ANTIBACTERIAL SUSCEPTIBILITY OF AVOCADO SEED OIL ON *CAMPYLOBACTER JEJUNI*

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A PROJECT WORK PRESENTED TO THE DEPARTMENT OF FOOD TECHNOLOGY, SCHOOL OF APPLIED SCIENCES AND TECHNOLOGY, AUCHI POLYTECHNIC AUCHI, EDO STATE.

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CERTIFICATION

This is to certify that this project work Titled Antibacterial Susceptibility Of Avocado Seed Oil On *Campylobacter Jejuni* was carried out by Afekhumeh Beauty Omoshife with Matric Number AST/2102060101 in partial fulfillment of the Award of Higher National Diploma (HND) in the Department of Food Technology, Auchi Polytechnic Auchi, Edo State.

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(Project Supervisor) Date

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(Head of Department)

DEDICATION

I want to specially dedicate this project work to God Almighty, the giver of life for his mercies and grace upon my life.

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ABSTRACT

The antibacterial susceptibility of avocado seed oil on *campylobacter jenjuni* was studied. Avocado seed oil on *campylobacter jejuni* revealed the inhibition of growth of this bacterium, through the susceptibility pattern to the extract was not uniform. The test organisms were sensitive to some of the oil concentrations and this range from 1.5mm to 22mm at all concentrations (25% and 50% respectively) *Campylobacter jejuni* A and B were both sensitive at all concentrations (25% and 50%) to the avocado seed oil extracted. The antibacterial susceptibility test that was carried out with the tested organisms using conventional antibiotics revealed that ofloxacin (5µg) and gentamicin (10 µg).

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

The avocado (Persea americana) belongs to the Lauraceae family of tropical and mediterrenian trees and shrubs. It is originated from Mexico and Central and South America; for thousands of years and until today.It has been a popular food, for treating skin eruptions and medicinal purposes due to its high nutrition content as well as for its therapeutic properties (Shruti, and Padma, 2015). It is a source of carbohydrate, protein, fiber, essential micronutrients for human consumption such as, polyphenols, fats, oils, vitamins (vit. C, E, K, B1, B2, B6, B9) and minerals (P, Na, Mg, K, Fe and Zn [Orhevba and Jinadu.2011]. Its low sugar content makes avocado very recommendable source of high energy food for those who are diabetic. It is highly consumed in the world due to the presence of unsaturated lipids and its relevance in improving and maintaining healthy heart and circulatory system (Maitera, Osemeahon, and Barnabas HL., 2014).

C. *jejuni* is the major species that caused infections than other pathogenic *Campylobacter* species (Liu et al., 2017) and also the major *Campylobacter* species that regularly cause diarrhea in human (Epps *et al.,* 2013). Infections caused by C. *jejuni* can develop into diverse severities such as mild and self-limiting diarrhea to hemorrhagic colitis and sometimes to meningitis and bacteremia (Burnham and Hendrixson, 2018; Dasti *et al.*, 2010).

C. *jejuni* infections are also associated with many secondary complications such as autoimmune neuropathy (Liu *et al.*, 2018), and inflammatory bowel disease (IBD) (Drenthen et al., 2011; Loshaj-Shala *et al.,* 2015). C. *jejuni* is the major *Campylobacter* species that cause disease in young people (Haddock *et al.*, 2010). C. *jejuni* infections can occur via various routes such as through direct contact with companion and farm animals or through waterborne or foodborne transmission (Domingues et al., 2012). C. *jejuni* is a commensal bacterial of chickens which inhabit the chicken intestines at a level >106–108 CFU/g of chicken faeces (Oh *et al.*, 2018) and chickens are the main vector for human *campylobacteriosis* (Hartley-Tassell *et al.*, 2018). C. *jejuni* consist of two subspecies; C. jejuni subsp. *jejuni* (Cjj) and C. *jejuni* subsp. doyley (Cjd) (Man, 2011). The main phenotypic feature generally used to differentiate Cjj from

Cjd strain is the inability of C. *jejuni* subsp. doyley to reduce nitrate and also, Cjd is also associated with high susceptibility to cephalothin.

Clinically, Cjd strain causes both enteritis and gastritis (Parker *et al.,* 2007). C. *jejuni* subsp. *jejuni* (Cjj) is the main bacterial cause of enteroinvasive diarrhea (Pacanowski et al., 2008) and the major symptoms of C. *jejuni* infections include severe enteritis, severe abdominal cramps, fever and bloody diarrhea with mucus (Biswas *et al.,* 2011). In addition, C. *jejuni* has also been reported to be associated with immunoreactive complications like Miller-Fisher syndromes (Dingle *et al.,* 2001).

There is a global tendency towards industrial fruit processing and, following such processes byproducts are normally discarded. However, these byproducts can cause ecological problems such as increased numbers of insects and rodents. Thus, studies to investigate the benefits of these byproducts as sources of food supplements or medicinal products are needed (Ramos, Jerz, Villanueva, LopezDellamary, Waibe, and Winterhalter., 2004). Different parts of avocado pear were used in traditional medications for various purposes including as an antimicrobia. Exploring the possible dietary and therapeutic potentials of especially underutilized agro-food wastes will in addition reduce the possible environmental waste burden (Egbuonu, Opara, Onyeabo, and Uchenna., 2008).

The seed of avocado is one of the under-utilized non-edible parts of the fruit, which are usually discarded as residues. Conducting a research on non-edible parts of fruits is an emerging trend, which may prove to be very profitable in the near future. Mostly, because it involves an important reduction in the production of wastes and the fact that the non-edible parts of many fruits like avocado have high levels of valuable bioactive compounds, particularly natural antioxidants (Vinha, Barreira, Castro, Costa, and Oliveira., 2013). The seed of avocado is redundant during the processing of the pulp. The seed waste may represent a severe ecological problem. However, at the same time, it may be of interest to industry as a source of bioactive compounds. Its chemical composition is comprised of phytosterols, fatty acids, triterpenes, and two new glucosides of abscisic acid. Biological activities of the avocado seed such as antioxidant, antihypertensive, fungicidal, larvicidal, hypolipidemic, and recently amoebicidal and giardicidal activities have been reported (Mensah, and Golomeke, 2015). According to several studies, the hypolipidemic effects of the avocado seed focused on methanolic extracts

(Wangensteen, Samuelsen, and Malterud., 2004) and aqueous extracts (Ozolua, Anaka, Okpo, and Idogun., 2009).

The result shows that, hypolipidemic effects provides an interesting alternative since the seed represents 13-18% (Omolara, Friday, and Chinelo., 2017) of the avocado fruit and is discarded during avocado pulp processing. Adeyemi et al states that, uses of avocado pear seed include use in the management of hypertension, diabetes, cancer and inflammation (Anaka, Ozolua, and Okpo., 2009). Several beneficial medicinal properties of compounds present in the avocado seed have been reported, which are related to the elevated levels of phenolic compounds (64% in seed, 23% in peel, and 13% in pulp). In addition, the seeds and peels of avocado also contribute 57% and 38% of the antioxidant capacities of the entire fruit, respectively (Wangensteen, Samuelsen, and Malterud., 2004). Thus, this review article was aimed at reviewing the proximate, functional, anti-nutrients and antimicrobial properties of avocado seed to aware basis for its possible dietary use and justification for its ethno-medicinal use.

Current consumer tendencies indicate that natural preservatives are preferred over synthetic compounds. Synthetic preservatives and additives are widely used in food products to maintain their quality; however, several studies have indicated that these compounds are toxic due to their constant intake (Amchova *et al.,* 2015, Beristaín-Bauza *et al.*, 2018). Therefore, researchers have been looking for new sources of natural compounds with antioxidant or antimicrobial activities and the evaluation of their application in food (Alañón *et al.,* 2017). Fruits and vegetables are a good source of phytochemical compounds; however, their by-products (peel, seed core, pomace, among others) are usually discarded, although they have, in many cases, more bioactive compounds than their edible parts (Barros *et al.,* 2017).

Avocado seeds are considered a waste product in the pulp and oil production and this represents about 12% of the fruit weight in Hass species (Ikhuoria and Malik, 2001). Research has shown that oil from the seed of avocado has health and human nutritional benefit to humanity. Roger (1999) reported that the avocado seed oil can be used to control obesity. Soong and Barlow (2004), showed that avocado seed oil containing antioxidant and phenolic compound. Antioxidant slows down the rate ageing by neutralising the radical elements produced in the body. Nwaogu *et al.,* (2008) reported that avocado seed is a good source of carbohydrate, protein, fat and some mineral elements such as calcium, phosphorus, potassium and magnesium. The quantity of the avocado seed oil used for nutritional and health purposes are

minute and are used occasionally. This does not allow the full utilisation avocado seed oil considering numerous tree plants in the country. Meanwhile, the industrial processing requires a tangible quantity of oil for its production, thereby, allows full utilisation of avocado seed oil.

1.2 Objectives of the Study

The aims and objectives of the work are:

1. To investigate antibacterial activity of avocado seed oil on *campylobacter Jejuni.*
2. Measure the degree of sensitivity of avocado seed oil *on campylobacter jununi*.

CHAPTER TWO
LITERATURE REVIEW

2.1 Avocado

Avocado (Persea americana Mill.) is a fruit native to Central America, grown in warm temperate and subtropical climates throughout the world. The pulp of this fruit contains about 60% oil, 7% skin, and approximately 2% seed (Tan, 2017). The main producers of avocado oil in the world are New Zealand, Mexico, the United States, South Africa, and Chile (Berasategi, 2012). Avocado oil has sparked a growing interest in human nutrition, food industry, and cosmetics. The lipid content, mainly of monounsaturated fatty acids, is associated with cardiovascular system benefits and anti-inflammatory effects (Carvajal-Zarrabal, 201).

There are no internationally defined parameters for avocado oil. The values that are commonly used are those recommended for olive oil. The quality standard for olive oil is available in the Codex Alimentarius and the International Olive Oil Council (IOC) (Avellone, 2017). Woolf (2014) proposed a classification for avocado oil based on its extraction method and fruit quality. Avocado oil of a higher quality, "extra virgin", corresponds to that produced from high-quality fruit, extracted only with mechanical methods, using a temperature below 50 _C and without the use of chemical solvents. "Virgin" avocado oil is produced with fruit of a lower quality (with small areas of rot and physical alterations), extracted by mechanical methods, using a temperature below 50 _ C and without the use of chemical solvents. "Pure" avocado oil is a type of oil for the production of which the quality of the fruit is not important; it is a bleached and deodorized oil, infused with the natural flavor of herbs or fruits. Finally, "mixed" avocado oil is combined with olive, macadamia, and other oils. Therefore, it presents sensory and chemical characteristics that are variable.

