göre daha yüksek ham protein, ham lif, NDF ve ADF değeri göstermiştir. In vitro gerçek sindirilebilirlik için türler ve numuneler arasında istatistiksel olarak önemli farklılıklar vardı ($P < 0.001$). Acı portakal posası, beş turunçgil türü arasında en yüksek in-vitro gerçek kuru madde sindirilebilirliğine ve NDF sindirilebilirliğine sahipti. Acı portakal ve limondan elde edilen agro-endüstriyel yan ürünler, geviş getiren hayvanlar için uygun maliyetli ve çevre dostu yem modifikasyonları elde etmek için besin değeri ve sindirilebilirliğin iyileştirilmesi ile ilgili daha ileri çalışmalar için dikkate alınması önemlidir.

Anahtar Sözcükler: Turunçgil türleri, yan-ürünler, kabuklar, posalar, besin değeri, sindirilebilirlik

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SYMBOLS AND ABBREVIATIONS

Symbols	**Meaning**
%	Percentage
°	Degrees
°C	Degrees celsius
cm	Centimeter
g	Gram
h	Hour
Kg	Kilogram
Kg/m3	Kilogram per cubic meter
L/kg	Liter per kilogram
L	Liter
min	Minute
mL	Milliliter
mm	Millimeter
mOsm/L	Milliosmole per liter
sec	Second
t	Tonnes
w/w	Weight by weight

Abbreviations	**Meaning**
ADF	Acid Detergent Fiber
ANOVA	Analysis of Variance
CF	Crude Fiber
CP	Crude Protein
DM	Dry Matter
EE	Ether Extract
IVTD	In-Vitro True Digestibility
NDF	Neutral Detergent Fiber
NDFD	Neutral Detergent Fiber Disappearance/Digestibility

TMR	Total Mixed Ration
XA	Ash Content

CHAPTER I

INTRODUCTION

1.1 Citrus Production and By-products

Citrus plants such as orange, lemon, mandarin, lemon, grapefruits, and bitter orange belonging to the family rutaceae (Rafiq et al., 2018) have been utilized and domesticated for years in different parts of the world. Global citrus production was estimated at 89 million tonnes in 2014 (USDA, 2014). About 26% of these are used in juice production. A lot of waste is generated from juice processing which makes up about 50% of the whole fruit weight. The waste often referred to as citrus-by-products are composed of citrus peels, pulps, and seeds.

In citrus production, oranges account for majority of production but there are also significant quantities of lemons, limes, and grapefruits produced. Turkey is among the top three citrus producing countries in the Mediterranean Basin and ranked ninth in the world (Figure 1.1), accounting for about 2.7% of the 137 million tonnes of citrus produced globally (FAO Stat, 2016).

Oranges are the main citrus fruit grown in Turkey, followed by mandarins, lemons, and grapefruits (Uzun and Yesiloğlu, 2012). One-third of total Citrus production globally, is used for juice and wine making (Li et al, 2006) with considerable amount of its peels and pomace resulting in waste.

Citrus peels and pomaces possess a large variety of bio-active compounds (secondary metabolites) that consists of phenolic compounds such as flavonoids (anthocyanins, proanthocyanins, hes-peridin, neohesperidin and naringin), condensed tanins and they are also a unique source of polymethoxyflavones (Li et al., 2006; Londono et al., 2010).

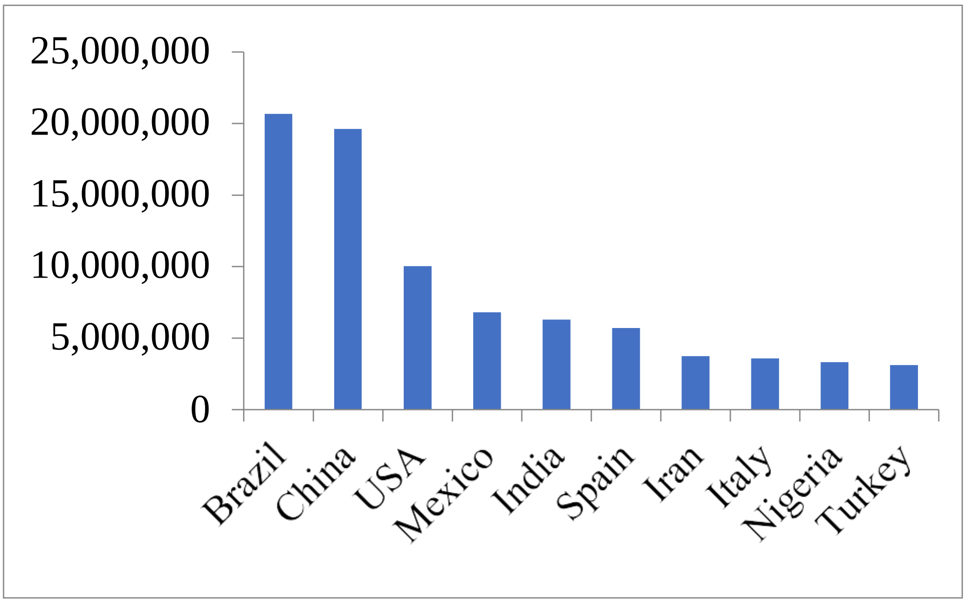

Figure 1.1. Top Ten Citrus Producing Countries. Source: USDA 2014

1.2 Uses and importance of citrus fruits and by-products

The countries in the Mediterranean accounts for 25% of citrus production in the world (Bampidis and Robinson, 2006). Part of the Citrus fruit yield, particularly oranges is used in canning industries for juice production. Citrus peels and pomaces are the major by-product from this process and are used as animal feed (Gohl, 1981).

Sheep keeping and cattle rearing are the dominant animal farming practiced in the Mediterranean and so the utilization of these agro-industrial by-products as part of animal feed is an area of interest (Volanis and Zoiopolous, 2003) to improve animal nutrition and production.

The potential of utilizing agro-industrial by-products from the peels and pomaces of citrus fruit in ruminant livestock nutrition is huge although currently underexploited (Tayengawa and Mapiye, 2018). In low to middle income countries, this is due to lack of finance, skill, technology, and infrastructure (Tayengawa and Mapiye, 2018). Understanding their nutritive benefits would be of advantage to improve animal nutrition and health, animal welfare, lowering of feed production cost, as well as reducing the amounts of solid organic wastes in the environment.

Further to this, the secondary metabolites in citrus by-products exhibit potent antioxidant, antimicrobial, anti-inflammatory, anti-tumor, anti-clotting, antiatherogenic and anti-stress properties (Murakami et al., 2000; Lin et al., 2011; Dhanavade et al.,2011; Middleton and Kandaswami, 1994, Montanari et al., 1998,Samman et al., 1996), making them a good and economical replacement for medicinal plants whose production is laborious and expensive (Nezarati and Maheri, 2016).

They are also useful in modulating rumen microbial population and diversity, volatile fatty acids production and methane production as well as on nitrogen metabolism. They also have beneficial effects on rumen degradation of dietary protein and absorption of amino acids in the small intestine Hassanpour et al (2011). They are a suitable provenance of pectin (Sakai and Okushima, 1980). They serve as non-caloric bulking agents, that enhance water and oil retention, improve emulsion and could prevent a wide range of diseases caused by oxidative stress (Alnaimy et al., 2017).

1.3 Research Aim and Hypothesis

Efforts have been made to study the nutritive value of citrus pomace and peels using different citrus species and ruminants at different physiological stages (Zoiopoulos et al., 2008) among other studies. However there remains knowledge gaps in the nutritive values of the five citrus species as comparatively assessed (peels and pomaces) in this study. In addition, there is limited knowledge on the in-vitro digestibility of these citrus peels and pomaces and their potential use for feed modification.

This study aims at evaluating the nutritive value and in-vitro digestibility of peels and pomaces of five citrus species *Citrus sinensis* (Sweet orange) *Citrus reticulata* (Mandarin orange), *Citrus aurantium* (Bitter orange), *Citrus limon* (Lemon), *Citrus paradisi* (Grapefruit). The hypotheses of this study are that:

a. Citrus pomaces have higher nutritive value than citrus peels
b. Citrus pomaces have higher in-vitro digestibility (IVTD (DM) & NDFD%) than citrus peels
c. Agro-industrial by-products from citrus species can be used to modify ruminant nutrition.

1.4 Importance of research

Ruminant feeding systems based on locally available agro-industrial by-product are an economic, environmentally friendly, and practical alternative to conventional ruminant feeds and as such there has been increased interest in evaluation of their nutritive value (Alnaimy et al., 2017). The use of cheap, available and abundant sources of agro-industrial feeds such as citrus peels and pomaces is a very important strategy to consider in ruminant nutrition (Benchaar et al 2008;Tajodini et al.2014).

This research is important in obtaining nutritive value and in-vitro digestibility of the peels and pomaces of different species of citrus to give right information to nutritionist and livestock producers towards replacing or at least modifying conventional feed ration of ruminants with the use of citrus by-products.

CHAPTER II

REVIEW OF LITERATURE

2.1 Citrus as an agro -industrial by-product

Citrus fruits are consumed much more than any other fruit in the world (Satari and Karimi, 2018). Turkey ranks ninth among the citrus producing countries in the world with oranges accounting for 43% of citrus production, followed by (tangerines) mandarins, lemons grapefruits as shown in Figure 2.1 (TurkSTAT, 2019). The east Mediterranean accounts for 70% of citrus production in Turkey, 43% of these are consumed as fresh fruits with about 4% of citrus production going into juice making (Zema et al., 2018). The Turkish citrus industry in 2017 alone was reported to have generated citrus waste of about 147,960 Tonnes (FAO Stat, 2017).

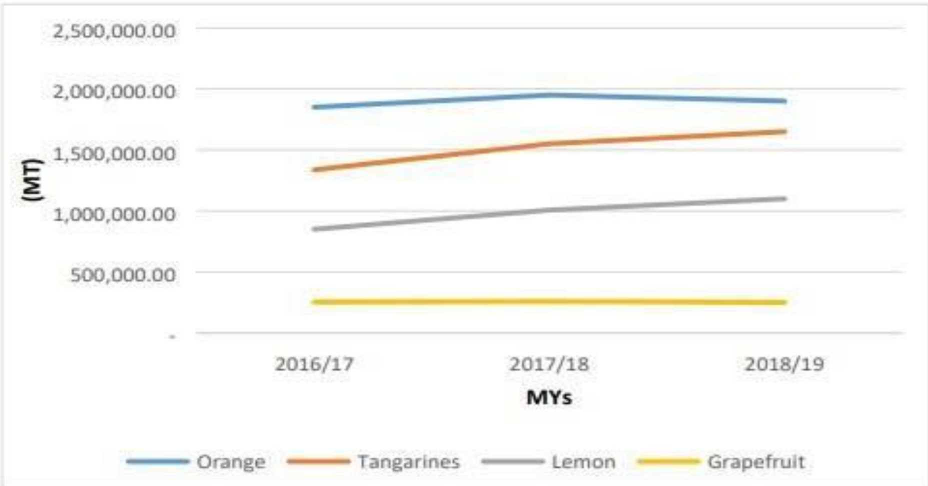

Figure 2.1. Turkish citrus production by products, marketing years (MY) 2016-2019 (TurkSTAT, 2019)

Citrus is the most produced and cultivated food in the world (Zema et al., 2018), Its fruits are largely consumed by humans and citrus processing is an important industry in the agro-industrial sector. It involves juice production; used in beverages and as food ingredients. Jam, marmalades, jelly, oils, essences, and food grade products (pectins and dietary fibers) are also produced. Peels and pomaces make up the bulk of citrus by-products generated, and it constitutes a major source of solid organic waste to the environment (Mamma and Christakopoulos, 2014).

Citrus pomace is a solid organic waste that consists of peels (60%-65% w/w), pulps (30%-35% w/w), different concentration of essential oils and seeds (Pascua and Carmonas,1980). Citrus cultivar and juice extraction system largely influences the total amount of citrus pomace that is generated as waste (Pascua and Carmonas, 1980; Marin et al., 2002). This ranges from 49% -69% w/w of the amount of fruit that is processed (Zema et al., 2018).

