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## Quality and safety assessment of selected edible oils and their antimicrobial activities against foodborne pathogens

Olasunmbo Abolanle Ajayi\*, Okiemute Ovokeroye Erhiano, Michael Olutoyin Afolabi

<sup>1</sup>Food Science and Technology Programme, College of Agriculture, Engineering and Science, Bowen University, P. M. B. 284, Iwo, Osun State, Nigeria.

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An investigative study on the quality (moisture, iodine, fatty acid, saponin, and alkaloids) of edible (olive, coconut, palm, and soybean) oils and antimicrobial activity were carried out on three important foodborne pathogens (*Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*). Four dilutions of edible oils were prepared (undiluted; 1:1; 1:10; and 1:20 oil:ethanol) and impregnated into the discs, using standard methods. Moisture content ranged from (0.05±0.02 to 0.65±1.01 %), saponification ranged from (38.78±6.02 to 95.76±6.15 mgKOH/g). Iodine ranged from (15.64±0.02 to 24.2±0.02 gI<sub>2</sub>/100g), free fatty acid (1.33±0.04 to 9.10±0.48 %) and peroxide value ranged from (24.00±0.00 to 51.67±5.8 mEq/kg). Selected oils tested positive for phytochemicals, except palm oil, which was negative for saponin. Zone of inhibition against selected foodborne pathogens *E. coli* were (8.0±0.0 to 13.5±2.1 mm), (8.0±0.0 to 9.5±0.71 mm) and (9.0±0.0 to 11.5±0.1 mm) *K. pneumoniae* (8.0±0.0 to 9.5±0.7 mm), (8.0±0.0 to 9.5±0.71 mm), (8.0±0.0 to 9.0±0.0 mm); *S. aureus* (7.0±0.0 to 9.0±0.0 mm), (7.8±0.0 to 9.0±0.0 mm) and (7.5±0.0 to 9.0±0.0 mm) for olive, coconut and soya oils respectively. Palm oil had no inhibitory effect against the isolates and the undiluted oils had more inhibitory effects than diluted oils. Olive, coconut, and soybean oils show inhibitory capability against selected foodborne pathogens.

**Keywords:** Antimicrobial activity, edible oils, heated, foodborne pathogens

### Introduction

Edible oils can be consumed raw or heated, when used in cooking. Edible oils except oleomargarine-based products are of plant origin (Belitz et al., 2009), and examples include coconut oil, palm oil, olive oil, castor oil, groundnut oil, which are derived from seeds and nuts of plants through different methods of extraction (Belitz et al., 2009).

Olive oil (*Olea europaea*) is the oldest vegetable oil with distinct flavour. It is used in the food industry as frying oil and salad dressing (Houshia et al., 2014). Furthermore, in some religious circles, it is used as anointing oil and as an added ingredient in some cosmetics and hair products. Olive oil has been documented to have antimicrobial capability against selected foodborne pathogens (Janakat et al., 2015).

Coconut oil has various applications in the food industry. It is used in the preparation of infant milk and as fat in ice cream preparation. It also has applications in other industries, such as pharmaceutical, cosmetic and plastic industries (Gopala et al., 2010). Virgin coconut oil inhibits pathogenic bacteria such as *Helicobacter* spp (Lieberman et al., 2006). It attacks and kills viruses that have a lipid (fatty) coating, such as herpes, HIV, hepatitis C, the flu virus and more according to Kabara (2000).

About 80- 90% of palm oil (*Elaeis guineensis*) produced is used in cooking, consumed raw with boiled yam in some cultures or as an ingredient in food products while about 10% is used in various industries such as biodiesel, cosmetics and pharmaceutical industry (Shimizu and Desrochers, 2012). Tocotrienol (a type of Vitamin E) is abundant in palm oil and is used in cosmetic industry to increase sunscreen efficiency by limiting penetration of UV rays into the skin which can cause cellular ageing (Ching, 2012). An *in vivo* study by Kabara et al. (1972) showed that palm oil did not have antibacterial activity against *Escherichia coli*.

Soybean (*Glycine max*) oil, a by-product of soybean meal, has recently been used as fuel in biodiesel production (Goldsmith, 2008). Increase in the consumption of processed food in Western diets to which oils are added for their taste, nutrition and cooking performance, has resulted in increase in the consumption of soybean oil in U.S, China and Brazil (Goldsmith, 2008). Soybean oil is used mainly in cooking as oil for frying, in salads, processed foods and recently, in biodiesel. Soybean oil has been documented to show antimicrobial activity against microorganisms such as *E. coli*, *T. rubrum* and *C. albicans* according to Tabassum and Vidyasagar (2014).