The Mexican norm (Di Stefano, 2017) states that the "crude oil of avocado" is a slightly amber-colored fatty liquid, obtained by physical extraction of the pulp and the seed of the fruit (Persea americana). "Pure" edible avocado oil is a product with at least 98.5% refined avocado oil.

2.2 Extraction Methods for Avocado Oil

The information presented in this section focuses on the process efficiency, improvement of production performance, and product quality as well as applications in the food industry. Considering the high humidity percentage of avocado (around 70 to 80%), the influence of the pulp drying method prior to oil extraction has been studied [Costagli, 2015]. The quality parameters (peroxide value, iodine value, amount of oleic acid, refractive index, electrical conductivity, content of carotenoids, chlorophyll, phenolic compounds, and antioxidant activity) have shown better results when the pulp is dried at 60 _C under vacuum, and the extraction is performed by the Soxhlet method. Meanwhile, the bioactive compounds were best preserved when the avocado pulp was dried at 60 _C with air ventilation and mechanical pressing [Krumreich, 2018]. On the other hand, avocado pulp oil, pressed and dried in a microwave, presented a better quality—determined by the acidity index, peroxide index, and oxidative stability—when compared with oil obtained by extraction with ethanol.

The composition of fatty acids did not di_er significantly when analyzing oil obtained by drying under microwaves or in a drying oven with forced air circulation (Santana, 2015). According to Chimsook and Assawarachan (2018), the studied drying method of the avocado pulp, prior to the extraction of the oil, does not significantly influence the composition of fatty acids. However, changes were determined in the antioxidant activity and vitamin E content of cold-pressed avocado oil (from Thailand). Higher antioxidant activities and a higher vitamin E content were observed in oil, the pulp of which was dried with hot air, when compared to oils obtained by an air-dried and vacuum process. This study is consistent with the fact that oils from the fortune avocado variety, obtained by the pulp drying lyophilization method, resulted in lower concentrations of _-tocopherol, squalene and _-sitosterol, as well as higher relative concentrations of campesterol and cycloartenol acetate, compared to oils obtained through hot air-drying processes [Dos Santos, 2014].

2.2.1 Cold Pressed Method

According to the CODEX STAN 19-1981 (2016), the method of extraction of edible vegetable oils is characterized by mechanical procedures, for example, extrusion or pressing, without the application of heat. In addition, the oil can only be purified by washing, sedimentation, filtration, and centrifugation.

In the cold pressing method, oil recovery is only obtained from the parenchyma cells of the pulp; its rupture begins in the first stages of grinding and it can be seen that the idioblastic

cells (oil carriers) remain intact during the process of extraction. The extraction yield increases when the pulp is beaten at 45.5 _C for 2 h [Yang, 2018]. In this method, a lower extraction yield is obtained, although with higher concentrations of _-tocopherol and squalene, as well as lower contents of campesterol and cycloartenol acetate, compared to the Soxhlet method [Valdés, 2019]. Drying by lyophilization and subsequent extraction by the Soxhlet method allows for a better extraction performance. However, when drying by lyophilization and extracting by cold pressing, oils with a greater concentration of antioxidants, and other bioactive compounds were obtained (Dos Santos, 2014).

2.2.2 Ultrasound-Assisted Aqueous Extraction Method (UAAE)

This method uses the cavitation forces produced by acoustic waves to break down the cell walls of the oil-containing cells. This process allows for the generation of an emulsion, which facilitates oil extraction. This method can be carried out using an ultrasonic bath or an ultrasonic horn transducer (Xuan, 2017). The high frequency ultrasound conditioning (0.4, 0.6, and 2 MHz, 5 min, 90 kJ/kg) of the avocado puree can improve the oil separation and potentially reduce the beating time in industrial processes, without a_ecting the quality of the oil. If this treatment is applied after shaking, the extractability of the oil increases by between 2% and 5%. The oils obtained from sonicated purees showed free fatty acids (FFA) and peroxide values below the levels of industrial specification (peroxide less than 20 meqO2/kg) and an increase in total phenolic compounds after a 2 MHz treatment (Martínez-Padilla, 2018). The ultrasound-assisted aqueous extraction (UAAE) of low virgin avocado oil in FFA, considered as virgin avocado oil, is that obtained by mechanical or natural means at low temperatures (<50 _C) and without chemical refining (Valdés, 2019). The optimal UAAE parameters to produce the highest extraction of virgin avocado oil was 6 mL/g water-dried pulp powder, 30 min of sonication time at 35 _C. The sonicated virgin avocado oil was lighter and had a higher level of unsaturated fatty acids, compared to the avocado oil extracted by the Soxhlet method (Corzzini, 2017).

2.2.3 Supercritical CO2 Method

This method of extraction is based on the use of supercritical fluids, substances that are, in certain circumstances, in a state in which they have intermediate properties between liquid and gas. Supercritical CO2 (scCO2) is a totally innocuous gas, which becomes a powerful solvent under conditions of pressure and at a temperature above its critical point (Valdés, 2019).

Extraction with scCO2 presents a higher performance at a pressure of 400 bar. The use of ethanol as a co-solvent favors the extraction of residual oil, benefiting the extraction of a fraction enriched in tocopherols (Corzzini, 2017).

Some authors (Barros, 2016, Barros, 2017) proposed the combined extraction of avocado oil and active compounds present in peppers (capsanthin) and tomatoes (lycopene) using scCO2 in order to enrich the avocado oil. For this, a fixed bed extractor was used, where the lipids and the desired active ingredient were subjected to the extraction process, simultaneously with scCO2. First, both the scCO2 and the oil extracted from the avocado passed through the avocado bed and then through the second bed, where the plant component to be co-extracted was found. The lipids obtained in the first chamber served as a co-solvent with scCO2 for extraction in the second chamber. In the case of the simultaneous extraction of edible avocado oil and the capsanthin (carotenoid) of red pepper, the higher concentration of oil improved the extraction yield of capsanthin. However, a less concentrated extract was obtained, since the carotenoid was diluted in the product. In the case of the extraction of avocado oil rich in lycopene, the extraction yield of lycopene increased as the proportion of avocado in the first extraction chamber increased, being the best condition for the extraction of lycopene present in tomato pomace at 400 bar and 50 _C.

Restrepo et al. (Restrepo, 2012) evaluated the quality of avocado oil extracted by Soxhlet, cold pressed, and scCO2 methods, determining the quality of the oil in terms of free fatty acid titration, peroxide index, iodine index, saponification, and specific gravity, according to the American Oil Chemists' Society (AOCS) standards. Extraction with supercritical fluids was the technique by which the highest yields and quality were obtained. Oils extracted by scCO2 were characterized as possessing a lower acidity index (0.48%), low oxidation of unsaturated fatty acids (16.87 meqO2/kg) and higher iodine index (80.18 cgI2/g), when compared with the other methods. In addition, extraction by cold pressing showed better results in terms of vitamin E content.

Regarding extraction by pressurized fluids, the extraction with the liquefied gas of compressed oil (LPG), constituted by a mixture of propane, n-butane, isobutane, ethane, and other hydrocarbons, showed a higher oil extraction performance in less time and with a lower solvent consumption than the scCO2 method. On the other hand, the oil obtained by compressed LPG presented higher concentrations of Stigmasterol, licopersene, palmitic acid, oleic acid, and

linoleic acid. However, scCO2 provided a higher yield in terms of antioxidant activity, which was determined by means of the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical assay [Abaide, 2017].

2.2.4 CO2 Subcritical Method

Extraction with sCO2 operates under the same principle as the scCO2 extraction, but with a temperature below 31.1 _C and CO2 pressure of 72.9 bar (Xuan, 2007). In this part of the review, a comparison of the physicochemical properties of avocado oil, extracted through sCO2, UAAE, and conventional solvent (AOAC 920.39 [2007]) will be analyzed. Extraction with sCO2 was performed at 27 _C and 68 bar CO2, UAAE was performed with 60 mL of distilled water, an ultrasonic power of 240Wand a frequency of 40 kHz for 30 min at 35 _C, followed by a final pressing. Compared to solvent extraction, oils extracted using sCO2 and UAAE had higher iodine index values but lower melting points, determined by slip, free fatty acid content, and saponification index values. The oils extracted by sCO2 and UAAE have a clear color and higher levels of unsaturated fatty acids than the oil extracted with hexane. Regardless of the extraction method, the main fatty acids in avocado oils were oleic and palmitic acids, while the main triacylglycerols in avocado oils were palmitoyl-dioleoyl-glycerol (POO; 22.48–23.01%) and palmitoyl- Oleoyl-linoleoyl-glycerol (POL; 17.64–18.23%) (Xuan, 2007).

2.2.5 Enzymatic Extraction

In order to improve the performance of extraction by centrifugation, the incorporation of enzymes, such as pectinases, -amylase, proteases, and cellulase, to avocado paste have been considered. The yield varies depending on the concentration and type of enzyme used and the reaction time and percentage of water used. It is emphasized that this method improves oil by up to 25 times, in comparison with the performance of a non-enzymatic centrifugation (Reddy, 2012).

2.2.6 Solvent Extraction

Reddy *et al*. (2018) compared four extraction methods to produce avocado oil (Hass and Fuerte variety). They analyzed: (1) the extraction with traditional solvent using Soxhlet (5.0 g of dried avocado sample with 250 mL of hexane for 24 h); (2) Ultrasonic Soxhlet extraction (5.0 g of dry avocado sample, sonicated in a water bath at 60 _C, with hexane as the solvent, for 1 h); (3) Soxhlet extraction, combined with a microwave treatment (avocado paste 5 mm thick, extended in the rotating plate of a domestic microwave oven, heated to the maximum power for

11 min, with 5 g of the resulting mass subsequently extracted by means of the Soxhlet method with hexane); and (4) extraction with supercritical fluid (Argon and scCO2 used as extraction fluids, extractions performed for 2 h, with a fluid flow rate of 2.8–3.5 mL/min).