2.2 Physico-chemical composition of citrus

The physico-chemical composition of citrus peel is dependent on cultivar, fruit cultivation and ripening stage (Kale and Adsule, 1995). It comprises insoluble carbohydrates (cellulose, pectin), soluble sugars (glucose, fructose, sucrose), acids (majorly citric and malic acids, also tartaric, benzoic, oxalic and succinic acids), lipids (oleic, linoleic, linolenic, palmitic, stearic acids, glycerol and phytosterol), mineral elements (nitrogen, calcium and potassium), volatile compounds (alcohols, aldehydes, ketones, ester, hydrocarbons), flavonoids (hesperidin, naringin), limonoids (limonin, isolimonin), essential oils (mainly, up to 95%, composed of dlimonene), enzymes (pectinesterase, phosphatase, peroxidase), pigments (carotene, xanthophylls) and vitamin (ascorbic acid, complex B vitamin, carotenoids) (Di Giacomo and Calvarano, 1987; Bampidis and Robinson, 2006).

Citrus fruit source and its processing methods largely affects the values of the chemical composition of citrus by-products (Ammerman and Henry, 1991). Citrus peels are highly acidic with a P^H of 3.5-5.8 (Bampidis &Robinson, 2006; Zema et al., 2018), high water retention capacity of 4.33 L/kg (DM) and an osmotic pressure of 79 mOsm/L of water (Giger-Reverdin, 2000) due to the hygroscopicity of pectins. The water content in the citrus peels varies. The fresh citrus peels (w/w) have water content of 90%, the pressed biomass has a water content of 80% and dried citrus peel has 8-10% (Zema et al., 2018).

It also varies in bulk density and hydration rate as this is dependent on the particle size and storage techniques. Bulk density is approximately 900kg/m3 for fresh peel, 200-300 kg/m3 for dried biomass down to 100 kg/m3 for pressed and compacted peel (Zema et al., 2018). The knowledge of the physical characteristics of citrus such as the bulk density and hydration rate of citrus peels; before feeding to ruminants is of importance as it impacts on the feed intake of TMR (Total mixed ration) due to rumen fill (Bampidis & Robinson, 2006).

2.3 Citrus as a feed supplement

It is useful in the production of biofuels. Several studies had evaluated the use of citrus peels in the production of biomethane and bioethane in biochemical processes although further work still needs to be done to overcome the toxic effect of the essential oil on microogranims (Zema,2018). The presence of flavonoids, citric acid and other phytochemicals in citrus peels also makes it a promising product for use in the cosmetic and pharmaceutical industries although it is not economically sustainable (Zema et al., 2018).

In agriculture, citrus peels are used as substrate for organic compost production or as soil conditioner (Zema et al., 2018). Also, the liquid that percolates after pressing the citrus peels are subjected to concentration to produce molasses which is used as animal feed (Gohl,1978; Kimball 1999). Shedding, liming, and pressing of the citrus peels gives it a uniform texture and composition which makes it more appealing for animals (Bampidis and Robinson, 2006; Kimball 1999; Gohl, 1978). In sheep nutrition, orange (*Citrus sinesis*) has been used as a food flavor (Ralphs et al., 1995).

The two clear options for citrus waste disposal are usually burning or adoption as feed supplements. Despite the potentials of citrus as feed additives, the limitation to its use remains its high moisture and low crude protein (Mamma and Christakopoulos, 2014). Citrus peels are usually conserved and stored through drying. They are first crushed into coarse pieces and then into small pieces. The biomass is mixed with lime to reduce hygroscopicity and further enhance drying. Drying takes place through direct steaming after pressing. Citrus molasses is produced from the liquid mass obtained, while the pressed and dried citrus peels and pomaces are milled (Zema et al., 2018) and used as fodder for livestock (Cevolani, 2016).

2.4 Nutritive value and digestibility of citrus by-products

In-vivo, in-situ and in-vitro techniques are the three methods for evaluation of feed digestibility. It is widely known that in-vivo technique of feed evaluation is the most accurate method, but the application is limited because it is laborious and time consuming (Oliveira et al., 2014). In-situ techniques are also important as Palangi et al (2013) used this method on four species (Orange, lemon, grapefruit, and Tangerine) of dried citrus pulp on two wethers and showed that citrus pulps have high energy feed potentials that can be adopted either for growth or lactation of ruminants.

Spanghero et al. (2010) compared in-situ and in-vitro methodologies with aim to determine precision and accuracy of digestibility parameters for fibrous feeds. They asserted that in-vitro techniques for digestibility studies are simple, fast, accurate, repeatable, and reproducible. In a more recent review of feed evaluation methods, Olowu and Yaman Fırıncıoğlu (2019) opined that the choice of evaluation techniques should be expanded beyond performance to include economy (associated costs) and environmental footprint.

Improving the nutritional value and digestibility of citrus by-products for utilization in ruminant nutrition is a subject of recent focus (Bampidis and Robinson, 2006) perhaps attributable to the global citrus production and its abundance as an agro-industrial waste (FAOstat,2017; Mamma and Christakopoulos, 2014).

Several literatures have reported varying nutritive values (Table 2.1) for the five species considered in this study. The gap with these reports is in that none was in a single study having the five species. More so, the reports on the digestibility of the five citrus species (Table 2.2) considered in this study are limited. The obvious gap is fewer digestibility studies conducted on these citrus species and differences in the digestibility of pomaces and peels of citrus species.

Table 2.1. Reported nutritive values for five citrus species (by-products)

Citrus Species	DM	XA	CP	CF	NDF	ADF	EE	References
Peels								
Bitter orange	NA	8.9	6.9	NA	NA	NA	1.06	Atta & El shenawi (2012)
Grape fruit	87.66	3.29	5.78	10.68	NA	NA	0.8	Vlaicu et al (2020),
Mandarin	96.21	3.96-10.03	2.16-8.55	7.14-27.89	NA	NA	1.57-2.97	Marin et al (2007), Magda et al (2008), Ghanem et al (2012), Gorinstein et al (2001), Figuerola et al (2005),
Lemon	90.42-96.99	2.52-6.26	5.87-9.42	12.47-15.18	NA	NA	0.48-4.98	Marin et al (2007), Janati et al (2012), Ghanem et al (2012), El-ghfar et al (2016)
Orange	88.9-96.86	2.56-4.24	1.79-9.06	6.3-13.9	10	7.6	0.8-4	Marin et al (2007), Magda et al (2008), Kammoun et al (2011), Ghanem et al (2012), M'hiri et al (2015), El-ghfar et al (2016), Castrica et al (2019), Vlaicu et al (2020)
Pulp/ Pomace								
Bitter orange	93.1	6.44	8.25	NA	21.23	17.04	2.6	Lashkari and Tagizadeh (2013)
Grape fruit	87.34-90.91	4-5.87	8.01-9.14	NA	16.66-20.9	13.08-17.6	1-2.38	Lashkari and Tagizadeh (2013), Palangi et al (2013)
Mandarin	88.72-91.02	4.8-6.78	6.64-6.99	NA	11.38-22	8.48-17.9	1.6-3.51	Lashkari and Tagizadeh (2013), Palangi et al (2013), Ozkan et al (2017)
Lemon	87.1-90.69	4.15-6.9	7.27-9.54	NA	16.68-21.97	15.1-19.45	1.03-3.49	Lashkari and Tagizadeh (2013), Palangi et al (2013), Nagarajaiah and Prakash (2016), Ozkan et al (2017), Beyzi et al 2018
Orange	88.73-93.2	1.2-5.5	4.26-8.68	NA	14.47-21.4	11.95-18.3	0.9-3.44	Lashkari and Tagizadeh (2012), Palangi et al (2013), Ozkan et al (2017), Beyzi (2018)

DM- Dry matter, **XA-**Ash content, **CP-** Crude protein, **CF-** Crude fiber,
NDF- Neutral detergent fiber **ADF-** Acid detergent fiber**, EE-** Ether extract

Table 2.2. Reported digestibility values for five citrus species (by-products)

Citrus Species	IVTD(DM)	NDF	NDFD%	References
Peels				
Bitter orange	87	NA	NA	Lashkari and Tagizadeh (2013)
Grapefruit	NA	NA	NA	
Mandarin	NA	NA	NA	
Lemon	NA	NA	NA	
Orange	NA	NA	NA	
Pulp/Pomace				
Bitter orange	84.8	NA	NA	Lashkari and Tagizadeh (2013)
Grapefruit	NA	NA	NA	
Mandarin	NA	NA	NA	
Lemon	85.76-91.2	NA	NA	Tripodo et al (2004), Lashkari and Tagizadeh (2013)
Orange	89.0-90.6	NA	57.6- 69.8	Aregheore (2000), Tripodo et al (2004), Lashkari and Tagizadeh (2013), Alnaimy et al (2017)

IVTD(DM)- In-vitro true digestibility dry matter **NDF**- Neutral detergent fiber
NDFD- Neutral detergent fiber disappearance/digestibility

Taiwo et al (1995) observed that unfermented citrus pulp had high glucose content with low levels of other nutrients, however when fermented for 61days there was production of lactic and acetic acids and this increased ammonia holding capacity of unfermented citrus pulp from 0.1NH_3/Kg DM in unfermented citrus pulp to a significant amount of 10.6NH_3/Kg DM and 16.4 NH_3/Kg DM in fermented citrus pulp without molasses and with molasses, respectively. Fermentation of citrus pulp also resulted in the increase of its buffering capacity by over 5000% without molasses and over 8000 % with molasses more than what was obtained for the unfermented citrus pulp.

Most citrus by-products are low in nitrogen content and neutral detergent fiber (NDF) however, the possibility of enhancing nitrogen content of citrus by-products through ammoniation with urea or supplementation in the ration shows that their nutritive value and digestibility can be enhanced to suit the animal’s diet (Bampidis and Robinson, 2006).

CHAPTER III

MATERIALS & METHODS

3.1 Procurement and sample preparation

The determination of the nutritive value and the in-vitro digestibility of five species of citrus peels and pomace were determined in the animal nutrition laboratory of the Ayhan Sahenk Faculty of Agricultural Science and Technologies in Niğde Ömer Halisdemir university, Turkey.

Five (5) kg each of *Citrus sinesis* (sweet orange), *Citrus limon* (lemon), *Citrus reticulata* (mandarin), *Citrus paradisi* (grapefruit) fruits were procured from the local market in Nigde city while five (5) kg of *Citrus aurantium* (bitter orange) was obtained from Adana in Turkey.

3.1.1 Preparation of citrus peels and pomaces

All citrus samples were at commercial maturity, edible and of good quality. 2.5 kg of each species of citrus fruits purchased were used for peel preparation while the other 2.5 kg was used for pomace preparation. The pomace was made up of the peels and the pulps. The fruits were washed and cut into half with a kitchen knife. A manual juice extractor was used to extract the juice from the citrus peels.

The sample for citrus peel preparation had their peels removed with the aid of a knife while the samples for citrus pomace were left with their peels after juice extraction. The peels and pomace were then cut into small pieces of about 1-2 cm and then placed in a labeled nylon bag. Each citrus sample was placed in a pre-weighed aluminum dish (tare) and weighed using an electric weighing scale.

The weighed samples were placed in the oven at a temperature of 50 ^{o}C for 48 h. After 48 h in the oven they were removed and placed on the bench for 24 h to cool. The samples were weighed and grinded using a 1mm sieve at a speed of 1400 rpm. They were then stored in pre-labelled plastic bags prior to chemical analysis.

3.2 Chemical Analysis

Citrus peels and pomaces were analyzed for dry matter (DM) and crude ash (XA) according to the AOAC (1995). The Nitrogen (N) content of peels and pomaces were determined using the Kjeldahl method (AOAC, 1995). Crude protein of citrus peels and pomaces were estimated with N*6.25. Crude fiber (CF), neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents of peels and pomaces were analyzed using the method of Van Soest (1991). All chemical analyses were carried out in duplicates.

3.2.1 Dry matter determination

Crucibles were cleaned and oven dried for 2 h to a constant weight (W1). 1 g each of peel and pomace sample from the five citrus species were weighed using an analytical balance and recorded as (W2) and placed inside the pre-weighed crucibles. The samples were then placed in the oven with the aid of a tong and allowed to dry at a temperature of 105 ^oC for 24 h.