In Nigeria, these edible oils are produced at the village level and not much attention is placed on the environmental conditions of the production areas, or the quality and hygeinic condition of the final product. The quality and safety of edible oil is very important to the health of consumers, hence WHO/FAO in (1999) set guidelines and standards for edible oils (moisture 0.2 %; acid value

0.6 mg/KOH/g; peroxide value of 10 mEQ/Kg) to minimize possible health hazards relating to adulteration. Recently, attention has shifted towards the use of plant oils such as coconut, olive, etc. for hair and skin care because they are acclaimed to have antimicrobial properties. This research aims to investigate whether edible oils from the open markets in Nigeria are of standard quality and if they possess antimicrobial activities against selected foodborne pathogens. **Materials and Methods**

Labeled edible oils (coconut, olive and soya bean) and un-labeled palm oil were procured from the open market in Iwo, Osun State and immediately transported to the Food Science and Technology Laboratory at Bowen University, Iwo, Osun State. These oils were stored in the dark at ambient ( $37 \pm 2$  °C) temperature until used. Pure culture of previously identified and labelled foodborne pathogens (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*) kept in nutrient agar slants were obtained from the Microbiology Laboratory, Bowen University.

### **Methods**

All analyses (except moisture content and antimicrobial activities were carried out in triplicates.

### **Physicochemical analyses**

Moisture content was determined according to AOCS method Ca 2b-38 (AOCS, 2017), while saponification value, iodine value, free fatty acid and peroxide value were performed according to Nielsen et al (2017).

### **Phytochemical Screening**

All phytochemical screenings were carried out in triplicates.

### **Alkaloids**

A few drops of Wagner's reagent was added to 3ml of sample and the formation of reddish/brown precipitate confirmed the presence of alkaloids (Longbap et al., 2018).

## Phenols

Phenol screening was carried out using Ferric chloride test. About 3 to 4 drops of ferric chloride solution were added to 3ml of sample and the presence of phenols was indicated by bluish black colouration (Hasbourne, 1973).

## Saponins

Following the method of Hasbourne (1973), 10mL of distilled water was added to 1ml of sample and shaken vigorously. The formation of foam stable for about 1 to 2 minF indicated the presence of saponins.

## Flavonoids

Flavonoids was performed according to Hasbourne (1973). About 3 drops of sodium hydroxide was added to 3ml of sample. The presence of flavonoids was indicated by the formation of yellow colouration.

## Antimicrobial analysis

### Preparation of bacteria inoculum

The pure culture of test organisms *E. coli*, *K. pneumoniae* and *S. aureus* previously identified and maintained in Nutrient agar slants, kept at room temperature ( $30 \pm 2^{\circ}\text{C}$ ) were further tested. Various biochemical analyses (Gram stain, catalase, indole, citrate, motility, MRVP and carbohydrate fermentation) studies were carried out to confirm the identity of the test organisms. After which they were sub-cultured overnight until the inoculum density equivalent to a 0.5 McFarland turbidity standard was obtained.

### Disk diffusion method

Disks of 6 mm in diameter were prepared from Whatman No. 1 filter paper and sterilized before use. Each isolate was streaked on to Mueller-Hinton agar plates in duplicates and allowed to air-dry for 10 minutes at room temperature. Triplicate discs impregnated with 20 $\mu\text{L}$  of edible oil samples of varying concentrations [undiluted; 1:1; 1:10; and 1:20 (v/v) oil:ethanol] were prepared, and the disks were aseptically placed on the Mueller-Hinton plates streaked with pathogens and incubated for 18 h at  $37^{\circ}\text{C}$ . Undiluted but heated ( $75^{\circ}\text{C}$ ) edible oils according to (Hasika et al., 2014) were also analyzed. Zones of inhibition were measured in mm after incubation overnight.

## Statistical Data Analysis

The data obtained was analysed using IBM SPSS Statistics Version 20. The mean and standard deviation were recorded.