The traditional Soxhlet extraction method yields the most reproducible results, whereas the microwave extraction showed a higher extraction yield and higher fatty acid content (69.94%). Meyer and Terry (2018) performed a sequential extraction and quantification of fatty acids and avocado sugars. The average oil yield using Soxhlet extraction, with ethanol as the solvent, was significantly higher than the oil obtained by homogenization with hexane, and the fatty acid profiles for the two methods were similar. As the maturity of the fruit increases, the extraction of oil is improved. After lipid removal, methanolic extraction was superior in terms of the sucrose and perseitol obtained, compared to extraction with 80% ethanol (v/v). The extraction of mannoheptulose was not affected by any of the solvents used.

The yield of avocado oil extraction has been assessed, comparing four extraction methods using solvents of different polarities. The extraction was performed using the Soxhlet method, with (1) petroleum ether, (2) homogenization with petroleum ether, (3) homogenization with a mixture of chloroform/methanol (2:1 v/v), and finally (4) extraction with chlorine-naphthalene and ball milling.

It was determined that methods that only use petroleum ether as an extraction medium presented lower yields (6–9% less) than the last two methods. Saponifiable residues were lower when the method using the chloroform/methanol mixture was employed. However, this method did not completely eliminate the residual oil from the fruit (Meyer, 2008).

Ortiz-Moreno (2019) analyzed the e_ect of four extraction methods on the chemical-physical quality of avocado oil, namely,

(1) The method of microwave extraction and manual pressing,

(2) Extraction with hexane using the Soxhlet methodology,

(3) Microwave extraction, combined with the Soxhlet methodology, using hexane as the solvent.

(4) Extraction with acetone.

The method with the highest oil extraction performance was the third method. The amount of trans fatty acids produced by the first method was the lowest and the latter method is also the one that generates the least physicochemical alterations.

When analyzing the effect of the drying method and avocado oil extraction process, ripe fruit,

independent of the drying method, presents a higher extraction performance than immature fruit. This is influenced by the enzymatic degradation of the cell wall of the parenchyma during maturation. Freeze drying improves the amount of oil extracted for the scCO2 extracts and, to a lesser extent, for the hexane extracts. Extraction with hexane has been shown to have a higher oil extraction yield than scCO2 due to the lower degree of selectivity of this solvent, which completely penetrates the plant material (Ortiz-Moreno, 2003).

Regarding the performance of the avocado oil extraction process, methodologies have been proposed for developing countries. One is carried outwith boiling petroleumether (30–60 _C) and another extraction with distilled water (avocado paste, diluted at a ratio of 3:1 and 5:1 (w/w), heated in a water bath of 75 to 98 _C, with subsequent centrifugation. In the extraction methods, calcium chloride, sodium chloride, calciumcarbonate and calciumsulfatewere used as extraction aids. The presence of inorganic salts at a low concentration improves the extraction performance, provided that it does not exceed 5%; otherwise, it has an adverse effect. Themost efficient extractions were obtained with a water/avocado ratio of 5:1, pH of 5.5 and centrifugal force of 12,300_ g, with the addition of 5% calcium carbonate or calcium sulfate. At higher heating temperatures (75–98 _C), the oil release time decrease. In addition, the gravity sedimentation for four days at 37 _C, followed by centrifugation, improves the oil extraction performance (Mostert, 2007).

Considering the use of organic solvents in avocado oil extraction processes could alter the quality of fatty acids by inducing the formation of trans isomers. Ariza-Ortega et al. (Bizimana, 1993) proposed the application of infrared spectroscopy by Fourier transform (FTIR) to study trans fatty acids in the avocado oils of the Hass, Fuerte, and Criollo varieties. For this, oil extraction was performed by centrifugation at 40 _C and extraction with hexane at 70 _C for 4 h. The method using centrifugation did not increase the deterioration of fatty acids. A strong band at 723 cm□1 was documented, which is attributable to the cis functional groups, where the green color was maintained. On the other hand, the infrared spectroscopy with Fourier transform (FTIR) analysis identified an absorption band, located at 968 cm□1, which is associated with fatty acids, with trans isomerism for the Fuerte variety extracted with hexane.

2.3 Procedures for the Conservation of Avocado Oil

The conservation of oils is a necessary issue to address, since it allows for increasing the useful life of the products. One of the efforts made to improve the conservation of avocado oil has been the use of physical techniques, such as the electric field.

The electric field (voltage 9 kV cm□1, frequency 720 Hz, time of 5 and 25 min) allows the polyphenol oxidase enzyme present in the avocado pulp to be inactivated, preserving the components present in the avocado oil. The modifications in the quality of the refined oil (established according to the acidity index, peroxides, and iodine) are minimal, considering the electric field method as an alternative for the addition of synthetic antioxidants [Ariza-Ortega,2010].

The oxidative stability (determined by finding the antioxidant activity reducing ferric ion, FRAP), during the storage of cold-extracted avocado oil in the presence of the oleoresins of Capsicum annuum L. (vegetable material rich in carotenoids), was assessed. It was determined that the optimal extraction of carotenoids was at a concentration of 1:3 (w/v: Capsicum annuum L/avocado oil) for 48 h in darkness at room temperature. The behavior of the oil under stronger conditions (45 _C, 30 days) showed the following characteristics: (1) the extracts were stable to lipid oxidation, with a Totox index total value of 27.34, (2) 85.6% of carotenoids were conserved, (3) 80.66% of the antioxidant activity was retained, and (4) there was a color change (DE) of 1.783. The oleoresins obtained by extraction with avocado oil can be considered as an economic and sustainable alternative for the extraction of carotenoids, with a good oxidative stability, compared with organic solvents (Cerecedo-Cruz, 2018).

2.4 Use of Analytical Techniques in the Quantification, Adulteration, and Contamination of Avocado Oil

There is growing interest among consumers in accessing quality and authentic products. Vegetable oils can suffer from contamination and/or adulteration, which causes the product to have components not specific to the oil. It is here that the development, implementation, and application of analytical technologies are very useful.

The components present in avocado oil, such as fatty acids and phytosterols, have been quantified, mainly by gas chromatography, coupled with a flame ionization detector (GC-FID). In addition, techniques, such as ultra-high-performance liquid chromatography (UHPLC), coupled with mass spectrometry (UHPLC-MS) or a photodiode array detector (UHPLC-PDA), as well as Inductively Coupled Plasma Mass Spectrometry (ICP-MS), have been used for the

identification and/or quantification of analytes, such as polyphenols, squalene and minerals, respectively (Cicero, 2018).

Other analytical techniques have been used for the qualitative determination of the components present in avocado oil, including 13C nuclear magnetic resonance spectroscopy (NMR), which has been used for the identification of its major components, including fatty acids (Retief, 2009). At the same time, 1H Nuclear magnetic resonance spectroscopy (1H-NMR) has been used for the detection of the minor components present in other vegetable oils (Castejón, 2014). Therefore, the development of new analytical methodologies for quantifying the analytes present in avocado oil represents a major challenge.

Rohman (2016) studied the purity of avocado oil, adulteratedwith palmoil and canola oil, through FTIR, combined with chemometric techniques. FTIR combined with multivariate calibrations can be used to detect and quantify the adulteration of avocado oil in binary mixtures with palm oil and canola oil.

The adulteration of avocado oil with soybean oil or grape seed oil can be determined using mid-infrared spectroscopy, combined with the statistical method of partial least squares discriminant analysis. This methodology allows for a simple and fast discrimination of avocado oil in binary mixtures and Tertiary oils. The frequency selected for the authentication of avocado oil was 1500–750 cm□1, with a precision of 100% for the analysis of the mixture of two oils and 93.3% for the mixture of three oils (Rohman, 2016).

Organophosphorus pesticides in samples of commercial avocado oil were determined using atmospheric pressure microwave-assisted liquid–liquid extraction (APMAE), with solid-phase extraction or low-temperature precipitation, as the clean-up step. The analysis was carried out by gas chromatography-flame photometric detection and gas chromatography-tandem mass spectrometry. Chlorpyrifos residues were detected in one of four samples of commercially packaged avocado oil, produced in Chile (Fuentes,2009). While spectroscopic techniques have focused on determining the adulteration of avocado oil with the presence of other types of vegetable oil, according to the literature reviewed here, there is a research deficiency related to the modification of the composition of avocado oil, including the study of its major components, such as triacylglycerides and/or fatty acids, in addition to its minority components, such as phytosterols, alkanes, aliphatic alcohols, polyphenols, and others. This could provide information for detecting the contamination of avocado oil with other oils of a different quality.

2.5 Technological Applications of Avocado Oil

At the industrial level, there is a constant demand for the production of healthy foods that can maintain their nutritional properties over time, as well as environmentally friendly technological solutions. Avocado oil is mainly sold for direct consumption due to its interesting contribution of fatty acids, vitamins, antioxidants, among other compounds. efforts have been made to develop products based on avocado oil. Arancibia (2018) propose the development of O/W nanoemulsions using the natural emulsifiers, lecithin and synthetic tween 80, systems that improve the characteristics with respect to traditional emulsions, such as (i) increased dispersibility of water in the encapsulated oils, which generates slightly turbid emulsions and an easy production, and (ii) a good physical and chemical stability, as well as a high bioavailability of its lipid components.

Another interesting technological application for avocado oil has been the production of structured lipids. Caballero et al. (2018) propose the elaboration of triacylglycerides of the MLM type, using regio-specific immobilized commercial lipases sn-1.3, where M corresponds to saturated medium-chain fatty acids (6–12 carbon atoms) at positions sn1 and sn3 of glycerol. L corresponds to saturated or unsaturated long-chain fatty acids (14–24 carbon atoms) in the sn2 position. The increased interest in this type of lipids is due to the low caloric intake (average caloric density for this family of lipids 5 kcal/g). According to the literature, there are no negative e_ects associated with the ingestion of MLM lipids for both animals and humans. Finally, avocado oil has also been used in the production of biodegradable polymers. Polyhydroxyalkanoates (PHAs) are linear polyesters, produced by a large number of bacteria under stress conditions, with di_erent thermal and mechanical properties, which depend on their molecular structure. Flores-Sanchez *et al*. (2019) prepared PHAs through a fermentative process using the bacterium C. nec*á*tor H-16 with avocado oil and fructose, as a carbon source. The highest yield in obtaining polymers was obtained when the addition of avocado oil was 20% v/v, which demonstrates the feasibility of using this oil as a renewable carbon source for the PHA production process.