After 24 h, they were removed from the oven and placed in the desiccator for 20 min to cool to room temperature. The weight of the crucibles containing the samples (peels and pomaces) were taken immediately and recorded as (W3). The dry matter was then calculated in percentage using Equation (3.1)

$$\% \text{ dry matter} = (W3 - W1)/W2 * 100 \quad (3.1)$$

3.2.2 Crude ash content

Crude Ash content was determined using the Muffle’s furnace. The crucibles containing the peel and pomace samples used to determine dry matter with weight W3 were placed in the muffle’s furnace for 4 h at a temperature of 600^oC. After ashing, the samples were removed and placed into the desiccator for 20 min to cool to room temperature. The final weight of crucible and sample was then taken and recorded as W4. The ash content of each citrus peels and pomaces was calculated using Equation (3.2). Organic matter was also calculated by subtracting the % ash content from 100%.

$$\% \text{ ash content} = (W4 – W1)/W2*100 \quad (3.2)$$

3.2.3 Crude protein

Crude protein of the samples was estimated using the Kjelroc digestion unit and Opsis liquid line Kjelroc analyzer made in Sweden. 1g each of peels and pomaces from the five citrus species was put into an Opsis tube in duplicates. Two kjedahl tablets and 12 mL of H_2SO_4 was added into each tube. The tubes were placed in the digester block and the protein digester was set at 420 ^oC for 60 min. The suction was regulated to neutralize the fumes that were released during the digestion process. After the heating period was completed, the block cooled for 15 min.

The protein content was then estimated using the Opsis liquid line Kjelroc analyzer through distillation and titration. Large kegs of distilled water, 40% sodium hydroxide solution, and 1% boric acid with mixed indicators (100 mg bromocresol green and 70 mg methyl red dissolved separately in 100 mL methanol) were attached to Kjelroc Analyzer. The machine was auto cleaned, and blank was determined before analyzing the samples for crude protein.

The Kjedahl program was set at % protein with a nitrogen factor of 6.25 i.e., Crude protein = N*6.25. The weight of sample (1g) in each tube was imputed into the machine display screen and the tubes with corresponding weights were analyzed for crude protein. The crude protein value for the sample in each tube was displayed on the screen on completion of titration and distillation. These values were recorded as crude protein content for the samples.

3.2.4 Crude fiber analysis

Crude fiber was estimated using ANKOM 200 fiber analyzer. Each of the 10 samples were run in duplicates. Twenty F57 filter bags were labelled with a solvent resistant marker. The bags were weighed (W1), and the balance was zeroed. One gram each of grinded peel and pomace of each species of citrus was weighed (W2) directly into the pre- weighed F57 filter bags. The bags were sealed using a heat sealer. A blank bag was also weighed and included in the run to determine blank bag correction.

The bags were soaked in petroleum ether for 10 min after which the solvent was discarded, and the bags were placed on a filter paper for 20 min to dry completely. The samples were then spread uniformly in the bag through shaking and flicking to prevent clumping. The bags were placed in the analyzer trays in threes and trays were stacked in the bag suspender. The bag suspender was placed in the fiber analyzer vessel with the suspender weight was placed on top to keep the suspender submerged.

2000 mL of 0.255N H_2SO_4 solution at ambient temperature was poured into the vessel. The vessel was closed, and extraction process was started by turning on the agitate and heat button for 40 min. After extraction, the solution was released from the vessel through the exhaust pipe and hot water rinse was performed twice for 5 min each. After the hot water rinse, the exhaust valves were closed and 2000 mL of 0.313N NaOH solution at ambient temperature was poured into the vessel.

The vessel lid was closed, the agitate and heating was started, and the samples were extracted for 40 min. At the end of the extraction, the solution was released through the exhaust pipe. The lid was opened, and 2000 mL of hot water was used to rinse the samples again twice for 5 min each. After completion of the rinse, the samples were removed, and excess water was pressed out with a gentle tap of the bags. The bags were then soaked in acetone for 5 min after which they were completely air-dried for 20 min.

The bags were placed in the oven to completely dry at a temperature of 105 ^oC for 2 h. They were removed from the oven after 2 h and placed in a desiccant pouch for 20 min to cool to ambient temperature. The bags were afterwards weighed and placed in pre-weighed crucibles They were placed in the muffles furnace for 2 h at 600^oC after which they were placed in the desiccator for 20 min to cool to room temperature. An analytical balance was then used to measure the weight and the loss of weight of organic matter (W3) was then determined. Percentage crude fiber was calculated with Equation (3.3).

$$\% \text{ Crude fiber} = 100 * (W3-(W1*C1))/W2 \qquad (3.3)$$

where: W1 = filter bag tare weight

W2 = Sample weight

W3 = Weight of organic matter (Loss of weight on ignition of bag and fiber), C1 = Ash corrected blank bag factor (loss of weight on ignition of blank bag/original blank bag)

3.2.5 Neutral detergent fiber

Neutral detergent fiber (NDF) was estimated using ANKOM 200 fiber analyzer. Twenty F57 filter bags were labelled with a solvent resistant marker and then weighed (W1) with an analytical balance. The balance was zeroed and 0.55 g each of citrus peel and pomace sample was weighed directly into the filter bag. The bags were tapped gently for uniform spreading of samples and elimination of clumping. The bags were then sealed within 4 mm of the top using a heat sealer. One blank bag was also weighed to include in the run for determination of blank bag correction (C1).

NDF solution was prepared using, 30g Sodium dodecyl sulfate, 18.61g ethylenediaminetetraacetic disodium salt, 6.81g Sodium borate, 4.56g Sodium phosphate dibasic (anhydrous), and 10.0 mL triethylene glycol mixed in 1 L distilled H2O to make up one litre solution. 20 g of Sodium sulphite (Na_2SO_3) and alpha-amylase was also used in the extraction process. The bags were placed into the bag suspender. Three bags were arranged per tray and the trays were stacked such that each tray was rotated at 120^o.

The bag suspender was then inserted into the fiber analyzer vessel and the suspender weight was placed on top to keep the bag suspender submerged. 2000 mL of ambient ND solution was prepared and poured into the fiber analyzer vessel. 20g of Na_2SO_3 and 4.0 mL of alpha amylase was added to the solution in the vessel. To start the digestion process, the agitate and heat button was turned on. Agitation was confirmed and the lid was closed. The timer was set to 75 min. At the end of extraction, the heat and agitate button was turned off and the drain valve was opened to allow exhaust of the hot solution before the lid was opened.

After releasing the solution, 2000 mL of water at 90^{o}C with 4.0 mL of alpha-amylase was added to the vessel for first and second rinses. Each rinse was for 5 min. Tap water without alpha-amylase was then used for the third rinse which was also for a 5 min duration. On completion of the rinsing process, the samples were removed, and the bags were tapped gently to remove excess water. The bags were soaked in acetone for 5 min and then completely air-dried for 20 min on filter paper. The bags were then placed in the oven to dry completely at 105 ^oC for 2 h after which, the bags were directly placed into a collapsible desiccant pouch for 20 min to cool to ambient temperature. With the use of an analytical balance, the bags were weighed (W3) and percentage NDF (as received basis) was calculated using Equation (3.4)

$$\% \text{ NDF (as received basis)} = (W3-(W1*C1))/W2*100 \quad (3.4)$$

Where W1=Bag tare weight,

W2 = Sample weight,

W3 = Dried weight of bag with fiber after extraction process,

C1 = Blank bag correction (final oven – dried weight divided by the original blank bag weight)

3.2.6 Acid detergent fiber

Acid detergent fiber (ADF) was also estimated using ANKOM 200 fiber analyzer. Twenty F57 filter bags were labelled with a solvent resistant marker and then weighed (W1) with an analytical balance. The balance was zeroed and 0.50 g (W2) each of citrus peels and pomaces sample was weighed directly into the filter bag. The bags were flicked gently for uniform spreading of samples and elimination of clumping. The bags were then sealed within 4 mm of the top using a heat sealer. A blank bag was also weighed to include in the run for determination of blank bag correction (C1).

The bags were placed on the trays in the bag suspender with three bags arranged on each tray. The trays were stacked such that each tray was rotated at 120^o. All the nine trays were placed in the bag suspender and the bag suspender was then inserted into the fiber analyzer vessel. The suspender weight was placed on top to keep the bag suspender submerged. ADF solution was prepared using, 20 g cetyl trimethylammonium bromide (CTAB) with 1 L of 1.00N H_2SO_4. 2 L of ADF solution was poured into the fiber analyzer vessel and the lid was closed. The digestion process was set for 75 min and it was started by pressing the agitate and heat button.

On completion of the digestion process, the solution was released through the exhaust pipe. The samples in the vessel were rinsed with hot water twice for 5 min each time. A third rinse was carried out using tap water also for a 5 min duration. After the rinse, the bags were removed, and excess water was pressed out from the bags by gentle tapping. Following this, the bags were soaked in acetone for 5 min after which they were removed and completely air-dried for 20 min on filter paper.

The bags were then placed in the oven for 2 h at 105 ^{o}C to dry completely. After 2 h, they were removed from the oven and immediately placed in a collapsible desiccant pouch for 20 min to cool to ambient temperature. The bags were weighed W3 and percentage ADF (as received basis) was calculated using Equation (3.5)

$$\% \text{ ADF (as received basis)} = 100 * (W3 - (W1*C1))/ W2 \tag{3.5}$$

Where W1 = Bag tare weight

W2 = sample weight

W3 = dried weight of bag with fiber after extraction process

C1 = Blank bag correction (running average of final oven- dried weight divided by original blank bag weight).

3.3 In-vitro true digestibility

In-vitro true digestibility (IVTD) of the five species of citrus peel and pomace samples were determined using DAISY II incubator by ANKOM Technology (Macedon, NY). Daisy II Incubator has four incubation jars with a capacity of 2000 mL each. 1600 mL buffer solutions and 400 mL rumen liquor was added into each jar. The filter bags were placed in the jars.

3.3.1 Preparation of filter bags and samples

Ankom F57 filter bags were rinsed in acetone for 3 min and completely air-dried. Each bag was then weighed, and the weight was recorded as (W1). The balance was zeroed, and 0.5 g of sample (W2) was weighed directly into the filter bag. Empty filter bags were weighed and sealed as blank for correction factor. Afterwards, the bags were heat sealed using an impulse bag sealer. The experiment was run in three batches with duplicates of each of the ten citrus samples (peels and pomaces).

3.3.2 Preparation of (combined Buffer Solution A and B)

Buffer A and Buffer B solutions were prepared a night before the incubation using the reagents and concentrations in Table 3.1. A volume of 266 mL of solution B (Basic solution) was added to 1330 mL of Solution A (Acidic solution). The exact amount of solution A to be added to solution B was adjusted with the use of a pH meter to obtain a final pH of 6.8 at 39^oC.

3.3.3 Preparation of Inoculum

The rumen liquor (inoculum) was collected postmortem from the rumens of two mature Holstein cattle that were slaughtered in a commercial abattoir in Niğde. Two 2000 mL thermos flask preheated at 39 ^oC and purged with CO_2 was used to store the collected rumen liquor. Two fistfuls of fibrous mat from the rumen of the cattle were added to one of the flasks after which the thermos flasks were taken to the laboratory. The rumen liquor was poured into a blender that was preheated to 39 ^oC.

Table 3.1. Composition of reagents for Buffer solution A and B

Reagents	**g/L**
(a) Buffer Solution A	
KH_2PO_4	10
$MgSO_4.7H_20$	0.5
NaCl	0.5
$CaCl_2.2H_20$	0.1
Urea (reagent grade)	0.5
(b) Buffer Solution B	**g/L**
Na_2CO_3	15
$Na_2S.9H_20$	1

The liquor was purged with CO_2 gas for 30 sec and then blended at a high speed for 30 sec. The blended digesta was then filtered through four layers of cheesecloth into a five-liter flask that was preheated at 39 ^{o}C. The residual rumen fluid in the other thermos was also filtered through four fresh layers of cheesecloth into the same five-liter flask. The rumen digesta in the five liters flask was continually purged with CO_2 until inoculum was transferred into the digestion jars.