## Results and Discussion

Physicochemical qualities of edible oils

The result of the quality assessments of the selected oil samples are presented in Table 1. Moisture content ranged from (0.05±0.01 to 0.65±1.01 %). Coconut oil had the highest value, but not significantly different from other oils in this study. Only coconut oil surpassed the WHO standard of (0.2 %) recommended for edible oil (WHO FAO, 1999). Moisture content of oil is a quality assessment of oil since rancidity is promoted by moisture, affecting the shelf life of all food products. High moisture content in oils could lead to hydrolytic rancidity in oils. Microorganisms also thrive well in the presence of high moisture content. Saponification value of the oils ranged from (38.78±6.02 to 95.76±6.15 mgKOH/g). Coconut oil had the highest saponification value and significantly different ( $p<0.05$ ) from palm, olive and soybean oil. The values reported for all the edible oils contradict the report of (117 to 329 mg/kg) for coconut from Boisa et al (2020); the value of palm oil (202.39 mg/g) and soybean oil (187.1 mg/g) observed by Wazeed et al (2023). Iodine value ranged from 15.64±0.02 to 24.23±0.02. The iodine value of the oils in this study contradicts the reports of Ahmad et al (2015), Boisa et al (2020) and Suryani et al (2020) for olive, coconut, and palm oils respectively. Oils with higher iodine value have more unsaturated fatty acids (Negash et al., 2019), and absorb more iodine (Nielsen et al., 2017)

**Table 1.** Physicochemical properties of selected edible oils

Samples	Quality Parameter				
	Moisture content (%)	SV (mgKOH/g)	IV (gI <sub>2</sub> /100g)	FFA/ Acid Value (oleic %)	PV (mEq/kg)
Olive oil	0.05 <sup>a</sup> ±0.02	48.56 <sup>b</sup> ±4.68	15.64 <sup>d</sup> ± 0.02	1.33 <sup>c</sup> ±0.04	51.67 <sup>a</sup> ±5.77
Coconut oil	0.65 <sup>a</sup> ±1.01	95.76 <sup>a</sup> ±6.15	21.57 <sup>b</sup> ± 0.43	2.54 <sup>b</sup> ±0.38	37.67 <sup>b</sup> ±0.58
Palm oil	0.13 <sup>a</sup> ±0.02	41.85 <sup>b</sup> ±8.86	24.23 <sup>a</sup> ± 0.02	9.10 <sup>a</sup> ±0.48	35.67 <sup>b</sup> ±1.15
Soya oil	0.19 <sup>a</sup> ±0.00	38.78 <sup>b</sup> ± 6.02	17.34 <sup>c</sup> ±0.43	1.64 <sup>c</sup> ±0.36	24.00 <sup>c</sup> ±0.00

SV- Saponification Value; IV- Iodine Value; FFA- Free Fatty Acid; PV- Peroxide Value. Test values are means with standard deviation. Duncan means with same letters within the column are not significantly different ( $p < 0.05$ ).

Free fatty acid value ranged from  $1.33 \pm 0.04$  in olive oil to  $9.10 \pm 0.48$  % in palm oil, and the value was significantly different ( $p < 0.05$ ) from the other oils. The free fatty acid value of soybean oil is within the range of Lanser et al (1991), but was higher than Eke-Ejiofor et al (2021)'s report (0.28 to 0.29 %). The free fatty acid value of coconut oil was similar. to the value of native cold-pressed coconut oil in Boisa et al (2020). Palm oil had higher value of free fatty acid than in Suryani et al. (2020) report. Thormar and Hilmarsson (2007), reported that the antimicrobial activity of fatty acids and monoglycerides depends on the number of carbon atoms and double bonds present in their fatty acid chain and that under certain conditions, they can inhibit the growth of vegetative forms of pathogenic bacteria. Peroxide value ranged from  $24.00 \pm 0.00$  to  $51.67 \pm 5.77$  mEq/kg. Olive oil had the highest peroxide value, and significantly different from other samples. The peroxide value of soybean oil was higher than the values found in Medina-Juárez and Gámez-Meza (2011). Peroxide value is a common test method for lipid oxidation (Nielsen et al., 2017). Oils with higher peroxide values would probably be unable to keep in storage for a long time. Saponification and iodine values according to WHO standard are (248-265 mgKOH/g oil; 6.3-10.6); (190-209 mg KOH/g oil, 50-55.0); and (189-195 mg KOH/g oil, 124-139) for coconut, palm and soyabean oil respectively The quality of the edible oils in this study did not meet WHO standards in all quality parameters except in moisture content.5

### Phytochemical properties

Alkaloids, phenols, and flavonoids were present in all the samples. Saponins were not found in palm oil (Table 2).