2.6 Composition of Avocado Oil

There is a growing interest in avocado oil, including the determination of the composition of

major and minor components. Therefore, for a total understanding of the nutritional and functional properties that this oil presents, it is important to consider the different varieties and parts of the fruit.

2.7 Characteristics According to the Variety and Origin of the Fruit

Avocado is a fruit grown mainly in warm temperate and subtropical climates throughout the world, so it is interesting to study how the climate and country of origin can affect the fruit quality and therefore, the oil. Thus, the oil from the fruit of the Hass variety, originating from crops from Mexico, Australia, the United States, and New Zealand, was characterized by a high content of 62% lipids, of which oleic (42–51%) and palmitic (20–25%) lipidswere present in a greater proportion. Among the predominant triacylglycerolswere OOO (21–34%) and OOP (19–24%), where O and P denote oleic and palmitic acids, respectively. On the other hand, Hass avocado oil from New Zealand contained a significant amount of natural pigments and unsaturated compounds, compared to oils from Mexico, Australia, and the United States .

Studies carried out in South America on the analysis and characterization of avocado oil showed a high content of monounsaturated fatty acids (69.4%) and a lower amount of polyunsaturated and saturated fatty acids, which were 16.6% and 14%, respectively. These studies indicate that avocado oil has a thermal stability close to 176 _C and has a lower concentration of total phenolic compounds than olive oil. Despite this, the antioxidant activity of avocado oil is similar to that of olive oil. Olive oil has a high concentration of polyphenols, such as tyrosol and hydroxytyrosol (Forero-Doria, 2014).

Galvão (2018) analyzed the fatty acid composition of the pulp, seed, and skin oil of the Fortuna, Collinson, and Barker varieties, indicating that there was a small variation in the composition of monounsaturated fatty acids in the skin oil among the cultivars. However, the seed oil of the Collinson variety was the best due to the lower SFA content. The SFA content for pulp oil corresponded to 22.3, 29.4 and 41.3% in the Fortuna, Collinson, and Barker varieties, respectively. In this sense, it was possible to a_rm that the pulp oil of the Fortuna and Collinson varieties presented a better quality, in terms of fatty acid profile, than the Barker variety.

In Mexico, avocado oil from six local creole varieties (BTancitaro, Irapuato, Org*á*nico, Puerto, San Jos*é*, and STancitaro) were analyzed and compared with oil from the Hass variety. It was observed that the Mexican creole genotypes had a greater thermal stability, properties resistant to oxidation, and a greater phenolic content, in comparison with the commercial oil

from the Hass variety. In addition, these varieties showed intense fluorescent peaks at 675 and 720 nm, as well as broad absorption bands centered at 465 and 510 nm, which can be used as an identification parameter for these oils (Forero-Doria, 2014). Yanty *et al.* (2019) indicated that the avocado oil originating from three Malaysian varieties was found in a significantly lower proportion than in the Australian Hass variety. In addition to being in a semi-solid form, all these oils had a higher proportion of oleic acid, although they also had different proportions of palmitic and linoleic acids. Regarding the composition of TAG for local varieties, the highest was POO, followed by POL, OOO, and PPO, while in the Hass variety, the distribution was OOO, followed by PPO, OOL, and POL. As a result of these different compositions in TAG, differences were found in the iodine index, melting point by slip and melting and solidification characteristics.

2.8. Avocado Seed Oil

In relation to avocado seed oil, Barrera-López and Arrubia-Vélez (Barrera-López, 2017) pointed out that the Lorena variety contained about 8.47% oil, and the unsaponifiable matter was 76.9%. The phytosterols quantified in a greater proportion were ergosterol, 5_-cholestane and stigmasterol. Avocado seed presents, in its composition, a large number of extractable polyphenols, which have attracted attention due to their high antioxidant capacity. It was determined that, with a higher power of the ultrasound (0–104 W) and increase of the temperature (20–60 _C), the polyphenol content and antioxidant capacity was increased [Segovia, 2016].

When performing a physicochemical analysis of the seed oil of the Hass variety, cultivated in Peru and obtained by the Soxhlet method, it was found that it had a high fatty acid profile in linoleic acid (48.77%) and linolenic acid (12.17%). While the antioxidant activity, determined by the DPPH method, was low, it was higher in the saponifiable fraction than in the unsaponifiable fraction, which was attributable to the presence of polyphenols and steroids. In addition, it was determined that the quality parameters, such as acidity, peroxide, saponification, iodine, and specific gravity indexes, were similar to those for extra virgin olive oil (Rengifo, 2015).

When comparing the composition of oil from the pulp and seed of the Fuerte variety, cultivated in the region of Northeastern Brazil, a great difference in the lipid content between the pulp and the seed can be seen (15.39% v.s. 1.87%, dry base). It was determined that the parameters of oil

quality, refractive index, gravity, and peroxide index were similar for both oils, but the iodine, acid index, and saponification index were higher in seed oil than in pulp oil. Gas chromatography showed that seed oil had a greater variety of fatty acids than pulp oil. Additionally, the fatty acid profile of the pulp was much more concentrated in monounsaturated fatty acids than that of seed, and conversely, the seed oil is much more concentrated in polyunsaturated fatty acids than pulp oil (Bora, 2001).

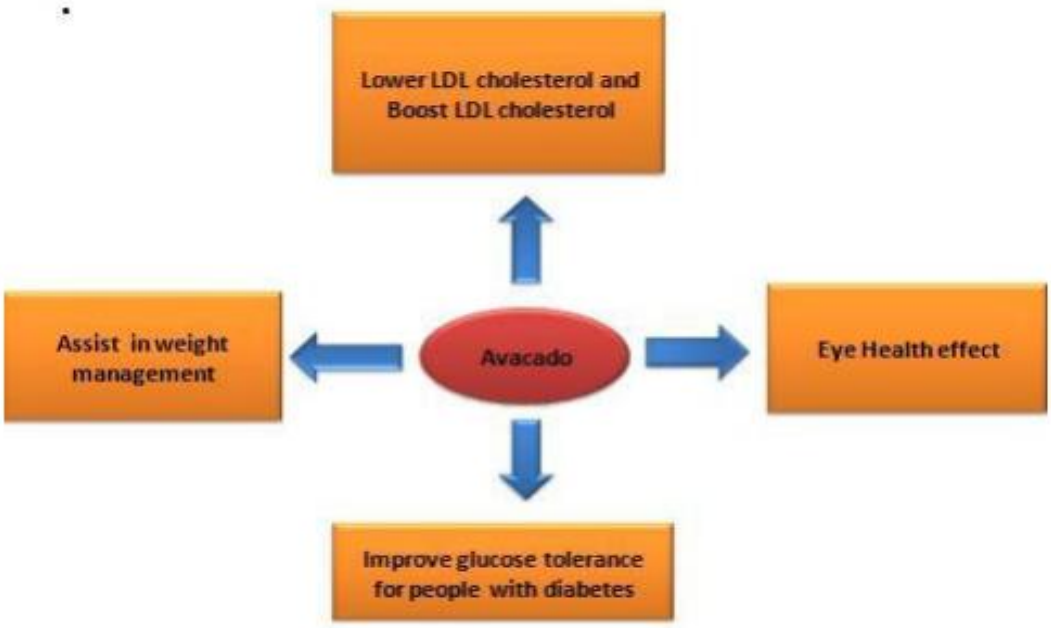
2.9 Biological Effects of Avocado Seed Oil

The presence of compounds with nutritional interest, such as unsaturated fatty acids (MUFA and PUFA), as well as compounds with biological activity, such as tocopherols, tocotrienols, phytosterols, carotenoids, and polyphenols, have made avocado oil of growing interest for research on the possible biological effects of avocado oil, with the aim of preventing and treating diseases through the diet of the population.

2.10 Human Health Effects of Avocado Seed Oil

A study in 13 healthy adults with a habitual hypercaloric and hyperlipidic diet, where butter was replaced by avocado oil extracted at 35 _C from the pulp alone, was conducted. The incorporation of avocado oil for a period of six days reflected an improvement in the postprandial profile of insulin, glycemia, total cholesterol, low-density lipoproteins, triglycerides, and inflammatory parameters, such as C-reactive protein (CRP) and interleukin-6 (Furlan, 2017). Avocado pulp oil (Mexican creole genotypes) has shown anti-inflammatory activity by inhibiting the enzymes COX 1 and COX 2 in a similar way to the drug, ibuprofen, and extra virgin olive oil [Espinosa-Alonso,2017]. Additionally, when avocado oil was added to vitamin B12 skin cream preparation, it was well tolerated and had the potential for long-term topical therapy of psoriasis (Stucker, 2001).

2.11 Health Benefits of Avocado Seed Oil



2.12 Avocado and Weight Management

The three studied looking the effect of avocado on a portion of weight management. The first study, examined the effects of avocados as part of an energy-confidential diet on serum lipids, weight loss, and vascular function in 55 overweight and obese people. The participants were randomly divided into two groups – one group consuming an energy restricted diet including 200g of avocado a day in place of 30g of other dietary fats, another group consuming an energy restricted control diet. Following six weeks, body weight, body mass index, and percentage of body fat all decrease drastically in both diet groups. The researchers concluded that 200g a day of avocado could be consumed in an energy-restricted diet without compromising weight loss when substituted for 30g of other dietary fats. A randomized crossover study found that overweight people adding half an avocado in lunch to increase their satiety by 25% and decreased their desire to eat by 30% for 3-5 hours next the meal. The 25 Adding avocado to lunch may help reduce mid meal snacking. A recent US analysis, found that avocado consumers had a lower body weight, Body Mass Index and waist fringe compared to those who did not eat avocado (Pieterse, 2005). The long-term trials as well as identify mechanisms for the role of avocados in weight management. The extract of avocado fruit inhibits the action of acetyl-CoA carboxylase, a key enzyme in the production of fat in the body (Fulgoni, 2013).