3.3.4 Incubation of samples

1600 mL of the resultant Buffer solution (A and B) and 400 mL of rumen inoculum was poured into each daisy II digestion jar after which each digestion jar was purged with CO2 for 30s. The Filter bags with sealed blank bags for correction factor (C1) were then evenly distributed into three daisy II jars. The digestion jars were purged with CO_2 and tightly sealed. All the digestion jars were placed into the pre-heated Daisy II incubator and incubated for 48h at a temperature of 39^{o}C±0.5.

After 48 h of incubation, the bags were removed from the digestion jars and rinsed with minimal mechanical agitation with cold tap water until water was clear to stop the microbial activity. The bags were placed in sealable bags and stored in the refrigerator before NDF determination. ANKOM200 fiber analyzer was used to determine NDF of the samples and the weight after determination of NDF was recorded as W3. The percentage in-vitro true digestibility on as received and dry matter basis was then calculated using Equations (3.6) and (3.7)

$$\% \text{ IVTD (as received basis)} = 100 - (W3-(W1*C1)/W2*100 \quad (3.6)$$

$$\% \text{ IVTD (DM basis)} = 100 - (W3-(W1*C1)/(W2*DM) *100 \quad (3.7)$$

Where W1 = Bag tare weight, W2 = Sample weight, W3 = Final bag weight after in-vitro and sequential ND treatment, C1 = Blank bag correction (final oven-dried weight/original blank bag weight)

3.3.5 Neutral detergent fiber

Neutral detergent fiber (NDF) of the digested samples was estimated using ANKOM 200 fiber analyzer. The bags containing the digested samples were placed into the bag suspender. Three bags were arranged per tray and the trays were stacked such that each tray was rotated and properly placed. The bag suspender was inserted into was then inserted into the fiber analyzer vessel and the suspender weight was placed on top to keep the bag suspender submerged. 2000 mL of ambient ND solution was prepared and poured into the fiber analyzer vessel. 20 g of Na2SO3 and 4.0 mL of alpha-amylase was added to the solution in the vessel.

To start the digestion process, the agitate and heat button was turned on. Agitation was confirmed and the lid was closed. The timer was set to 75 min. At the end of extraction, the heat and agitate button was turned off and the drain valve was opened to allow exhaust of the hot solution before the lid was opened. After releasing the solution, 2000 mL of water at 90 ^{o}C with 4.0 mL of alpha -amylase was added to the vessel for first and second rinses. Each rinse was for 5 min. Tap water without alpha-amylase was then used for the third rinse which was also for a 5 min duration.

On completion of the rinsing process, the samples were removed, and the bags were tapped gently to remove excess water. The bags were soaked in acetone for 5min and then completely air-dried for 20 min on filter paper. The bags were then placed in the oven to dry completely at 105 ^{o}C for 2 h after which, the bags were directly placed into a collapsible desiccant pouch for 20 min to cool to ambient temperature. With the use of an analytical balance, the bags were weighed (W3) and percentage NDF (as received basis) was calculated using Equation (3.8)

$$\% \text{ NDF (as received basis)} = (W3-(W1*C1))/W2*100 \qquad (3.8)$$

Where W1=Bag tare weight, W2 = Sample weight, W3 = Dried weight of bag with fiber after extraction process, C1 = Blank bag correction (final oven – dried weight divided by the original blank bag weight)

3.4 Statistical Analysis

Statistical validity of normality, homogeneity and independence for this research was ensured by subjecting the results of chemical parameters to Kruskal-Wallis' non-parametric one-way ANOVA while Tukey's multiple comparison test for ANOVA was used to analyse the effects of species, peels, and pomaces on in-vitro digestibility.

All analyses were carried out on Jamovi, a statistical analysis package. Statistical significance was reported at $P \leq 0.001$ and results obtained from these analyses were reported as tables and graphs.

CHAPTER IV

RESULTS

4.1 Chemical analysis (Nutritive value)

There was no statistical significance in the mean of the dry matter, ash content, crude protein, crude fiber, neutral detergent fiber, and acid detergent fiber obtained for the peels and pomaces of the five citrus species at ($P > 0.05$) using the Kruskal Wallis, one-way ANOVA on ranks (a non- parametric method). By ranking the means (Table 4.1a and 4.1b), bitter orange peels had highest dry matter among citrus peels while mandarin pomace had the highest dry matter among citrus pomaces. Also, bitter orange had the highest ash content while lemon had the highest crude protein, crude fiber, neutral detergent fiber, and acid detergent fiber for both the peels and pomaces. Appendix I shows the comprehensive result of analysis for chemical parameters of all samples.

4.1.1 Dry matter

All the citrus peels and pomaces had dry matter of over 91%. Bitter orange peels and mandarin peels had higher dry matter compared to the peels of other citrus species and lemon peels had the lowest dry matter of 91.46% (Table 4.1a). Among the pomaces of citrus species, mandarin had the highest dry matter while lemon had the lowest.

4.1.2 Ash content

The ash content of the five citrus peels and pomaces ranged from 3.57% – 5.52% (Table 4.1a). Bitter orange species generally had the highest ash content for both peels and pomaces among the five citrus species. Conversely, grapefruit peels and pomaces showed the lowest ash content.

4.1.3 Crude protein

The peels and pomaces of the citrus species ranged from 2.7%- 5.78%. Lemon peels and pomace had the highest crude protein values of 7.38% and 7.78% (Table 4.1a). The lowest crude protein values of 3.65% and 2.7 % were found in bitter orange peels and pomace, respectively.

4.1.4 Crude fiber

Crude fiber of the five species of the citrus peels and pomaces ranged from 10.60 - 15.25% as seen in Table 4.1b. Lemon peels and pomace had the highest crude fiber value. The lowest crude fiber was estimated in bitter orange peels and in grapefruit pomace.

4.1.5 Neutral detergent fiber

The Neutral detergent fiber (NDF) for the peels and pomaces of the five citrus species ranged from 13.58% - 20.51% as seen in Table 4.1b. Lemon peels and pomace had the highest NDF of 20.51% and 20.21% respectively. Orange peels had the lowest NDF compared to peels of other species while among pomaces, grapefruit had the lowest NDF of 13.58%.

4.1.6 Acid detergent fiber

Acid detergent fiber (ADF) for the peels and pomaces of the five citrus species ranged from 10.77% -14.97%. The highest ADF values of 14.97% and 14.63% was found in lemon peels and pomaces. Orange had the lowest ADF among peels while grapefruit had the lowest among pomaces.

Table 4.1a. Chemical composition of peels and pomaces of five Citrus Species (%)

Unit	Species	Samples	Codes	DM	XA	CP
1	Orange	Pomace	OPP	91.68±0.02	4.33±0.008	5.48±0.02
2	Orange	Peel	OPE	91.67±0.09	4.51±0.02	4.98±0.05
3	Lemon	Pomace	LPP	91.11±0.16	5.1±0.02	**7.78±0.03**
4	Lemon	Peel	LPE	91.46±0.001	5.01±0.01	**7.38±0.14**
5	Mandarin	Pomace	MPP	**93.08±0.01**	3.91±0.004	5.71±0.03
6	Mandarin	Peel	MPE	**93.52±0.02**	4.29±0.01	4.59±0.13
7	Grapefruit	Pomace	GPP	91.56±0.04	3.57±0.04	4.62±0.04
8	Grapefruit	Peel	GPE	92.09±0.03	3.72±0.03	4.76±0.12
9	Bitter Orange	Pomace	BOPP	91.58±0.02	**5.52±0.04**	3.65±0.09
10	Bitter Orange	Peel	BOPE	**93.58±0.04**	**5.28±0.003**	2.7±0.61

Chemical parameters key:DM = Dry matter; XA = Ash content; CP = Crude protein; CF = Crude fiber NDF = Neutral detergent Fiber; ADF = Acid detergent Fiber

Table 4.1b. Chemical composition of peels and pomaces of five Citrus Species (%)

Unit	Species	Samples	Codes	CF	NDF	ADF
1	Orange	Pomace	OPP	10.6±0.50	14.54±0.15	10.85±0.003
2	Orange	Peel	OPE	11.8±0.10	16.33±0.25	12.13±0.20
3	Lemon	Pomace	LPP	**15.25±1.25**	**20.21±0.15**	**14.97±0.06**
4	Lemon	Peel	LPE	**14.65±0.55**	**20.51±0.01**	**14.63±0.02**
5	Mandarin	Pomace	MPP	12.1±1.70	14.6±0.08	11.33±0.09
6	Mandarin	Peel	MPE	12.55±1.85	17.23±0.90	12.63±0.17
7	Grapefruit	Pomace	GPP	10.15±0.55	13.58±0.10	10.77±0.07
8	Grapefruit	Peel	GPE	12.65±0.85	17.45±0.04	12.93±0.05
9	Bitter Orange	Pomace	BOPP	11.7±0.10	15.78±0.10	12.41±0.06
10	Bitter Orange	Peel	BOPE	13.5±0.20	18.05±0.05	14.09±0.02

4.2 In-vitro true digestibility of citrus peels and pomaces

There were significant effects of species and samples for the in-vitro dry matter digestibility IVTD(DM), neutral detergent fiber (NDF) and neutral detergent fiber disappearance (NDFD%) between the five species of citrus in this research.

Table 4.2. In-vitro Digestibility of peels and pomaces of five Citrus Species (%)

Unit	Species	Samples	Codes	IVTD(DM)	NDF	NDFD
1	Orange	Pomace	OPP	$94.8{\pm}0.38^{b}$	$5.29{\pm}0.39^{f}$	$63.8{\pm}2.39^{ab}$
2	Orange	Peel	OPE	$94.3{\pm}0.38^{b}$	$5.79{\pm}0.39^{ef}$	$64.6{\pm}2.39^{ab}$
3	Lemon	Pomace	LPP	$92.1{\pm}0.38^{d}$	$8.15{\pm}0.39^{d}$	$59.7{\pm}2.39^{b}$
4	Lemon	Peel	LPE	$90.1{\pm}0.38^{e}$	$9.32{\pm}0.39^{a}$	$54.6{\pm}2.39^{bc}$
5	Mandarin	Pomace	MPP	$93.2{\pm}0.38^{c}$	$6.87{\pm}0.39^{e}$	$52.9{\pm}2.39^{c}$
6	Mandarin	Peel	MPE	$92.7{\pm}0.38^{cd}$	$7.35{\pm}0.39^{de}$	$57.3{\pm}2.39^{bc}$
7	Grapefruit	Pomace	GPP	$94.7{\pm}0.38^{b}$	$5.44{\pm}0.39^{f}$	$60.0{\pm}2.39^{b}$
8	Grapefruit	Peel	GPE	$92.0{\pm}0.38^{d}$	$8.25{\pm}0.39^{c}$	$52.7{\pm}2.39^{bc}$
9	Bitter Orange	Pomace	BOPP	$96.4{\pm}0.38^{a}$	$3.74{\pm}0.39^{g}$	$76.3{\pm}2.39^{a}$
10	Bitter Orange	Peel	BOPE	$91.6{\pm}0.38^{de}$	$8.51{\pm}0.39^{b}$	$52.8{\pm}2.39^{bc}$

Note: a-g superscripts represent points of statistical significance at $P \leq 0.001$

4.2.1 In-vitro true dry matter digestibility

Lemon had the lowest in-vitro dry matter digestibility of 90.9% of the peel samples while orange (94.3%) had the highest of the peel samples at ($P \leq 0.001$). Among the pomace samples, lemon had the lowest in-vitro dry matter digestibility of 92.1% while bitter orange had the highest (96.4%). At $P \leq 0.001$, there was a significant effect among the five citrus species.

The high statistical significance between species was seen with bitter orange vs lemon; grapefruit vs lemon; grapefruit vs orange; lemon vs mandarin; lemon vs orange; and mandarin vs orange. Also, between the samples (peel and pomace) there was highly significant effect ($P \leq 0.001$).

4.2.2 Neutral detergent fiber

Orange had the lowest NDF of 5.79% among the peel samples while lemon samples (9.32%) had the highest within the same sample at (P ≤0.001). Among the pomace samples, bitter orange had the lowest NDF of 3.74% while lemon had the highest (lemon) at (P ≤0.001).