**Table 2.** Phytochemical properties of selected oil(s)

Samples	Phytochemical properties			
	Alkaloids	Phenols	Saponins	Flavonoids
Olive oil	+	+	+	+
Coconut oil	+	+	+	+
Palm oil	+	+	-	+
Soya oil	+	+	+	+

+ = presence of the phytochemical; - = absence of the phytochemical

Phytochemicals are bioactive substances found in plants, and associated with antioxidative activities of plants (Suffredini et al., 2004). They are also associated with the plants ability to respond to microbial attacks (Akpomie et al., 2020). Al-Bayati and Suleiman (2008) reported that alkaloids had inhibitory effect against most bacteria. Studies have been carried out to test the antimicrobial activity of saponins (Maneemegalai and Naveen, 2010). Flavonoids are said to have antioxidant abilities (Noroozi et al., 1998). Phenols are known for their antioxidative and antimicrobial properties especially those contained in olive oil (Markovic et al., 2019). Flavonoids, alkaloids and saponins were not found in coconut oil in a study done by Boisa et al (2020).

### Antimicrobial activity of edible oil against selected foodborne pathogens

Undiluted edible (olive, coconut and soybean oil) showed greater antimicrobial activity against the Gram negative enteric bacteria compared to the Gram positive in this study (Table 3). Palm oil showed the least activity against all selected pathogens as the measurement of the zone of inhibition of bacterial growth were <6.5 mm. Solvent, ethanol was observed to have inhibitory effect on the pathogens and the 1:1 olive oil:ethanol and soybean:ethanol show effective antimicrobial activity against *E. Coli*. Further dilutions gave conflicting zones of inhibition for *Klebsiella* spp. None of the oils were observed to be particularly effective on *Staphylococcus* (Table 3). The result shows that olive, coconut, and soybean oils have antimicrobial activity against *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. Palm oil did not show any antimicrobial activity.

**Table 3.** Inhibition zone of isolates using various concentration of edible oils

Edible oil	Concentration	Zone of inhibition (mm)		
		<i>E. coli</i> *	<i>K. pneumoniae</i> *	<i>S. aureus</i> **
Olive oil	<b>Undiluted</b>	13.5 <sup>a</sup> ±2.1	8.0 <sup>b</sup> ±0.0	7.0 <sup>b</sup> ±0.0
Coconut oil		9.5 <sup>a</sup> ±0.7	9.5 <sup>a</sup> ±0.7	9.0 <sup>a</sup> ±0.0
Palm oil		<6.5	<6.5	<6.5
Soya oil		11.5 <sup>a</sup> ±0.7	9.0 <sup>b</sup> ±0.0	7.5 <sup>b</sup> ±0.7
Olive oil	<b>1:1</b>	9.5 <sup>a</sup> ±0.7	9.0 <sup>a</sup> ±0.0	9.0 <sup>a</sup> ±0.0
Coconut oil		8.0 <sup>a</sup> ±0.0	8.0 <sup>a</sup> ±0.0	7.8 <sup>a</sup> ±0.4
Palm oil		<6.5	<6.5	<6.5
Soya oil		11.0 <sup>a</sup> ±0.0	8.0 <sup>b</sup> ±1.4	8.0 <sup>b</sup> ±0.0 <sup>b</sup>

<b>1:10</b>			
Olive oil	8.0 <sup>b</sup> ±0.0	9.5 <sup>a</sup> ±0.0	8.0 <sup>a</sup> ±0.0
Coconut oil	8.0 <sup>a</sup> ±0.0	8.0 <sup>a</sup> ±0.0	7.8 <sup>a</sup> ±0.4
Palm oil	<6.5	<6.5	<6.5
Soya oil	9.5 <sup>a</sup> ±0.7	8.0 <sup>b</sup> ±0.0	8.0 <sup>a</sup> ±0.0
<b>1:20</b>			
Olive oil	8.0 <sup>a</sup> ±0.0	9.5 <sup>a</sup> ±2.1	8.0 <sup>a</sup> ±0.0
Coconut oil	9.0 <sup>a</sup> ±0.0	8.5 <sup>a</sup> ±0.8	8.0 <sup>a</sup> ±0.0
Palm oil	ND	ND	ND
Soya oil	9.0 <sup>a</sup> ±0.0	8.0 <sup>b</sup> ±0.0	9.0 <sup>a</sup> ±0.0
+Control	10.5±0.0	10.0±0.0	8.5±0.0
-Control	6.0±0.0	6.0±0.0	6.0±0.0

\* Gram negative foodborne pathogen; \*\* Gram positive foodborne pathogen; Values represent means ±SE (standard errors) (n = 3). Same letter across the row are not significantly different (p<0.05), using Duncan separation.