2.13 Improve glucose tolerance for people with diabetes type 2 diabetes

The two studied that the effects of avocado in those with type 2 diabetes mellitus, were reduced blood glucose levels in 13 out of the 15 participants with Type 2 diabetes, and noted only five of the 15 had clinically significant reductions. A small randomised crossover study involving 12 women with Type 2Dibetes, found that after four weeks each of a high monounsaturated fat diet (with an avocado a day, eating a third at each meal) and a high carbohydrate diet, both diets caused a minor cholesterol lowering effect with no major changes in High Density Lipid cholesterol. The avocado diet was associated with a greater decrease in blood triglycerides and glycemic control was similar with both diets. Researchers concluded that including avocado in the diet of those with T2D could help reduce cholesterol and triglycerides without compromising blood glucose control. Clearly, more research in the area of Type 2 diabetes is needed, but there have also been interesting Type 2 diabetes, preliminary animal studies that suggest that extracts of avocado leaf and seed may improve blood glucose control.

2.14 Help the Absorption of Colorful Carotenoids Necessary for Eye Health

The macula lutea is a "yellow spot" in the center of the retina responsible for central vision. Macular degeneration is the result of age related damage and it impacts central vision. The macula is yellow because it is rich in lutein, carotenoids and zeaxanthin that are thought to combat light-induced damage caused by free radicals. Carotenoids help to reduce the risk of macular degeneration and are transported to the macula by High Density Lipid cholesterol. Avocados provide a affluence of benefits as they contain carotenoids and help to boost High Density Lipid cholesterol and their healthy fats absorb fat soluble carotenoids from other foods. Research shows that avocado to a salad or salsa increases the absorption of carotenoids from other salad vegetables fivefold (Brai, 2007). Carotenoids are considered to be pro Vitamin A and converted to vitamin A in the body. Vitamin A is a necessary fat-soluble vitamin that is required for normal reproduction, vision and immune function. In animal products Preformed vitamin A is found while carotenoids are largely found in plant foods and eggs (Widomska, 2014). The recently exposed that eating of avocado with other vegetables rich in carotenoids boosts both the absorption of carotenoids and their conversion to vitamin A. Particularly, avocado improved the absorption of beta carotene from tomatoes and enhanced the efficiency of conversion to vitamin A. In the case of carrots, absorption was increased sixfold and the efficiency of conversion of vitamin A (Widomska, 2014).

2.15 Dosage

Given by mouth Avocado oil/soy oil unsaponifiable fraction has been studied for osteoarthritis treatment of the knee at 300 to 600 mg daily dosage and osteoarthritis treatments use at 300 mg/day in 2 other studies where indicative relief was obtained (Meyers, 2014).

2.16 Adverse reactions

The Manifestation allergy of avocado may be limited to the throat and mouth (oral allergy syndrome, itching mouth, swollen tongue, and throat) or oral symptoms with generalized symptoms (eg, wheezing, abdominal cramping, chest tightness, diarrhoea). Cross-sensitivity has been shown with melons (eg, cantaloupe), peaches, tomatoes, potatoes, bananas, chestnuts and kiwi fruits. Crosssensitivity has also been seen in patients with natural rubber latex (e.g. latex gloves) allergy and avocados. This cross-sensitivity is called the "latexfruit syndrome" . An IgE-mediated inflammatory mechanism has been shown to be similar in producing an allergic reaction to latex, bananas, and avocados.

2.17 Side effect and safety

Avocado is mostly safe for most people when the fruit is eaten in food average amounts. When taken by mouth seems to be possibly safe as a medicine for up to 2 years or when applied to the skin for up to 3 months. It normally has few side effects, and those people who used a specific avocado, vitamin B12, cream for psoriasis reported mild eager. Remain in mind that avocado has a lot of calories because of its fat content.

2.17.1 Pregnancy and breast-feeding

There is not adequate steadfast in sequence about the protection of taking avocado as medicine if you are pregnant or breastfeeding. Stay on the safe side and a stick of to the foods amount.

2.17.2 Latex allergy

The People who are sensitive to latex can have an allergic reaction to avocado.

2.18 *Campylobacteriosis*

Campylobacteriosis is a food poisoning caused by genus *Campylobacter*. *Campylobacter* belong to a distinct group of specialized bacteria designated rRNA superfamily VI of Class *Proteobacteria* (Allos, 2011). *Campylobacter* species are slender Gram-negative rod-shaped, spiral-shaped with single or pair of flagella. Some *Campylobacter* species have multiple flagella such as C. showae while some species are non-motile like *C. gracilis* (Acke, 2018). *Campylobacter* species are indole negative, oxidase positive, hippurate positive, catalase positive, nitrate positive and glucose utilization negative (Pal, 2017). *Campylobacter* species are

closely related group of bacteria that principally colonise the gastrointestinal tracts of different animals (El-Gendy *et al.,* 2013).

Campylobacter species are enormous significance due to the increase in number of species implicated in animals and human's infections (Jamshidi *et al.,* 2008; Kaakoush *et al.,* 2015*).* Since its first identification, the number of pathogenic *Campylobacter* species that causes animal and human infections are largely classified through phylogenetic means with few as 500–800 bacteria ingestion dose resulting to human disease (Frirdich *et al.,* 2017; Kaakoush *et al.,* 2009). Nonetheless, report has shown that *Campylobacter* doses of 100 cells or less have been linked with human infections (Tribble *et al.,* 2010). *Campylobacter* species have also been reported to be implicated in various human systemic infections including septic thrombophlebitis, endocarditis, neonatal sepsis, pneumonia (Alnimr, 2014), bloodstream infections (BSIs) (Morishita *et al.,* 2013), acute colitis of inflammatory bowel disease and acute appendicitis (Lagler *et al.,* 2016). Other major post-infections that significantly add to *Campylobacter* disease burden include severe demyelinating neuropathy, Guillain-Barr_e syndrome (GBS) (Scallan *et al.,* 2015), sequelae and Miller-Fisher syndrome (MFS) (Skarp *et al.,* 2016).

Campylobacter species are also associated with series of gastrointestinal infections like colorectal cancer and Barrett's esophagus (Man, 2011). In small group of patients, *Campylobacter* species have also been reported to be associated with extragastrointestinal infections such as brain abscesses, meningitis, lung infections, bacteremia and reactive arthritis (Man, 2011). *Campylobacter* is a significant zoonotic causes of bacterial food-borne infection (Hsieh and Sulaiman, 2018) and farm animals are the major reservoir of *Campylobacter* species and the major cause of *campylobacteriosis* (Grant *et al.,* 2018). Worldwide, farm animals are also the major cause of both bacteria food poisoning (Del Collo *et al.,* 2017) and *Campylobacter* foodborne gastrointestinal infections (Seguino et al., 2018).

Campylobacter foodborne infection is a problem and an economic burden to human population which caused about 8.4% of the global diarrhea cases (Connerton and Connerton, 2017). *Campylobacter* foodborne infection is a global concern because of the emerging *Campylobacter* species involved in both human infections and *Campylobacter* foodborne outbreaks (CDC, 2014). *Campylobacter* foodborne outbreak is defined as *Campylobacter* infection that involve more than two or more persons as a result of consumption of

Campylobacter contaminated foods (Mungai *et al.,* 2015). Majority of *campylobacteriosis* cases are not recognized as outbreaks rather as sporadic episode involving a single family group (Del Collo et al., 2017).

Campylobacteriosis is a collective name of infections caused by pathogenic *Campylobacter* species and is characterized by fever, vomiting, watery or bloody diarrhea (Scallan *et al.,* 2015). In general, *Campylobacter* infections are predominantly common in certain age group such as children (below 4) and the aged (above 75) (L_evesque **et al.,** 2013). Other group of people at high risk of *Campylobacter* infections are immunocompromised individuals, hemoglobinopathies patients and those suffering from inflammatory bowel disease (Kennedy *et al.,* 2004).

In the 1970's with the development of suitable selective media, it was established that *Campylobacter jejuni,* and to a lesser extent *Campylobacter coli,* are a major cause of diarrhoeal illness, rivaling and even surpassing *Salmonella* in importance in many countries, *Campylobacter laridis, C. Concisus* and *C. hyointestinalis* have also been isolated occasionally from patients with diarrhoea and *C pylori,* now calsscied and *Helicobacter Pylori,* has been associated with gastritis and stomach and duodenal ulcers

In addition, the risks of *Campylobacter* infections are higher in high income nations than in low income nations (Platts-Mills and Kosek, 2014). In low income nations, a number of environmental sources pose a high risks of human *Campylobacter* infections (Lee *et al.,* 2013); and most outbreaks are caused by consumption of poultry meats and poultry products (Taylor *et al.,* 2013). Poultry meats include meats from laying hens, turkeys, ostriches, ducks and broilers (Epps *et al.,* 2013), and poultry meats and it product cause about 60–80% of the global *campylobacteriosis* cases (EFSA, 2015).

2.19 Isolation and Identification

Although most of the isolation procedures and media used were designed for C. *Jejuni,* they are also suitable for *C. coli* and *C. laridis.* Pathogenic *campylobacters* have a reputation for being difficult to grow but in fact their nutritional requirements are not particularly complex and they can be grown on a number of peptone, based media including nutrient broth. Where problems can sometimes arise is in their sensitivity to oxygen and its reactive derivatives. Although pathogenic *campylobacters* possess catalase and superoxide dismutase, the accumulation of peroxides and superoxide in media during storage or incubation can inhibit

growth. For this reason an incubation atmosphere of 5 – 6% oxygen with about 10% carbon dioxide and media containing oxygen scavenging compounds such as blood, pyruvate, ferrous salts, charcoal and metabisulfite are commonly used.

A number of selective enrichment media are used which include cocktails of antibiotics such as polymyxin B, trimethoprim and others as selective agents. In many cases cells isolated from food or other environmental sources have been sublethally injured as a result of stresses such as freezing, drying or heating and, as a result, are more sensitive to antibiotics and toxic oxygen derivatives. This can mean that they will not grow on the usual selective media unless allowed a period for recovery and repair in which case a resuscitation stage of 4 h at 37^{o} C in a non selective environment is recommended.

After selective enrichment for 24 and 48h under microaerobic conditions at 42 – 43^{o} C, samples are streaked on to selective plating media. These normally contain a nutrient – rich basal medium supplemented with oxygen scavengers such as blood and /or FBP (a mixture of ferrous sulfate, sodium metabisulfite, and sodium pyruvate) and a cocktail of antibiotics similar to those used for selective enrichment. It is important to store pre prepared media under nitrogen, at 4^{o} C and away from light to reduce the build up of toxic oxide.