At P ≤0.001, there was a significant effect among the five citrus species. The high statistical significance between species was seen with bitter orange vs lemon; grapefruit vs lemon; lemon vs mandarin; lemon vs orange and mandarin vs orange. Also, between the samples (peel and pomace) there was highly significant effect (P ≤0.001).

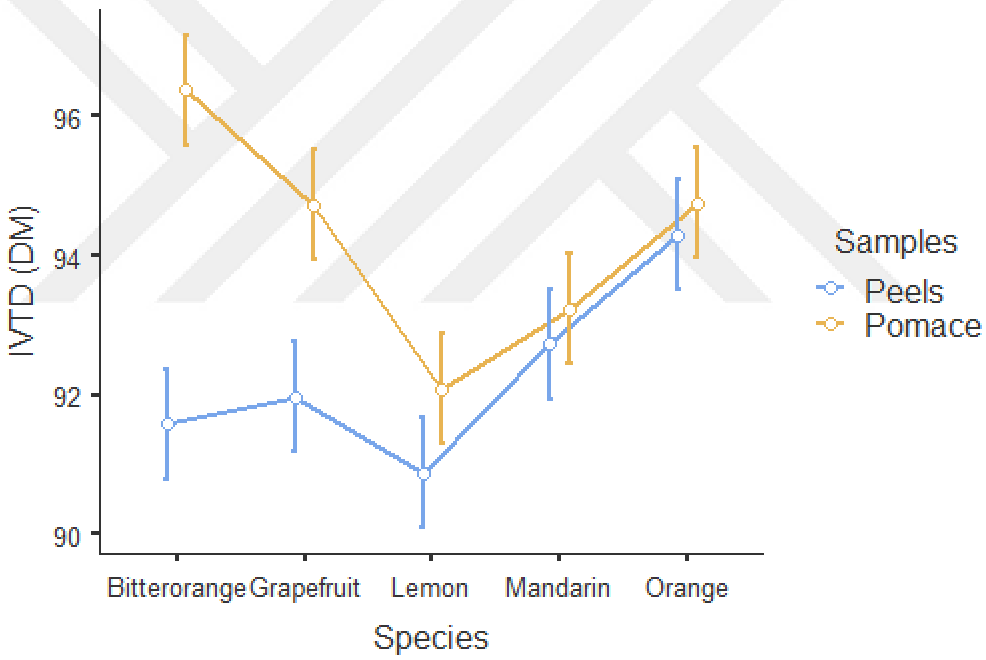

Figure 4.1. Graph showing the in-vitro true dry matter digestibility of five citrus species and samples

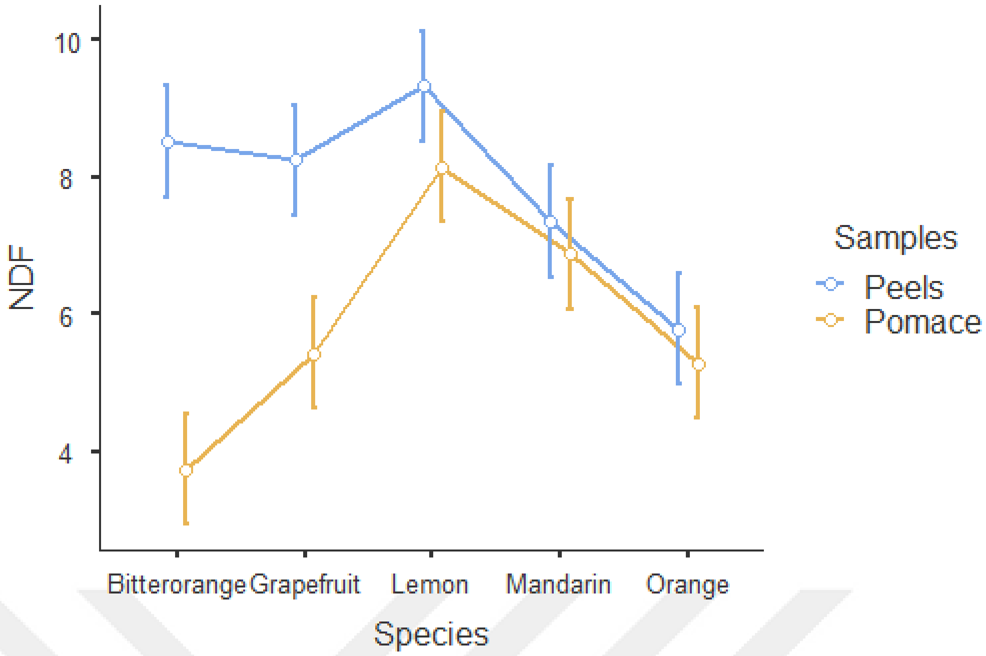

Figure 4.2. Graph showing the neutral detergent fiber (NDF) of five citrus species and samples

4.2.3 Neutral detergent fiber digestibility

Grapefruit had the lowest Neutral detergent fiber disappearance (NDFD%) of 52.7% of the peel samples while orange (64.6%) had the highest of the peel samples at ($P \leq 0.001$). Among the pomace samples, mandarin had the lowest NDFD% of 52.9% while bitter orange had the highest (76.3%) at ($P \leq 0.001$).

At $P \leq 0.001$, there was a significant effect among the five citrus species. The statistical significance between species was seen with bitter orange vs grapefruit; bitter orange vs lemon; grapefruit vs orange; lemon vs orange; and mandarin vs orange. Also, between the samples (peel and pomace) there was highly significant effect ($P \leq 0.001$).

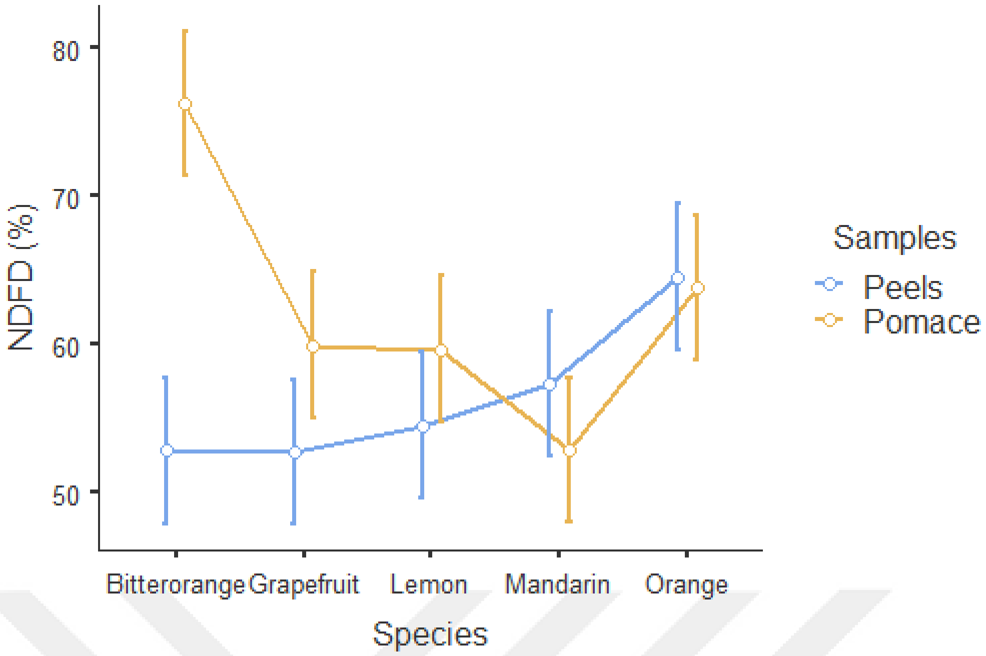

Figure 4.3: Graph showing the in-vitro NDFD% of five citrus species and samples

CHAPTER V

DISCUSSION

5.1 Chemical analysis (Nutritive value)

This study shows that citrus pomaces do not have higher nutritive value than citrus peels as there was no statistical significance in the mean of dry matter, ash content, crude protein, crude fiber, neutral detergent fiber, and acid detergent fiber obtained for the peels and pomaces of the five citrus species at ($P > 0.05$). Further to this, the peels of bitter orange and mandarin had the highest dry matter (Table 4.1) amongst all the peels and pomaces together.

Lemon peels also had the highest NDF (Table 4.1) value amongst the peels and pomaces of all the citrus species hence the hypothesis that citrus pomace have higher nutritive value than peel is rejected. The differences in the values obtained for the chemical parameters of the different citrus peels and pomaces can be due to the fruit source, maturity stage and methodology (Ammerman and Henry, 1991).

5.1.1 Dry matter

The dry matter value obtained for bitter orange peels, 91.58% was lower than as reported by Lashkari and Tagizadeh (2013). However, the dry matter obtained for grapefruit peel was higher than as obtained in the study conducted by Vlaicu et al (2020). Similarly, grapefruit pomace had higher dry matter than the range reported in previous studies (Lashkari and Tagizadeh, 2013; Palangi et al., 2013). Mandarin peels in this study had lower dry matter than as reported by Marin *et al* (2007) and the pomace was higher than the range of 88.72% -91.02%, reported by Lashkari and Tagizadeh (2013) and Palangi et al (2013).

Lemon peels was within the range reported by El-ghfar et al (2016) and Ghanem *et al* (2012) while the lemon pomace dry matter was higher than as reported by Lashkari and Tagizadeh (2013) and Palangi et al (2013). The dry matter of orange peels obtained in this study was within the range obtained by Vlaicu et al (2020), El-ghfar et al (2016) and

Ghanem et al (2012). Similarly, orange pomace fell within the range of previous studies (Palangi et al. 2013; Lashkari and Tagizadeh, 2013; Nagarajaiah and Prakash, 2016).

5.1.2 Ash content

In this study, the ash content obtained for bitter orange peels was lower than as obtained by Atta and El-Shenawi (2012). Also, the bitter orange pomace was higher than the 5.52% reported by Lashkari and Tagizadeh (2013). In grapefruit peel, the ash content was like the report of Vlaicu et al (2020). However, the ash content for the grapefruit pomace was lower than the range in previous studies (Palangi et al. 2013; Lashkari and Tagizadeh, 2013). The ash content of 4.29% obtained for mandarin peels was within the range (3.96% - 10.03%) reported by Ghanem et al. (2012), Magda et al. (2008) and Marin et al. (2007). Mandarin pomace however had a lower ash content (3.91%) than the range (4.8% - 5.57%) reported by Palangi et al (2013), Lashkari and Tagizadeh (2013) and Ozkan et al (2017).

The ash content obtained for lemon peel fell within range of previous studies (Marin et al. 2007; Ghanem et al. 2012; El-ghfar et al. 2016; Janati et al. 2012). Lemon pomace also had ash content that was within the range of values (4.15% - 6.9%) obtained by Nagarajaiah and Prakash (2016), Ozkan et al (2017), Lashkari & Tagizadeh (2013) and Palangi et al (2013). Orange peels had ash content (4.51%) that was higher than the reported range (Marin et al. 2007; Vlaicu et al. 2020; M'hiri et al. 2015; Kammoun et al. 2011; Ghanem et al. 2012; El-ghfar et al. 2016; Castrica et al. 2019). The ash content of orange pomace (4.33%) was however within the range of values (1.2% - 5.5%) reported by Nagarajaiah and Prakash (2016), Ozkan et al (2017), Palangi et al (2013) and Lashkari and Tagizadeh et al (2013).

5.1.3 Crude protein

In this study, bitter orange peels had lower crude protein of 2.7% and differed from Atta and El-Shenawi (2012). Similarly, the pomace of bitter orange had crude protein of 3.65% which was lower than the value as obtained by Lashkari and Tagizadeh (2013). Also, grapefruit peels had lower crude protein than as reported by Vlaicu et al (2020). Further to this, grapefruit pomace had crude protein of 4.62% which was lower than the reported range, 8.01% -9.14% (Palangi et al. 2013; Lashkari and Tagizadeh, 2013).

Mandarin peel had crude protein of 4.59% in this study, which was within the range of 2.16% - 8.55% as reported by Magda et al (2008), Marin et al (2007) and Ghanem et al (2012). Crude protein of 5.71% was obtained for mandarin pomace which was lower than the range (6.64% - 6.99%) obtained by Lashkari and Tagizadeh (2013), Palangi et al (2013) and Ozkan et al (2017).