Polyphenols in olive oil have been investigated to be responsible for antimicrobial roles (Piroddi et al., 2017; Rigacci and Stefani, 2016). Oleuropein has been shown to have antimicrobial activity against both Gram-negative and Gram-positive bacteria although hydroxytyrosol has higher toxicity to bacteria cells than oleuropein (Bisignano et al., 1999). Pang and Chin (2018) exerts that oleocanthal, another phenol in olive oil, exhibits antimicrobial activity against *E. coli*, *S. enterica*, *L. monocytogenes*, *Helibacter pylori*, *S. aureus* and *Enterococcus faecalis*. Though individual phenols have inhibitory effect against pathogens, a greater inhibitory effect occurs when they are combined together (Lee and Lee, 2010) as seen in a study carried out on phenol-rich olive mill waste (Obied et al., 2007). According to Capiono et al (2001), some researchers opine that olive leaf has more polyphenol content than olive oil and olive fruit. This can explain the greater zone of inhibition by olive leaf extract in a study done by Himour et al (2017).

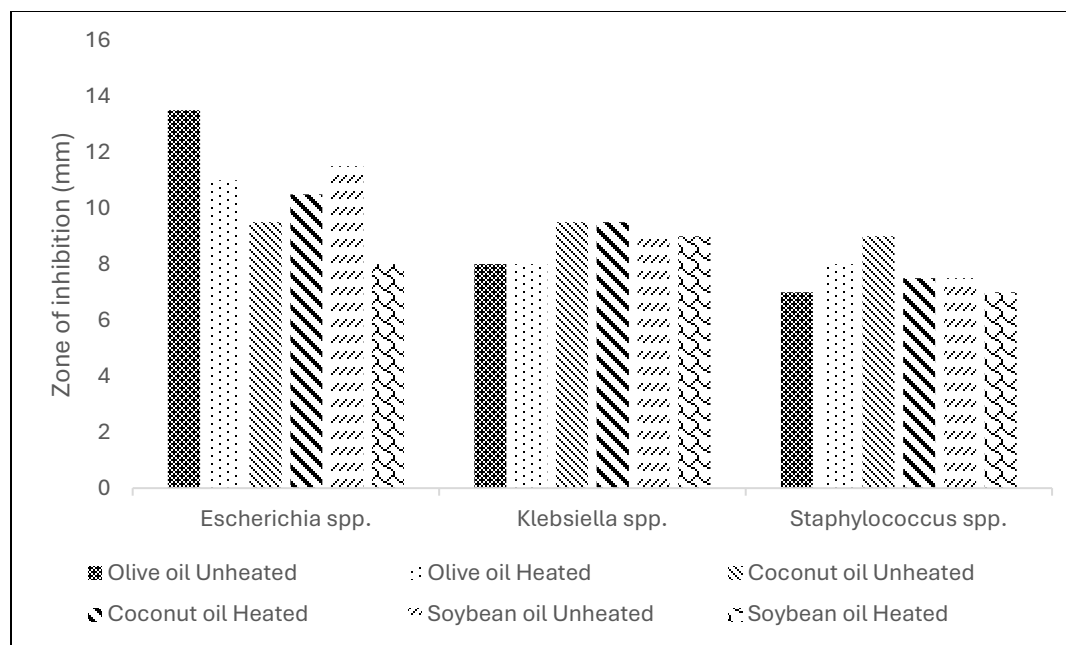
Since most edible oils are rarely consumed raw the oils were heated to simulate cooking and the antimicrobial effect was analyzed. Figure 1 shows a comparison between the undiluted, unheated and undiluted, heated samples. Heated olive and soybean oils showed slight reduction in the zone inhibition for *E. coli* and *Klebsiella*. However, heated olive oil had an increased activity against *S. aureus*, indicated by the zone of inhibition. Heated coconut oil was also effective against *E. coli*. There was no difference in the zone of inhibition between unheated and heated palm oil. Overall, the variations in the zone of inhibition between unheated and heated oils were minimal and not

significant. It is unclear how long the oils have been extracted, the storage conditions and if adulterated or unhygienically produced, and may contain pathogens (Seiyaboh et al., 2018). Since all of these factors may contribute to the reduction of polyphenols and other antioxidants present in the oils and inadvertently the antimicrobial capacity of the products.