2.20 Association with Foods

Campylobacter infection can be acquired by a number of routes. Direct transmission person – to – person or from contact with infected animals, particularly young pets such as kittens or puppies, has been reported, as have occasional waterborne outbreaks. However food is thought to be the principal vehicle.

As a common inhabitant of the gastrointestinal tract of warm blooded animals, campylobacter in inevitably finds its way on to meat when carcasses are contaminated with intestinal contents during slaughter in the abattoir, the incidence of campylobacter positive beef carcasses in Australia was found to decrease from 12.3% to 2.9% on chilling and a similar survey of pig carcasses in the UK found to a decrease from 59% down to 2%. This is primarily a result of the sensitivity of *campylobacter* to the dehydration that takes place on chilling. Subsequent butchering of red – meat viability will decline more slowly.

Poultry carcasses which cool more rapidly due to their size suffer less surface drying when air – chilled and this, probably coupled with the surface texture of poultry skin, enhances

survival. Surveys in Australia, the UK and the USA have found 45%, 72% and 80% respectively of chilled poultry carcasses at the abattoir to contain *campylobacter*.

The incidence of *campylobacters* on retail means in several countries has been found to vary from 0 – 8.1% for red meats and from 23.1 – 84% for chicken. Adequate cooking will assure safety of meats but serious under cooking or cross contamination from raw to cooked product in the kitchen are thought to be major routes of infection.

Despite it frequent occurrence in poultry, eggs do not appear to be an important source of campylobacter. Studies of eggs from flocks colonized with *C. jejuni* have found the organism on around 1% of egg shells or the inner shell and membranes. Prolonged survival on the dry eggs surfaced is unlikely and egg albumin has been shown to be strongly bactericidal.

2.21 *Campylobacter jejuni*

C. *jejun*i is a motile, microaerophilic, zoonotic, thermophilic bacterial considered as the leading cause of worldwide foodborne bacterial gastroenteritis (Taheri et al., 2019). It's a member of the genus *Campylobacter* with polar flagella and helical morphology that is used for movement through viscous solutions including the mucus layer of the gastrointestinal tract (Lertsethtakarn *et al.,* 2011). C. *jejuni* is the major enteric pathogen that displays significant strain-to-strain dissimilarities in their pathogenicity patterns (Hofreuter *et al.*, 2006).

2.22 Pathogenicity of Campylobacter Species

Campylobacter species are of economic importance as they constantly cause foodborne infections due to diverse genes involved in its pathogenicity (Bolton, 2015). *Campylobacter* pathogenicity is based on the virulence factors (Larson *et al.,* 2008) and these virulence factors are multi-factorial in nature and the ability of these bacteria to survival and resist physiological stress also contributes to its pathogenicity (Casabonne *et al.,* 2016; Ketley, 1995). The various virulence related mechanisms displayed by *Campylobacter* species includes invasive properties, oxidative stress defence, toxin production, iron acquisition and its ability to remain viable but non-culturable state (Bhavsar and Kapadnis, 2006).

Campylobacter invasion, adherence and colonization also add to the pathogenicity of these groups of bacteria (Backert et al., 2013). Other virulence factors of *Campylobacter* include; secretion of some sets of proteins, translocation capabilities and flagella-mediated motility (Biswas *et al.,* 2011).

2.23 Toxin Production

Campylobacter produce different type of cytotoxins and cytolethal distending toxin (CDT) is one of these toxins (Schulze et al., 1998). CDT is a tripartite toxin that is made up of three subunits encoded by the cdtA, cdtB and cdtC genes. Cytolethal distending toxin activity is determined by these three cdt cluster genes (Martinez *et al.,* 2006). These three cdt cluster genes are all needed for these toxins to be active (Asakura *et al.,* 2008). The cdtA and C genes are heterodimeric toxin subunits responsible for toxin binding and internalization of the host cell while cdtB is the subunit which encodes for the toxic/active components of the toxin (Abuoun *et al.,* 2005). Cytolethal distending toxins induce diarrhea in both humans and animals by intrusive with the division of cells in the intestinal crypts (Carvalho *et al.,* 2013).

2.24 Invasion

Invasion is another virulence mechanism in *Campylobacter* that is carried out by the flagella which also function as an export apparatus in the secretion of non-flagella proteins during host invasion (Poly and Guerry, 2008). There are many virulence genes that are involved in *Campylobacter* invasion mechanism and the products of these genes including flagellin C (flaC) and invasion antigens (cia) genes.

These genes are transported into the host cell's cytoplasm with the aid of flagella secretion system which is vital for invasion and colonisation (Konkel *et al.,* 2004). The secretion of invasion antigens and invasion protein B (ciaB) are also important virulence proteins synthesized by *Campylobacter* species which help in the epithelial cells invasion and adhesion of the host gastrointestinal tract (Casabonne *et al.,* 2016). Other important virulence genes and proteins synthesized by *Campylobacter* species including the 73-kDa protein involved in adhesion, the invasion antigen C protein involved in full invasion of INT-407 cells, invasion associated protein gene (iamA) implicated in invasion and virulence, the periplasmic protein HtrA responsible for full binding to the epithelial cells, the HtrA chaperone implicated in full folding of out outer membrane protein, the CiaI gene implicated in intracellular survival (Bolton, 2015) and pldA and hcp genes responsible for the expression of invasion (Iglesias-Torrens *et al.,* 2018).

2.25 Transmission routes of Campylobacter infection

Campylobacter species majorly colonized the intestine of poultry, European blackbirds, cattle, sheep, ostriches, cats, dogs and pigs (Dearlove *et al.,* 2016). These bacteria are shed in the faeces of these animals into the environment (Goni *et al.,* 2017). *Campylobacter* can also spread

to person by direct contact to animals such as pets (ESR, 2016; Westermarck, 2016), with dog owners at high risk of *Campylobacter* infection (Gras *et al.*, 2013).

Beside pets, other domestic animals such as cattle are also regularly colonized by *Campylobacter* species and persons working with these animals are also at high risk of *Campylobacter* infection (Hansson *et al.*, 2018). Other sets of people at high risk of *campylobacteriosis* include farms and abattoirs workers who sometimes do not practice hand-washing and food safety habits (Aung *et al.*, 2015). However, identification and understanding the transmission routes of *Campylobacter* infections is crucial for its prevention and control (Newell *et al.*, 2017). The common and major route/pathways of *campylobacteriosis* includes through faecal-oral routes (Rosner *et al.*, 2017), through consumption of contaminated undercooked meats or through consumption of contaminated food/water (Grzybowska-Chlebowczyk *et al.*, 2013).

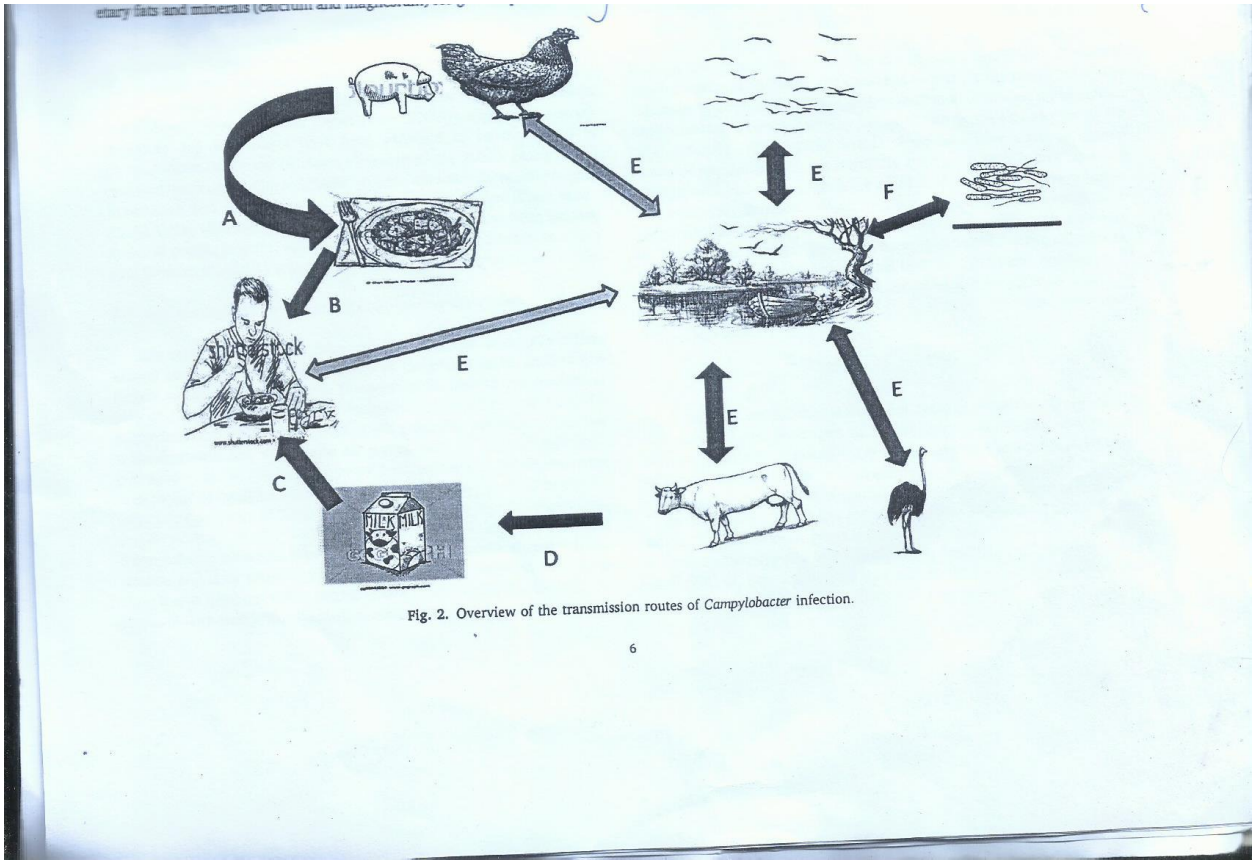


Fig. 2. Overview of the transmission routes of *Campylobacter* infection.