In this study, lemon peels had crude protein of 7.38% which was within the range of 5.87% - 9.42% reported by Ghanem et al (2012), Figuerola et al (2005), El ghfar et al (2016), Masmoudi et al (2008) and Janati et al (2012). Lemon pomace had crude protein of 7.78% which was within the range of 7.27% - 9.54% (Nagarajaiah and Prakash, 2016; Palangi et al. 2013; Ozkan et al. 2017; Lashkari and Tagizadeh, 2013). Orange peels had a crude protein of 4.98% which was within the range of 1.79% - 9.06% (Ghanem et al. 2012; Magda et al. 2008; Castrica et al. 2019; Vlaicu et al. 2020; El-ghfar et al. 2016; Kammoun et al. 2011; M'hiri et al. 2015). Similarly, orange pomace had crude protein of 5.48% which was within the range of 4.26% - 8.68% reported by Nagarajaiah and Prakash (2016), Ozkan et al (2016), Lashkari and Tagizadeh (2013), and Palangi et al (2013).

5.1.4 Crude fiber

In this study, grapefruit peels had crude fiber of 12.65% which was higher than the value (10.68%) reported by Vlaicu et al (2020). Mandarin peels had crude fiber of 12.55% which was within the range of 7.14% - 27.89% reported by Ghanem et al. (2012) and Magda et al (2008). Lemon peels had crude fiber of 14.65% which falls within the range of 12.47% -15.18% obtained by El-ghfar et al (2016), Gorinstein et al (2001) and Janati et al (2013). Orange peels had crude fiber of 11.8% which falls within the range of 6.3% - 13.9% by Marin et al (2007), Vlaicu et al (2020), El-ghfar et al (2016), Magda et al (2008) and Gorinstein et al (2001). It is noteworthy that no known literature captured the crude fiber content of the citrus pomaces for the five citrus species hence provides opportunity for further research.

5.1.5 Neutral detergent fiber

Bitter orange pomace had an NDF of 15.78% which was higher than the value reported by Lashkari and Tagizadeh (2013). Grapefruit pomace had NDF of 13.58% which was lower than the range of 15.78% - 21.23% reported by Lashkari & Tagizadeh (2013), Palangi et al (2013). In mandarin pomace, the NDF was within the range (11.39% - 22%) reported by Lashkari and Tagizadeh (2013), Ozkan et al (2017), Palangi et al (2013). Lemon pomace had NDF of 20.21% and was within the reported range (Lashkari and Tagizadeh, 2013; Palangi et al. 2013; Ozkan et al. 2017).

Orange pomace had an NDF of 14.54% which falls within the range of 14.47% - 21.4% reported by Lashkari and Tagizadeh (2013), Ozkan et al (2017), Palangi *et al* (2013). Orange peels had NDF of 16.33% which was higher than the value 10.0% obtained by Castrica (2019). Generally, it also appeared that literatures on the neutral detergent fiber of citrus peels are limited.

5.1.6 Acid detergent fiber

In this study, bitter orange pomace had ADF of 12.41% which was lower than the value of 17.04% reported by Lashkari and Tagizadeh (2013). Grapefruit pomace had ADF of 10.77% which was lower than the range of 13.08% - 17.6% reported by Lashkari and Tagizadeh (2013) and Palangi et al (2013). Mandarin pomace had ADF of 11.33% which fell within the range of 8.48% - 17.9% reported by Lashkari and Tagizadeh (2013), Ozkan et al (2017) and Palangi et al (2013). ADF obtained for lemon pomace was 14.97% which was lower than the range of 15.1% -19.45% obtained by Palangi et al (2013), Lashkari and Tagizadeh (2013), Ozkan et al (2017), Beyzi et al (2018).

Orange pomace had ADF of 10.85% which was lower than the range of 11.95% - 18.3% reported by Lashkari and Tagizadeh (2013), Beyzi et al (2018), Ozkan et al (2017), Palangi et al (2013). The ADF obtained for orange peels (12.13%) was higher than the value of 7.6% reported by Castrica et al (2019). It also appeared that limited studies were carried out on the peels of the five citrus species used in this research.

5.2 In-vitro true digestibility

In this study, pomaces had higher in-vitro dry matter digestibility across the five citrus species (Figure 4.1). Mandarin and orange peels had higher NDF digestibility than their corresponding pomaces while bitter orange, grapefruit and lemon peels had lower NDF digestibility than corresponding pomaces hence the hypothesis that citrus pomaces have higher in-vitro digestibility than peel is rejected. Variations in the in-vitro digestibility values obtained from several studies on citrus by-products may be due to the different types of in-vitro digestibility methods used (Lashkari and Tagizadeh, 2013).

5.2.1 In-vitro dry matter digestibility

The in-vitro true dry matter digestibility IVTD (DM) of bitter orange pomace in this study was 96.38% which was higher than the IVTD (DM) of 84.8% reported by Lashkari and Tagizadeh (2013). Lemon pomace had in-vitro dry matter digestibility of 92.08% which falls within the range of 85.76% - 91.2% reported by Lashkari and Tagizadeh (2013) and Tripodo et al (2004). Orange pomace had in-vitro dry matter digestibility of 94.76% which was higher than the range of 89.03% - 90.6% reported by Lashkari and Tagizadeh (2013) and Tripodo et al (2004).

Generally, there appeared to be limited studies and literature on the in-vitro dry matter digestibility of citrus peels. In this study, bitter orange peels had in-vitro dry matter digestibility of 91.58%, grapefruit peels had IVTD(DM) of 91.97%, mandarin peels had IVTD (DM) of 92.73%, lemon peels had in-vitro dry matter digestibility of 90.88% and orange peels had IVTD(DM) of 94.3%

5.2.2 Neutral detergent fiber

In this study, citrus peels had higher NDF than citrus pomaces across all five species (Figure 4.2). Limited studies have been carried out on the NDF of citrus peels and pomaces. The peels of bitter orange, grapefruit, mandarin, lemon, and orange had NDF of 8.51%, 8.25%, 7.35%, 9.32%, 5.79% respectively. Bitter orange, grapefruit, mandarin, lemon, and orange pomaces had NDF of 3.74%, 5.44%, 6.87%, 8.15% and 5.29% respectively.

5.2.3 Neutral detergent fiber digestibility

Orange pomace had NDFD% of 63.82% which was within the range of values (57.6% - 69.8%) reported by Alnaimy et al (2017) and Aregheore et al (2000). There have been limited studies on the NDFD% of citrus peels and other citrus pomaces. The pomaces of bitter orange, grapefruit, were higher compared to the peels of same species (Figure 4.3). Conversely, mandarin, and orange peels had higher NDF digestibility.

5.3 Using citrus for ruminant feed modification

Generally, citrus peels and pomaces used in this study had in-vitro dry matter digestibility that ranged from 85% - 96.57% and crude protein ranging from 2.7% - 7.78% which indicates that they are high in soluble carbohydrates, low in crude protein and can be considered as a non-forage energy source for ruminants (Lashkari and Tagizadeh, 2013; Mamma and Christakopoulos, 2014). The low NDF values ranging from 13.58% - 20.51% obtained for the citrus peels and pomace also indicates that they are rich in soluble sugars that would be good for ruminants (Aregheore, 2000). In this study, NDFD% range of 52.74% - 76.28% (Table 4.2) obtained for the five species of citrus peels and pomaces indicate that they are a good source of digestible NDF which is important for increasing dry matter intake and milk production in lactating dairy cows (Zoioupoulous et al., 2008).

Further to this, the high amount of soluble and readily fermentable carbohydrates in addition to their readily digestible Neutral detergent fiber in citrus peels and pomaces shows that they have high nutritive value (Alnaimy et al., 2017). They can also be considered as partial replacements for starch rich concentrates such as cereal grains because they increase Neutral detergent fiber and acid detergent fiber digestibility coefficients when used (Alnaimy et al., 2017). Dried citrus peels and pomaces can also be safely included in beef, growing cattle, and lactating ruminant rations to replace energy sources without adverse effect on milk yield and animal health (Fegeeros et al., 2005; Alnaimy et al., 2017).

The hypothesis that citrus peels and pomaces as an agro-industrial by-product can be used to modify ruminant feed is accepted. However, within the limits of this study, none of the five species have shown distinction in nutritive value and digestibility. Citrus peels and pomace are high in moisture (Mamma and Christakopoulos, 2014) and cannot be stored for long. Therefore, to increase and improve their potential as an agro-industrial by product for use in modification of ruminant nutrition, it needs to be conserved through drying or ensiling with other dry materials (Zoioupoulous et al., 2008; Scerra et al., 2001).

5.4 Further research

This study has opportunities for further research into understanding the nutritive values of mixed citrus species and their in-vitro digestibility. Also, in-vitro and in-vivo studies of citrus modified feeds and effects of fermentation on improving nutritive value and digestibility should be further done. These studies can be done with clear considerations for dairy ruminant nutritional needs differently from meat producing ruminants.

CHAPTER VI

CONCLUSION

There were no statistically significant species and sample effects among the five citrus species for the chemical analysis. However, lemon peel and pomace showed higher crude protein, crude fiber, NDF and ADF compared to other species. There were statistically significant differences among the species and sample for in-vitro digestibility. The bitter orange pomace had the highest in-vitro true dry matter digestibility and NDF digestibility of the five citrus species.

The use of cheap, available, and abundant sources of agro-industrial feeds such as citrus peels and pomaces is a very important strategy to consider in ruminant nutrition. This research is important to providing additional knowledge on the nutritive value and in-vitro digestibility of the peels and pomaces of different citrus species as well as a vista of opportunities to researchers and nutritionist on the potentials of these agro-industrial by-products to replace or at least modify conventional feed ration of ruminants.

By implication, the vast number of citrus species and varieties globally as well as the diverse use will continue to be a good source of agro-industrial wastes. These would enable farmers and researchers to explore without limits and boundaries the opportunities for improved ruminant ration, cost effectiveness and environmental friendliness.

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APPENDIX I: COMPREHENSIVE RESULTS OF STATISTICAL ANALYSIS FOR CHEMICAL PARAMETERS OF FIVE CITRUS SPECIES

Group Descriptives

	Codes	N	Mean	SD	SE
ADF	BOPE	2	14.09	0.03998	0.02827
	BOPP	2	12.41	0.08998	0.06363
	GPE	2	12.93	0.08214	0.05808
	GPP	2	10.77	0.10423	0.07370
	LPE	2	14.63	0.03554	0.02513
	LPP	2	14.97	0.09542	0.06747
	MPE	2	12.63	0.25092	0.17743
	MPP	2	11.33	0.13050	0.09228
	OPE	2	12.13	0.28981	0.20492
	OPP	2	10.85	0.00531	0.00376
NDF	BOPE	2	18.05	0.07663	0.05419
	BOPP	2	15.78	0.14355	0.10151
	GPE	2	17.45	0.06274	0.04437
	GPP	2	13.58	0.14814	0.10475
	LPE	2	20.51	0.01941	0.01372
	LPP	2	20.21	0.22485	0.15900
	MPE	2	17.23	1.28363	0.90767
	MPP	2	14.60	0.12248	0.08661
	OPE	2	16.33	0.35377	0.25015
	OPP	2	14.54	0.22416	0.15850
Crude protein	BOPE	2	2.70	0.86833	0.61400
	BOPP	2	3.65	0.13789	0.09750
	GPE	2	4.76	0.17466	0.12350
	GPP	2	4.62	0.06647	0.04700
	LPE	2	7.38	0.21001	0.14850
	LPP	2	7.78	0.04525	0.03200
	MPE	2	4.59	0.19658	0.13900
	MPP	2	5.71	0.05515	0.03900
	OPE	2	4.98	0.07778	0.05500
	OPP	2	5.48	0.03465	0.02450
DM	BOPE	2	93.58	0.05664	0.04005
	BOPP	2	91.58	0.03970	0.02807
	GPE	2	92.09	0.04320	0.03055
	GPP	2	91.56	0.06682	0.04725
	LPE	2	91.46	0.00123	8.68e-4
	LPP	2	91.11	0.23917	0.16912
	MPE	2	93.52	0.03079	0.02177
	MPP	2	93.08	0.02272	0.01606
	OPE	2	91.67	0.13848	0.09792
	OPP	2	91.68	0.03029	0.02142