The result derived from this study shows that coconut oil has inhibitory effect on *S. aureus* and *E. coli* which is in line with Akpomie et al (2020). However, it did not align with that of Abbas et al (2017) where coconut oil had more inhibitory effect on *S. aureus* than *E. coli*. Lauric acid, a major fatty acid in coconut oil, is said to have antibacterial activities and the antimicrobial activity of coconut oil can be attributed to this (Ugbogu et al., 2006).

Palm kernel oil, known to have antimicrobial effect against a number of pathogens (*E. coli*, *S. aureus*, etc.) according to the report of Floriana et al (2015), is derived from the same parent plant as palm oil (*Elaeis guineensis*). Neither heating or diluting the oil had any effect on the antimicrobial activity in this study. This might be because the palm may have been adulterated, a common practice in Nigeria (Abdullahi et al., 2023). Lack of inhibitory effect of palm oil on *S. aureus* and *E. coli* aligns with a research work conducted by Ekwenye and Ijeomah (2005).

There is less documentation on the antimicrobial capability of soybean oil as compared to the previously mentioned oils. Soybean oil inhibited all pathogens, which aligns with the study done by Tabassum and Vidyasagar (2014). The differences in cell wall structures in Gram positive and Gram-negative bacteria might be the reason for the difference in inhibitory effects of the various oils and the variations between the Gram negative bacteria can be as the result of difference in outer membrane proteins (Winfield and Groisman, 2004; Hobb et al., 2009; Silhavy et al., 2010). Altemimi et al (2017), stated that Gram negative bacteria were more susceptible to crude extracts compared to Gram-positive bacteria and this may explain the variations in inhibitions between this study and previous studies.



**Figure 1:** Zone of inhibition (mm) of unheated and heated oils against foodborne pathogens

#### Correlation Coefficients among Analyzed Factors

Results in Table 4 shows the correlation coefficients among the type of edible oil quality properties such as moisture, saponification, peroxidase, free fatty acid, iodine values and the zone of inhibition observed in undiluted oil samples. It was observed that the highest correlation coefficient was found between the peroxide value and the antimicrobial activity against *E. coli* ( $r^2 = -.961$ ); type of oil and PV ( $r^2 = -.953$ ); followed by moisture content and type of oil ( $r^2 = 0.942$ ); peroxide value also had strong correlation with iodine value ( $r^2 = .850$ ). In general, there were strong correlation coefficient of antimicrobial activity of the undiluted edible oils against the selected foodborne pathogens.

**Table 4.** Correlation coefficients among type of edible oils, quality properties and pathogens

Variables	Type of oil	Moisture	SV	PV	FFA	IV	<i>E.coli</i>	<i>K. pneumo</i>	<i>S. aureus</i>
Type of oil		.942	-.390	-.953	.263	.256	-.188	-.241	-.241
Moisture			-.539	-.839	.193	.077	-.170	-.244	-.244
SV				.149	-.212	.251	.415	.521	.521
PV					-.127	-.250	-.002	.037	.037
FFA						.850	-.961	-.931	-.931

SV=saponification value; PV= peroxidase value; FFA= free fatty acid; IV= iodine value

## Conclusion

The research showed that olive, coconut, palm and soybean oils contain alkaloids, phenols and flavonoids. Palm oil did not indicate the presence of saponins unlike olive, coconut and soybean oils. Olive, coconut and soybean oils had antimicrobial activities against the selected foodborne pathogens (*Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*). These oils had their greatest inhibitory effect against *E. coli* with the oils being most effective when undiluted and unheated. Palm oil was not observed to show antimicrobial activity against any of the selected pathogen in this study. Measured zones of inhibition of heated edible samples were compared to the unheated samples, there were variations in the antimicrobial activities of olive, coconut and soybean oils with pathogens after heating. Further statistical analysis indicated a strong correlation between type of oil, quality properties and the zone of inhibition of selected pathogens.

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