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Figure 2: Overview of the transmission routes of *campylobacter* infection

2.25 Milk as a route of *Campylobacter* transmission

Worldwide, there is a rise in the consumption of unpasteurized milk as a result of its health benefits compared to pasteurized milk (Sugrue *et al.,* 2019). Despites the health benefits in the consumption of unpasteurized milk, there is a great concern to the health risk its pose to human (Baars *et al.,* 2019). Milk is a white liquid and a nutrient-rich food produced in the mammary glands of mammals. It's a source of protein, dietary fats and minerals (calcium and magnesium) for growth particularly in children (O'Callaghan *et al.,* 2019). Milk is consumed either unpasteurized or pasteurized and mammals that produced milk for human consumption includes sheep, buffalo, goats, cows, yak and camel and the highest proportions of commercially produced milks are from cows (Quigley *et al.,* 2013).

Milk is considered germ-free when secreted in the alveoli of the udder (Vacheyrou *et al.,* 2011). Fresh milk drawn from animals naturally possess a short lived antibacterial system that display 'germicidal' or 'bacteriostatic' properties but bacterial growth is inevitable after sometimes except it undergoes heat treatment or freezing (Sarkar, 2016). Milk is a good substrate for bacteria growth (Hudson *et al.,* 2014) and it's reported to be among the major transmission routes for *Campylobacter* to humans (El-Zamkan and Hameed, 2016).

Milk is natural foods that has no protection against external contamination and can easily be contaminated when separated from it source (Neeta *et al.,* 2014). Milk contamination generally occurs from environmental sources such as water, grass, milking equipments, feed, air, teat apex, soil and other sources (Coorevits *et al.,* 2008). It's believed that the occurrence of *Campylobacter* species in raw milk samples is from faecal contamination (Oliver *et al.,* 2005).

Campylobacter species have been detected in cow milk (Del Collo *et al.,* 2017 *et al.,* 2017), and different *Campylobacter* species that have been detected in milk samples from different mammals including C. *Jejuni* detected in buffalo and cow milk (Modi *et al.,* 2015) and C. *coli* identified in cow milk (Rahimi *et al.,* 2013).

Campylobacter species have also been detected in bulk tank milk where these milks are stored (Bianchini *et al.,* 2014), and *Campylobacter* species reported to have been detected from milk samples from the bulk tank include C. *lari*, C. *jejuni* and C. *coli* (Del Collo *et al.,* 2017). Globally, several cases of illness and deaths have been reported to occur via consumption of contaminated raw milk and its products (Hati *et al.,* 2018), and in many countries, milk-borne pathogens are of public health concern (Amenu *et al.,* 2019).

2.27 Meat as a Route of *Campylobacter* Transmission

Worldwide, consumption of meats is steadily increasing and meats are sometimes contaminated with microorganisms but bacteria contaminations may sometimes occur from animal microbiota, equipment surfaces and water (Vihavainen *et al.,* 2007). The bacteria from animal's microbiota that majorly contaminate meats include pathogenic Salmonella and *Campylobacter* species and these two bacteria species are majorly responsible for human gastroenteritis as a result of consumption of contaminated undercooked meat (Rouger *et al.,* 2017). *Campylobacter* contamination remains the major cause of bacterial food-borne infection and the major reservoir of these bacteria species are poultry (Wieczorek *et al.,* 2015). Infections caused by poultry consumption represents about 50–70% of the global *Campylobacter* infections cases (Seliwiorstow *et al.,* 2015), and poultry is define as meats from chicken, turkey, duck and goose (Szosland-Fałtyn *al.,* 2018).

Beside poultry, *Campylobacter* species have also been detected in other meat typss such as pork and beef (Hussain et al., 2007; Korsak *et al*., 2015), et mutton (Nisar *et al.,* 2018) and in camel, lamb and chevon (Rahimi *et al.,* 2010). *Campylobacter* species that have been isolated and detected in meat samples include C. *coli* and C. *jejuni* identified in poultry meat (Mezher *et al.,* 2016), C. *coli*, C. *lari*, C. *jejuni* and C. *fetus* detected in mutton samples (Sharma *et al.,* 2016), and C. *jejuni* and C. coli detected in pork, beef and lamb (Wong *et al.,* 2007). Isolation and detection of these bacteria species from meats samples position them as one of the major transmission route (Duarte *et al.,* 2014).

2.28 Epidemiological information of *Campylobacter* Outbreaks

The reports in the incidences of *Campylobacter* outbreaks differs among countries and the true nature of the global occurrence rate is largely unknown (*WHO, 2013*). The reasons for lack of true incidences rate of *Campylobacter* outbreaks includes underreporting of *Campylobacter* infection cases, differences in the reporting systems, difficulties with diagnosis and differences in surveillance in case of outbreaks (Hansson *et al.,* 2018). *Campylobacter* outbreaks are usually either from waterborne or foodborne infection involving several persons (Frost *et al.,* 2002), and majority of *Campylobacter* outbreaks are usually from animal origin (Wilson *et al.,* 2008).

Although, in low income countries, *Campylobacter* outbreaks are majorly from environmental sources such as streams and river where many people depend on these water bodies as their major drinking water source (Clark *et al.,* 2003; Platts-Mills and Kosek, 2014).

Beside involvement of water sources in human infection in low income countries, water sources have also been reported to be implicated in *Campylobacter* outbreaks in high income countries such as Norway (Jakopanec *et al.,* 2008), New Zealand (Bartholomew *et al.,* 2014), Canada (Clark *et al.,* 2003), Finland (Kuusi *et al.,* 2004) and Denmark (Kuhn *et al.,* 2017). *Campylobacter* milk borne infection and outbreaks have also been reported in several high and low income countries (García-S_anchez *et al.,* 2017). Some of the countries with records of *campylobacteriosis* outbreaks including the Netherlands (Bouwknegt *et al.,* 2013), Israel (Weinberger *et al.,* 2013), China (Chen *et al.,* 2011), Japan (Kubota *et al.,* 2011), India (Mukherjee *et al.,* 2013), Sweden (Lahti *et al.,* 2017), Mexico (Zaidi *et al.,* 2012) and the United States (Geissler *et al.,* 2017; Gilliss *et al.,* 2013). Also, other nations where there have been records of *Campylobacter* outbreaks includes Canada (Keegan *et al.,* 2009; Ravel *et al.,* 2016), British Columbia (Stuart *et al.,* 2010), Australia (Kaakoush *et al.,* 2015; Unicomb *et al.,* 2009), the United Kingdom (Tam *et al.,* 2012), Belgium (Braeye *et al.,* 2015), Denmark (Nielsen *et al.,* 2013), Germany (Hauri *et al.,* 2013), Norway (Steens *et al.,* 2014), Poland (Sadkowska-Todys and Kucharczyk, 2014), New Zealand (Berger, 2012; Sears *et al.,* 2011), Madagascar (Randremanana *et al.,* 2014), Malawi (Mason *et al.,* 2013), Kenya (O'Reilly *et al.,* 2012; Swierczewski *et al.,* 2013), Iceland and Estonia (Skarp *et al.,* 2016), Guatemala (Benoit *et al.,* 2014) and Peru (Lee *et al.,* 2013).

2.29 Prevention and Treatment of *Campylobacter* Infections

Prevention of *Campylobacter* infections can be directly applied to humans by different ways including sewage sanitary conditions, provision of portable water, vaccine usage, public awareness concerning the significance of pasteurization of milk, proper cooking of food from animal origins and the use of therapeutics in case of infections (Hansson *et al.,* 2018). Prevention of *Campylobacter* infections can also be directed on animals by phage treatment (Borie *et al*., 2014), probiotics, prebiotics, and by improved biosecurity such as the provision of good water quality at farm level and also by monitoring the regular use of antibiotics in animal husbandry. Another vital preventive measure that will help lower the level of these bacteria is the withholding of feed from poultry for about 12 h before slaughter (Hansson *et al.,* 2018).

Campylobacter infections are sometimes self-limiting but in most cases fluid and electrolyte replacement are major supportive measures for the treatment of this infection (Guarino *et al.,* 2014). Beside fluid and electrolyte replacement, antibiotics are used when

symptoms pesist and antibiotics treatments are most effective when started within three days after onset of illness.

Nonetheless, antibiotics are regularly used in *Campylobacter* infected patients with diarrhea, high fever or patients with other severe illness like weakened immune systems, AIDS, thalassemia, and hypogammaglobulinemia (CDC, 2016). Antibiotics drugs of choice for the treatment of *campylobacteriosis* includes fluoroquinolones, aminoglycosides, tetracycline, macrolides, betalactams (Bolton, 2015) and erythromycin (Bardon *et al.,* 2009). Other useful alternative antibiotics drugs of choice include ciprofloxacin, vancomycin (Bruzzese *et al.,* 2018) and quinolones (Gilber and Moellering, 2007).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Materials

Filter paper
Nutrient agar
MacKonkey agar
Cotton wool
Methylated spirit
Alcohol
Fuel paper
Avocado oil
Test tubes
Distilled water
Bursen burner
Flat bottom flask
Inoculation loop
Petri dishes
Wire loop

Autoclave

3.2 Sample Collection

Persea americana seed used in this study were purchased from Uchi Market, Etsako West Local Government Area, Auchi in Edo State.

3.3 Source of Microorganism

The organism was collected from the intestinal tract of chicken which was purchased from a local market (Uchi Market) in Auchi, Edo State.

3.4 Methodology

The avocado was cut using a knife and the peels were removed while the raw materials sizes were reduced manually. It was thereafter dried for seven (7) days until its moisture content was removed. The dried avocado seeds were milled using a hydraulic press to obtain a smooth mixture. The oil was extracted using soxhlet extractor and this was done using N-hexane as the solvent.

3.5 Serial Dilution

Serial dilution was used in standard plate counts because the number of bacteria in the sample (intestinal tract of chicken) is unknown. The sample was diluted to obtain a number of CFU (Colony forming units) that supplies statistically significant result. After dilutions were prepared, an amount of liquid was spread over the surface of an agar plate (MacKonkey and nutrient agar) and then incubated to allow for bacteria growth. CFU count from these diluted plates were used to calculate the number of bacteria and cells in the original (undiluted) plate.

Syringe and flat bottom flask were sterilized using a bursen burner. The agar was poured on petri dishes after cooling and allowed to solidify for thirty minutes and placed in an incubator.