Group Descriptives

	Codes	N	Mean	SD	SE
Ash	BOPE	2	5.28	0.00514	0.00364
	BOPP	2	5.52	0.05837	0.04128
	GPE	2	3.72	0.04889	0.03457
	GPP	2	3.57	0.06618	0.04680
	LPE	2	5.01	0.01635	0.01156
	LPP	2	5.10	0.03640	0.02574
	MPE	2	4.29	0.02490	0.01760
	MPP	2	3.91	5.89e-4	4.16e-4
	OPE	2	4.51	0.03018	0.02134
	OPP	2	4.33	0.01149	0.00813
Crude Fiber	BOPE	2	13.50	0.28284	0.20000
	BOPP	2	11.70	0.14142	0.10000
	GPE	2	12.65	1.20208	0.85000
	GPP	2	10.15	0.77782	0.55000
	LPE	2	14.65	0.77782	0.55000
	LPP	2	15.25	1.76777	1.25000
	MPE	2	12.55	1.20208	0.85000
	MPP	2	12.10	2.40416	1.70000
	OPE	2	11.80	0.14142	0.10000
	OPP	2	10.60	0.70711	0.50000

APPENDIX II: COMPREHENSIVE RESULTS OF STATISTICAL ANALYSIS FOR IN-VITRO DIGESTIBILITY OF FIVE SPECIES

A. In-vitro True Dry Matter digestibility IVTD(DM)

	Sum of Squares	df	Mean Square	F	p	η²	η²p	ω²
Species	43.1	4	10.769	17.9	< .001	0.342	0.705	0.321
Samples	37.7	1	37.702	62.8	< .001	0.299	0.677	0.293
Species ✻ Samples	27.2	4	6.802	11.3	< .001	0.216	0.602	0.196
Residuals	18.0	30	0.600					

Homogeneity of Variances Test (Levene's)

F	df1	df2	p
1.31	9	30	0.275

Normality Test (Shapiro-Wilk)

Statistic	p
0.993	0.996

Post Hoc Comparisons - Species

Comparison							
Species		Species	Mean Difference	SE	df	t	p_{tukey}
Bitterorange	-	Grapefruit	0.632	0.387	30.0	1.632	0.489
	-	Lemon	2.501	0.387	30.0	6.456	< .001
	-	Mandarin	0.995	0.387	30.0	2.569	0.102
	-	Orange	-0.550	0.387	30.0	-1.419	0.621
Grapefruit	-	Lemon	1.868	0.387	30.0	4.823	< .001
	-	Mandarin	0.363	0.387	30.0	0.936	0.880
	-	Orange	-1.182	0.387	30.0	-3.051	0.035
Lemon	-	Mandarin	-1.506	0.387	30.0	-3.887	0.004
	-	Orange	-3.050	0.387	30.0	-7.874	< .001
Mandarin	-	Orange	-1.545	0.387	30.0	-3.987	0.003

Note. Comparisons are based on estimated marginal means

Post Hoc Comparisons - Sample

Comparison							
Sample		Sample	Mean Difference	SE	df	t	p_{tukey}
Peels	-	Pomace	-1.94	0.245	30.0	-7.93	< .001

Note. Comparisons are based on estimated marginal means

Post Hoc Comparisons - Species ✻ Sample

Comparison									
Species	Sample		Species	Sample	Mean Difference	SE	df	t	p_{tukey}
Bitterorange	Peels	-	Bitterorange	Pomace	-4.7948	0.548	30.0	-8.7521	< .001
		-	Grapefruit	Peels	-0.3915	0.548	30.0	-0.7145	0.999
		-	Grapefruit	Pomace	-3.1387	0.548	30.0	-5.7292	< .001
		-	Lemon	Peels	0.7066	0.548	30.0	1.2897	0.948
		-	Lemon	Pomace	-0.4998	0.548	30.0	-0.9122	0.995
		-	Mandarin	Peels	-1.1495	0.548	30.0	-2.0981	0.542
		-	Mandarin	Pomace	-1.6552	0.548	30.0	-3.0212	0.117
		-	Orange	Peels	-2.7197	0.548	30.0	-4.9643	< .001
		-	Orange	Pomace	-3.1742	0.548	30.0	-5.7939	< .001
	Pomace	-	Grapefruit	Peels	4.4033	0.548	30.0	8.0375	< .001
		-	Grapefruit	Pomace	1.6561	0.548	30.0	3.0229	0.117
		-	Lemon	Peels	5.5013	0.548	30.0	10.0417	< .001
		-	Lemon	Pomace	4.2950	0.548	30.0	7.8398	< .001
		-	Mandarin	Peels	3.6453	0.548	30.0	6.6539	< .001
		-	Mandarin	Pomace	3.1396	0.548	30.0	5.7309	< .001
		-	Orange	Peels	2.0751	0.548	30.0	3.7878	0.020
		-	Orange	Pomace	1.6206	0.548	30.0	2.9582	0.133
Grapefruit	Peels	-	Grapefruit	Pomace	-2.7473	0.548	30.0	-5.0146	< .001

Post Hoc Comparisons - Species ✻ Sample

Comparison									
Species	Sample		Species	Sample	Mean Difference	SE	df	t	p_{tukey}
		-	Lemon	Peels	1.0980	0.548	30.0	2.0042	0.603
		-	Lemon	Pomace	-0.1083	0.548	30.0	-0.1977	1.000
		-	Mandarin	Peels	-0.7580	0.548	30.0	-1.3836	0.923
		-	Mandarin	Pomace	-1.2637	0.548	30.0	-2.3066	0.414
		-	Orange	Peels	-2.3282	0.548	30.0	-4.2497	0.006
		-	Orange	Pomace	-2.7827	0.548	30.0	-5.0793	< .001
	Pomace	-	Lemon	Peels	3.8453	0.548	30.0	7.0189	< .001
		-	Lemon	Pomace	2.6390	0.548	30.0	4.8170	0.001
		-	Mandarin	Peels	1.9893	0.548	30.0	3.6311	0.030
		-	Mandarin	Pomace	1.4836	0.548	30.0	2.7080	0.216
		-	Orange	Peels	0.4191	0.548	30.0	0.7649	0.999
		-	Orange	Pomace	-0.0354	0.548	30.0	-0.0647	1.000
Lemon	Peels	-	Lemon	Pomace	-1.2063	0.548	30.0	-2.2019	0.477
		-	Mandarin	Peels	-1.8560	0.548	30.0	-3.3878	0.053
		-	Mandarin	Pomace	-2.3617	0.548	30.0	-4.3109	0.005
		-	Orange	Peels	-3.4262	0.548	30.0	-6.2540	< .001
		-	Orange	Pomace	-3.8807	0.548	30.0	-7.0836	< .001
	Pomace	-	Mandarin	Peels	-0.6497	0.548	30.0	-1.1859	0.969
		-	Mandarin	Pomace	-1.1554	0.548	30.0	-2.1090	0.536
		-	Orange	Peels	-2.2199	0.548	30.0	-4.0520	0.010
		-	Orange	Pomace	-2.6744	0.548	30.0	-4.8816	0.001
Mandarin	Peels	-	Mandarin	Pomace	-0.5057	0.548	30.0	-0.9231	0.994
		-	Orange	Peels	-1.5702	0.548	30.0	-2.8661	0.160
		-	Orange	Pomace	-2.0247	0.548	30.0	-3.6957	0.025

Post Hoc Comparisons - Species ✻ Sample

Comparison									
Species	Sample		Species	Sample	Mean Difference	SE	df	t	p_{tukey}
	Pomace	-	Orange	Peels	-1.0645	0.548	30.0	-1.9431	0.642
		-	Orange	Pomace	-1.5190	0.548	30.0	-2.7727	0.191
Orange	Peels	-	Orange	Pomace	-0.4545	0.548	30.0	-0.8296	0.997

Note. Comparisons are based on estimated marginal means

Estimated Marginal Means - Species ✻ Sample

				95% Confidence Interval	
Sample	Species	Mean	SE	Lower	Upper
Peels	Bitterorange	91.6	0.387	90.8	92.4
	Grapefruit	92.0	0.387	91.2	92.8
	Lemon	90.9	0.387	90.1	91.7
	Mandarin	92.7	0.387	91.9	93.5
	Orange	94.3	0.387	93.5	95.1
Pomace	Bitterorange	96.4	0.387	95.6	97.2
	Grapefruit	94.7	0.387	93.9	95.5
	Lemon	92.1	0.387	91.3	92.9
	Mandarin	93.2	0.387	92.4	94.0
	Orange	94.8	0.387	94.0	95.5

B. NDF

	Sum of Squares	df	Mean Square	F	p
Overall model	111.9	9	12.432	19.9	< .001
Species	46.9	4	11.724	18.7	< .001
Samples	37.9	1	37.887	60.5	< .001
Species ✻ Samples	27.1	4	6.775	10.8	< .001
Residuals	18.8	30	0.626		

Homogeneity of Variances Test (Levene's)

F	df1	df2	p
1.34	9	30	0.258

Normality Test (Shapiro-Wilk)

Statistic	p
0.992	0.994

Post Hoc Comparisons – Species

Comparison							
Species		Species	Mean Difference	SE	df	t	p_{tukey}
Bitterorange	-	Grapefruit	-0.715	0.396	30.0	-1.806	0.389
	-	Lemon	-2.605	0.396	30.0	-6.586	< .001
	-	Mandarin	-0.987	0.396	30.0	-2.495	0.119
	-	Orange	0.592	0.396	30.0	1.496	0.573
Grapefruit	-	Lemon	-1.891	0.396	30.0	-4.779	< .001
	-	Mandarin	-0.272	0.396	30.0	-0.688	0.957
	-	Orange	1.306	0.396	30.0	3.302	0.019
Lemon	-	Mandarin	1.618	0.396	30.0	4.091	0.003
	-	Orange	3.197	0.396	30.0	8.081	< .001
Mandarin	-	Orange	1.579	0.396	30.0	3.990	0.003

Note. Comparisons are based on estimated marginal means

Post Hoc Comparisons – Sample

Comparison							
Sample		Sample	Mean Difference	SE	df	t	p_{tukey}
Peels	-	Pomace	1.95	0.250	30.0	7.78	< .001

Note. Comparisons are based on estimated marginal means

Post Hoc Comparisons - Species ✻ Sample

Comparison									
Species	Sample		Species	Sample	Mean Difference	SE	df	t	p_{tukey}
Bitterorange	Peels	-	Bitterorange	Pomace	4.768	0.559	30.0	8.523	< .001
		-	Grapefruit	Peels	0.265	0.559	30.0	0.473	1.000
		-	Grapefruit	Pomace	3.075	0.559	30.0	5.496	< .001

Post Hoc Comparisons - Species ✻ Sample

Comparison									
Species	**Sample**		**Species**	**Sample**	**Mean Difference**	**SE**	**df**	**t**	**p_{tukey}**
		-	Lemon	Peels	-0.809	0.559	30.0	-1.445	0.902
		-	Lemon	Pomace	0.366	0.559	30.0	0.655	1.000
		-	Mandarin	Peels	1.158	0.559	30.0	2.069	0.561
		-	Mandarin	Pomace	1.637	0.559	30.0	2.926	0.142
		-	Orange	Peels	2.726	0.559	30.0	4.873	0.001
		-	Orange	Pomace	3.225	0.559	30.0	5.765	< .001
	Pomace	-	Grapefruit	Peels	-4.504	0.559	30.0	-8.050	< .001
		-	Grapefruit	Pomace	-1.694	0.559	30.0	-3.027	0.116
		-	Lemon	Peels	-5.577	0.559	30.0	-9.969	< .001
		-	Lemon	Pomace	-4.402	0.559	30.0	-7.868	< .001
		-	Mandarin	Peels	-3.611	0.559	30.0	-6.454	< .001
		-	Mandarin	Pomace	-3.131	0.559	30.0	-5.597	< .001
		-	Orange	Peels	-2.042	0.559	30.0	-3.650	0.028
		-	Orange	Pomace	-1.543	0.559	30.0	-2.758	0.197
Grapefruit	Peels	-	Grapefruit	Pomace	2.810	0.559	30.0	5.023	< .001
		-	Lemon	Peels	-1.073	0.559	30.0	-1.918	0.658
		-	Lemon	Pomace	0.102	0.559	30.0	0.182	1.000
		-	Mandarin	Peels	0.893	0.559	30.0	1.596	0.840
		-	Mandarin	Pomace	1.372	0.559	30.0	2.453	0.332
		-	Orange	Peels	2.462	0.559	30.0	4.400	0.004

Post Hoc Comparisons - Species ✻ Sample

Comparison									
Species	**Sample**		**Species**	**Sample**	**Mean Difference**	**SE**	**df**	**t**	p_{tukey}
		-	Orange	Pomace	2.961	0.559	30.0	5.292	< .001
	Pomace	-	Lemon	Peels	-3.883	0.559	30.0	-6.941	< .001
		-	Lemon	Pomace	-2.708	0.559	30.0	-4.841	0.001
		-	Mandarin	Peels	-1.917	0.559	30.0	-3.427	0.048
		-	Mandarin	Pomace	-1.438	0.559	30.0	-2.570	0.275
		-	Orange	Peels	-0.348	0.559	30.0	-0.623	1.000
		-	Orange	Pomace	0.151	0.559	30.0	0.270	1.000
Lemon	Peels	-	Lemon	Pomace	1.175	0.559	30.0	2.100	0.541
		-	Mandarin	Peels	1.966	0.559	30.0	3.515	0.039
		-	Mandarin	Pomace	2.446	0.559	30.0	4.371	0.005
		-	Orange	Peels	3.535	0.559	30.0	6.318	< .001
		-	Orange	Pomace	4.034	0.559	30.0	7.211	< .001
	Pomace	-	Mandarin	Peels	0.791	0.559	30.0	1.414	0.913
		-	Mandarin	Pomace	1.270	0.559	30.0	2.271	0.435
		-	Orange	Peels	2.360	0.559	30.0	4.218	0.007
		-	Orange	Pomace	2.859	0.559	30.0	5.110	< .001
Mandarin	Peels	-	Mandarin	Pomace	0.479	0.559	30.0	0.857	0.997
		-	Orange	Peels	1.569	0.559	30.0	2.804	0.180
		-	Orange	Pomace	2.068	0.559	30.0	3.696	0.025
	Pomace	-	Orange	Peels	1.089	0.559	30.0	1.947	0.639
		-	Orange	Pomace	1.589	0.559	30.0	2.839	0.169
Orange	Peels	-	Orange	Pomace	0.499	0.559	30.0	0.892	0.996

Post Hoc Comparisons - Species ✻ Sample

Comparison								
Species	Sample	Species	Sample	Mean Difference	SE	df	t	p_{tukey}

Note. Comparisons are based on estimated marginal means

Estimated Marginal Means - Species ✻ Sample

				95% Confidence Interval	
Sample	Species	Mean	SE	Lower	Upper
Peels	Bitterorange	8.51	0.396	7.70	9.32
	Grapefruit	8.25	0.396	7.44	9.05
	Lemon	9.32	0.396	8.51	10.13
	Mandarin	7.35	0.396	6.55	8.16
	Orange	5.79	0.396	4.98	6.59
Pomace	Bitterorange	3.74	0.396	2.94	4.55
	Grapefruit	5.44	0.396	4.63	6.24
	Lemon	8.15	0.396	7.34	8.95
	Mandarin	6.87	0.396	6.07	7.68
	Orange	5.29	0.396	4.48	6.09

C. NDFD%

	Sum of Squares	df	Mean Square	F	p	η^2	η^2p	ω^2
Species	659	4	164.9	7.19	< .001	0.250	0.489	0.213
Sample	376	1	375.6	16.38	< .001	0.142	0.353	0.132
Species ✻ Sample	920	4	230.0	10.03	< .001	0.348	0.572	0.311
Residuals	688	30	22.9					

Homogeneity of Variances Test (Levene's)

F	df1	df2	p
1.43	9	30	0.219

Normality Test (Shapiro-Wilk)

Statistic	p
0.985	0.853

Post Hoc Comparisons – Species

Comparison							
Species		Species	Mean Difference	SE	Df	t	p_{tukey}
Bitterorange	-	Grapefruit	8.210	2.39	30.0	3.429	0.014
	-	Lemon	7.434	2.39	30.0	3.105	0.031
	-	Mandarin	9.446	2.39	30.0	3.945	0.004
	-	Orange	0.366	2.39	30.0	0.153	1.000
Grapefruit	-	Lemon	-0.776	2.39	30.0	-0.324	0.997
	-	Mandarin	1.235	2.39	30.0	0.516	0.985
	-	Orange	-7.845	2.39	30.0	-3.276	0.021
Lemon	-	Mandarin	2.011	2.39	30.0	0.840	0.916
	-	Orange	-7.069	2.39	30.0	-2.952	0.044
Mandarin	-	Orange	-9.080	2.39	30.0	-3.792	0.006

Note. Comparisons are based on estimated marginal means

Post Hoc Comparisons – Sample

Comparison							
Sample		Sample	Mean Difference	SE	df	t	p_{tukey}
Peels	-	Pomace	-6.13	1.51	30.0	-4.05	< .001

Note. Comparisons are based on estimated marginal means

Post Hoc Comparisons - Species ✻ Sample

Comparison									
Species	**Sample**		**Species**	**Sample**	**Mean Difference**	**SE**	**df**	**t**	**p_{tukey}**
Bitterorange	Peels	-	Bitterorange	Pomace	-23.4346	3.39	30.0	-6.9212	< .001
		-	Grapefruit	Peels	0.1054	3.39	30.0	0.0311	1.000
		-	Grapefruit	Pomace	-7.1197	3.39	30.0	-2.1027	0.540
		-	Lemon	Peels	-1.7129	3.39	30.0	-0.5059	1.000
		-	Lemon	Pomace	-6.8530	3.39	30.0	-2.0240	0.590
		-	Mandarin	Peels	-4.4743	3.39	30.0	-1.3214	0.941
		-	Mandarin	Pomace	-0.0691	3.39	30.0	-0.0204	1.000
		-	Orange	Peels	-11.7277	3.39	30.0	-3.4636	0.044
		-	Orange	Pomace	-10.9758	3.39	30.0	-3.2416	0.073
	Pomace	-	Grapefruit	Peels	23.5401	3.39	30.0	6.9523	< .001
		-	Grapefruit	Pomace	16.3149	3.39	30.0	4.8184	0.001
		-	Lemon	Peels	21.7217	3.39	30.0	6.4153	< .001
		-	Lemon	Pomace	16.5816	3.39	30.0	4.8972	0.001
		-	Mandarin	Peels	18.9603	3.39	30.0	5.5997	< .001
		-	Mandarin	Pomace	23.3655	3.39	30.0	6.9008	< .001
		-	Orange	Peels	11.7070	3.39	30.0	3.4575	0.045
		-	Orange	Pomace	12.4588	3.39	30.0	3.6796	0.027
Grapefruit	Peels	-	Grapefruit	Pomace	-7.2251	3.39	30.0	-2.1339	0.520
		-	Lemon	Peels	-1.8184	3.39	30.0	-0.5370	1.000

Post Hoc Comparisons - Species ✻ Sample

Comparison									
Species	**Sample**		**Species**	**Sample**	**Mean Difference**	**SE**	**df**	**t**	**p_{tukey}**
		-	Lemon	Pomace	-6.9585	3.39	30.0	-2.0551	0.570
		-	Mandarin	Peels	-4.5798	3.39	30.0	-1.3526	0.932
		-	Mandarin	Pomace	-0.1745	3.39	30.0	-0.0515	1.000
		-	Orange	Peels	-11.8331	3.39	30.0	-3.4948	0.041
		-	Orange	Pomace	-11.0812	3.39	30.0	-3.2727	0.068
	Pomace	-	Lemon	Peels	5.4068	3.39	30.0	1.5968	0.839
		-	Lemon	Pomace	0.2666	3.39	30.0	0.0787	1.000
		-	Mandarin	Peels	2.6454	3.39	30.0	0.7813	0.998
		-	Mandarin	Pomace	7.0506	3.39	30.0	2.0823	0.553
		-	Orange	Peels	-4.6080	3.39	30.0	-1.3609	0.930
		-	Orange	Pomace	-3.8561	3.39	30.0	-1.1389	0.976
Lemon	Peels	-	Lemon	Pomace	-5.1401	3.39	30.0	-1.5181	0.874
		-	Mandarin	Peels	-2.7614	3.39	30.0	-0.8156	0.998
		-	Mandarin	Pomace	1.6438	3.39	30.0	0.4855	1.000
		-	Orange	Peels	-10.0147	3.39	30.0	-2.9577	0.133
		-	Orange	Pomace	-9.2629	3.39	30.0	-2.7357	0.205
	Pomace	-	Mandarin	Peels	2.3787	3.39	30.0	0.7025	0.999
		-	Mandarin	Pomace	6.7840	3.39	30.0	2.0036	0.603
		-	Orange	Peels	-4.8746	3.39	30.0	-1.4397	0.904

Post Hoc Comparisons - Species ✻ Sample

Comparison									
Species	Sample		Species	Sample	Mean Difference	SE	df	t	p_{tukey}
		-	Orange	Pomace	-4.1227	3.39	30.0	-1.2176	0.964
Mandarin	Peels	-	Mandarin	Pomace	4.4053	3.39	30.0	1.3010	0.946
		-	Orange	Peels	-7.2533	3.39	30.0	-2.1422	0.514
		-	Orange	Pomace	-6.5015	3.39	30.0	-1.9201	0.656
	Pomace	-	Orange	Peels	-11.6586	3.39	30.0	-3.4432	0.046
		-	Orange	Pomace	-10.9067	3.39	30.0	-3.2212	0.077
Orange	Peels	-	Orange	Pomace	0.7519	3.39	30.0	0.2221	1.000

Note. Comparisons are based on estimated marginal means

Estimated Marginal Means - Species ✻ Sample

				95% Confidence Interval	
Sample	Species	Mean	SE	Lower	Upper
Peels	Bitterorange	52.8	2.39	48.0	57.7
	Grapefruit	52.7	2.39	47.8	57.6
	Lemon	54.6	2.39	49.7	59.4
	Mandarin	57.3	2.39	52.4	62.2
	Orange	64.6	2.39	59.7	69.5
Pomace	Bitterorange	76.3	2.39	71.4	81.2
	Grapefruit	60.0	2.39	55.1	64.9
	Lemon	59.7	2.39	54.8	64.6
	Mandarin	52.9	2.39	48.0	57.8
	Orange	63.8	2.39	58.9	68.7

CURRICULUME VITAE

Oyinkansola Olubunmi Olowu (Nee Olaniyan) was born on …./…./…. in ….. She completed her high school education from Federal Polytechnic Staff Secondary School in Bida Niger state Nigeria in 2002 and excelled at the West African Senior Secondary Certificate Examinations (Science major). She joined Ladoke Akintola University of Technology, Ogbomoso Oyo state Nigeria in 2005 to pursue higher education and graduated in 2010 with Bachelor of Technology (Honours) in Environmental Biology, Second Class Upper Category. She worked as an agricultural science teacher in 2011 under the compulsory National Youth Service Corp program of Nigeria and worked in the client service unit of the Bridge Clinic Lagos between 2012 and 2019.

Oyimkansola Olubunmi Olowu enrolled in the Graduate School of Natural and Applied Sciences, Department of Animal Production and Technologies at Niğde Ömer Halisdemir University, Niğde, Turkey to pursue her MSc degree under the supervision of Dr. Sema Yaman Firincioğlu. She has two publications to her credit which are, Azeez M.A., Aremu C.O., and Olaniyan O.O (2013) Assessment of genetic variation in accessions of sesame (Sesamum indicum L.) and its crosses by seed protein electrophoresis, *Journal of Agroalimentary Processes and Technologies*, 19 (4), 383-391 and secondly, Olowu, O.O and Yaman Fırıncıoğlu, S. (2019). Feed Evaluation Methods: Performance, Economy and Environment, *Eurasian Journal of Agricultural Research*, 3 (2), 48-57. She is a native English speaker, proficient with three other local Nigerian languages (Yoruba, Hausa and Nupe) and learning the Turkish language.