3.6 Procedure

Different percentage of alcohol oil were prepared:

2.5% mil oil to 7.5mil alcohol

5% mil oil to 5% mil alcohol

7.5% mil oil to 2.5% mil alcohol

10% mil oil to 0% mil alcohol

Campylobacter jejuni is colourless

3.7 Antibacterial Subseptibility Test (Disc Diffusion Test)

60 micro liters of the sample enters the 5mm hole in the Nutrient Agar and MacKonkey agar prepared.

Antibacterial susceptibility test was done by taking a sample from the infected site and detect the possible drug resistance in common pathogen and to assure susceptibility for drug of choice for particular infections.

Antibacterial sensitivity testing is the measurement of the susceptibility of bacteria to antibiotics. It is used because bacteria may have resistance to some antibiotics.

Sensitivity testing usually occur in a medical laboratory and uses culture media method that expose bacteria to antibiotics or genetics that test to see if bacteria have genes that confer resistance. Culture method often involves measuring the diameter of areas without bacterial growth called zone of inhibition.

The minimum inhibitory concentration of the antibiotics is the lowest concentration of the antibiotics that stops the growth of bacteria.

The avocado seed oil was used to carry out susceptibility testing with different percentage of oil to alcohol.

CHAPTER FOUR

RESULT AND DISCUSSION

4.1 Result

Result for the antibacterial properties of *Persea americana* seed oil on *Campylobacter jejuni* is presented below:

Figure 1 shows the diagrammatical representation of *Campylobacter jejuni* activities of avocado seed oil using different anti biotics. Figure 2 shows the minimum inhibitory contration of avocado seed oil against *Campylobacter Jejuni* and figure 3 show anti microbial susceptibility of antibiotics; Ceftazidime (30µg), Nitrofurantoin (1.0µg) ofloloxacin (5 µg), Gentamicin (10µg) against *campylobacter jejuni.* Plate 1 shows the effect of avocado seed oil on *campylobacter jejuni* and plate 2, antibacterial subsceptibility of conventional antibiotics to *campylobacter jejuni.*

Table 4.1: Zone of inhibition (mm) of *Persea americana* seed oil at different diffusion ranges

Dilution	**Zone of inhibition**
25%	10.5mm
50%	22mm

The table above shows that the test of *Campylobacter jejuni* on *Persea americana* seed oil at 25% dilution ratio has a zone of inhibition of 10.5mm while at 50% has a zone of inhibition of 22mm. This signifies that *Campylobacter jejuni* reacts more on *Persea americana* seed oil at a higher diffusion ratio compared to lower diffusions.

Table 4.2: Mean zone of inhibition (mm) of *Persea americana* seeds oil against *Campylobacter jejuni* using different antibiotics

Antibiotics	**Zone of inhibition**
Ceftazidime	2mm
Nitrofurantoin	5mm
Ofloxacin	13.5mm
Gentamicin	8mm

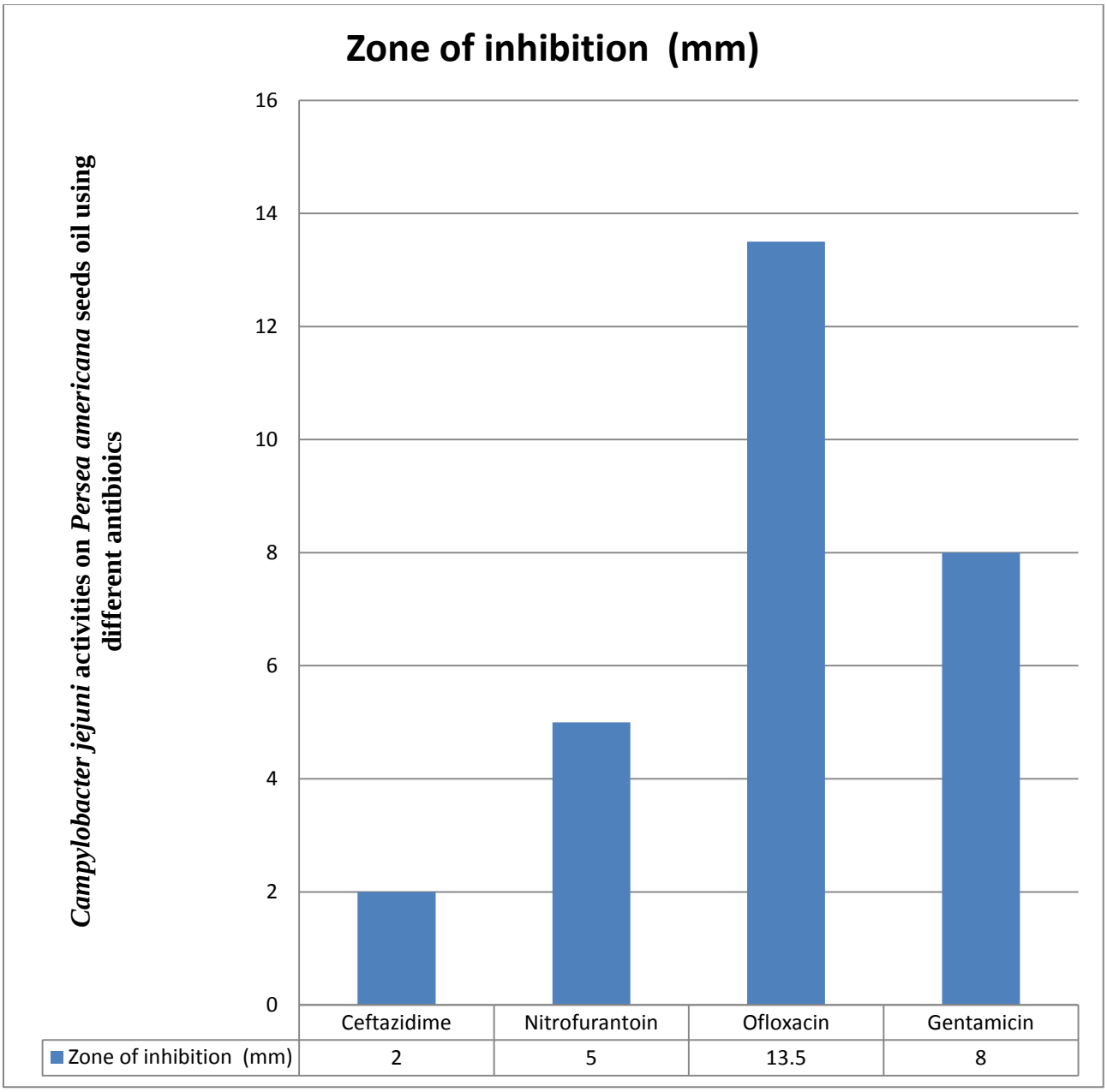

Fig 4.1: Diagrammatical representation of *Campylobacter jejuni* activities of *Persea americana* seeds oil using different antibiotics

Fig 4.2: Antibacterial effects of Avocado seed oil on *Campylobacter Jejuni*

4.3: Antibacterial effects of antibiotics on *Campylobacter Jejuni.*

4.2 Discussion

Plant extracts are sources of a variety of biotechnology products. Therefore, countless studies have been conducted in order to evaluate characteristics of these extracts, which can be used for the treatment of diseases, due to their antimicrobial, antifungal, analgesic, anti-inflammatory and antitumor activities (Qing-Yi *et al*., 2005; Leite *et al*., 2009; Rodríguez-Carpena *et al*., 2011). Among the commonly evaluated properties, the antibacterial activity has received special attention, and numerous studies

have been conducted, including different avocado extracts (Gomez-Flores *et al*., 2008; Castro *et al*., 2010; Rodríguez-Carpena *et al*., 2011).

About 80% of the world population in Africa depends on traditional medicine for primary health care. *Persea americana* (Avocado) is among the useful plant used for traditional medicine in Africa. Different parts of these plant bodies, extracted with different types of solvent have been used by researchers for investigating its properties.

However, although widely used, there are not yet any standardization methods to analyze the antimicrobial activity of extracts of natural products (Ostrosky *et al*., 2008). The Disk diffusion test is indicated by the FDA (Food and Drug Administration/USA) and established as standard by the CLSI (Clinical Laboratory Standard Institute/USA, 2010), and, therefore, was the method chosen to conduct this study.

Extraction solvent is N-hexane and this indicates that the active constituents of the oil have more ability to be dissolved in organic medium. The result of this study highlights that the organic solvent extracts exhibited greater antimicrobial principles were either polar or non-polar and they were extracted only through the organic medium.

The analysis was done using diverse antibiotics to check their degree of resistance to microbes (*Campylobacter jejuni*). The result revealed that Ofloxacin has the highest zone of inhibition at 13.5mm, followed by Gentamicin and Nitrofurantoin with zone of inhibition 8mm and 5mm respectively. Ceftazidime has the least zone of inhibition of 2mm.

Ofloxacin having the highest zone of inhibition indicates that the active constituents of the oil have more ability to be dissolved when reacting with Ofloxacin that other antibiotics used in the study. This means that infections caused by this organism could be managed effectively using a single dose of Ofloxacin. This result is similar to the findings of Umeaku *et al.* (2018). Ceftazidime having the least zone of inhibition against *Campylobacter jejuni* shows that, amongst the tested antibiotics, it possess the least antibacterial activities against the selected isolates. This implies that more concentration of Ceftazidime needs to be applied for potency purpose.

CHAPTER FIVE
CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The study revealed that *Persea Americana* seed oil extract exhibited pronounced antibacterial activities against the tested pathogenic organism; *Campylobacter jejuni*. From the above evidence, it is clear that the oil extract of the studied plant possesses antibacterial therapeutic properties especially when extracted with N-hexane and could serve as alternative therapy for some microbial infections. This therefore supports the folklore usage of the studied plant and the traditional knowledge of local users for treating bacteria and suggests that medicinal plants could be more economical and safe in treating these bacteria.

5.2 Recommendations

The followings are the recommendations;

1. Proper hygiene
2. Subjecting food to heat treatment
3. Separation of raw and coked meat
4. Keep food at safe temperatures
5. Birds should be kept in closed housing conditions
6. Good hygienic slaughtering practice reduces the contamination of carcasses by faeces.
7. Training in hygienic food handling for abattoir workers and raw meat producers is essential to keep contamination to a minimum

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APPENDIX

NUTRIENT AGAR

14g was dissolved in 500ml of distilled water

MACKONKEY AGAR

25.7g was dissolved in 500mi distilled water